Investigating novel TRAIL sensitizing drugs for Glioblastoma multiforme

by

Filiz Şenbabaoğlu

A Thesis Submitted to the
Graduate School of Engineering
in Partial Fulfillment of the Requirements for
the Degree of

Master of Science
in
Bio-medical Sciences and Engineering

Koc University

June 2014

Koc University

Graduate School of Sciences and Engineering

This is to certify that I have examined this copy of a master's thesis by

Filiz Şenbabaoğlu

and have found that it is complete and satisfactory in all respects,
and that any and all revisions required by the final
examining committee have been made.

Committee Members:

_____

Tuğba Bağcı-Önder, Ph. D. (Advisor)

_____

Burak Erman, Ph. D.

_____

Özgür Öktem, M. D.

Date: _____

ABSTRACT

Glioblastoma multiforme (GBM) is the most aggressive and frequent type of primary brain tumor. Even though only 1% of adult cancers are GBMs, their malignant nature makes them the fourth greatest cause of cancer deaths. Treatment of GBM is extremely difficult due to several factors. First, the tumor cells, despite their relatively rapid cell cycle, are quite resistant to conventional therapies. In addition, the blood–brain barrier (BBB) permeability makes it difficult to achieve the desired effect of chemotherapeutics without dose–limiting systemic side effects Current treatment options for GBM include surgery, radiation therapy and chemotherapy. However, development of resistance to these approaches is frequent. For this purpose, new treatment options are needed for GBM patients.

Apoptosis, programmed cell death, plays a central role in the development and homeostasis of multicellular organisms. Two main characterized apoptosis pathways are defined in mammalian cells: extrinsic and intrinsic pathways. Intrinsic pathway includes the activity of mitochondria and is controlled by Bcl-2 protein family members. Extrinsic pathway is mediated by death receptors, which are a subgroup of the tumor necrosis factor (TNF) receptor superfamily. In cancers, including GBMs, apoptosis pathway is suppressed, and tumor cells adopt pathways to evade it. However, re-activating apoptosis by using extrinsic ligands can be a prominent therapeutic approach. As such, TNF-related apoptosis-inducing ligand (TRAIL) has the ability to induce apoptosis in tumor cells, but it does not have cytotoxicity on normal cells, which makes it a perfect candidate for GBM treatment.

Although TRAIL is a prime therapeutic candidate, many cancer cells are intrinsically resistant and/or acquire resistance to TRAIL. The mechanism of TRAIL resistance is still not completely solved, but it is believed to be due to dysregulations at multiple levels from death receptors to executor caspases within the apoptotic pathway. TRAIL resistance can be overcome by combination therapies. Combination of TRAIL with chemotherapeutics, radiation or other novel therapeutic drugs is a promising approach based on several pre-clinical observations.

In this thesis, we describe our approach of identifying novel TRAIL-sensitizing drugs among known and approved chemicals and utilizing high throughput screening (HTS) method. HTS is a method used for drug discovery, where large numbers of drug-like compounds are tested using robotic methods, data processing and control software, to identify promising leads.

Drug repurposing is the application of known drugs to other uses, which takes off years required to develop a new drug and provides a drastic economic benefit. In addition, a repurposed drug already comes with the systemic toxicity knowledge, hence reducing cytotoxicity problems. With this in mind, a HTS with a chemical library consisting of 1200 Food and Drug Administration (FDA) approved drugs were utilized for investigating TRAIL sensitizers for GBM. Accordingly, we identified a class of drugs, called cardiac glycosides, with a novel TRAIL sensitizing function for GBM cells. We also investigated the downstream changes in apoptosis pathway components upon the application of this new class of drugs. Our results suggest that combination of cardiac glycosides and TRAIL can be a promising therapeutic approach for GBM patients.

ÖZET

Glioblastoma multiforme (GBM) beyin tümörleri arasında en sık görülen ve en agresif primer beyin tümörüdür. Yetişkinlerde görülen kanserler arasında sadece %1'lik dilim GBM' e ait olsa da, kötü huylu yapısı nedeniyle tüm kanser ölümleri arasında dördüncü sıradadır. GBM tümörlerinin tedaviye yüksek direnç göstermesi, beyinde bulunan ve sınırlı geçirgen özelliğe sahip Kan-Beyin Bariyeri'nden (KBB) geçişi kemoterapi tedavisinin zorlaştırması gibi faktörlerden dolayı GBM tedavisi güçleşmektedir. Ayrıca hücreler mevcut tedavi biçimleri olan cerrahi müdahale, radyoterapi ve kemoterapiye çok hızlı direnç gösterdikleri için bu tür tedaviler GBM hastalarının yaşam sürelerini ve kalitesini yeterince arttıramamaktadır. Tüm bu sebeplerle GBM tümörlerinde yeni tedavi yaklaşımlarına ihtiyaç duyulmaktadır.

Apoptoz, çok hücreli organizmaların gelişimi ve homeostazında önemli bir rol oynayan programlı hücre ölümüdür. Memeli hücrelerinde tanımlanmış iki temel apoptoz yolağı vardır: Ölüm Reseptörleri Yolağı ve Mitokondriyal Yolak. Mitokondriyal apoptoz yolağı mitokondri aktivitesi içerir ve Bcl-2 protein ailesi üyeleri tarafından kontrol edilir. Ölüm reseptörleri yolağı ise tümör nekroz faktörü (TNF) reseptör üst ailesinin bir alt grubudur ve ölüm reseptörleri tarafından yönetilir. Kanser hücrelerinde apoptozda görev alan bu yolaklar baskılanmaktadır. Bu nedenle, dışarıdan ligandlar kullanılarak ölüm reseptörü yolağını aktive etmek önemli bir tedavi yaklaşımı olarak gözükmektedir. TNF-aracılı apoptoz-indükleyici ligand (TRAIL)'ın normal hücreler üzerindeki sitotoksik etkisi az olmakla beraber, kanser hücrelerinde apoptozu tetikleyebilmektedir. Bu nedenle TRAIL, GBM tedavisi için önemli bir adaydır.

TRAIL öncü bir tedavi adayı olmasına rağmen, pek çok kanser hücrelerinde TRAIL'e direnç görülmektedir veya bu hücreler zamanla TRAIL'e direnç kazanmaktadırlar. TRAIL direnç mekanizmaları tam olarak çözülememiş olsa da direncin ölüm reseptörlerinin düzensizliğinden, apoptoz yolağı içinde önemli bir rolü olan kaspaz enzimlerine kadar birçok nedenden kaynaklandığı düşünülmektedir. Kanser hücrelerindeki TRAIL direnci; kemoterapi, radyasyon veya diğer tedavi amaçlı kullanılan ilaçların kombinasyonu ile aşılabilmektedir.

Bu tezde, Yüksek Verimli Tarama (High Throughput Screening, HTS) yöntemi ile bilinen ve onaylı kimyasal kütüphanesi kullanılarak TRAIL direncini aşan ilaçlar belirlenmiştir. Bilinen ilaçların kullanımının yeniden yönlendirilmesi yeni bir ilacın kullanımı ve onaylanması için gereken süreyi ve ekonomik boyutu azaltacaktır. Buna ek olarak, onaylanmış ilaçların sistemik toksisite üzerinde bilinen etkileri de büyük bir avantaj sağlamaktadır. Bu düşünceyle GBM

hücrelerinde, Amerikan Gıda ve İlaç İdaresi (FDA) tarafından onaylanmış 1200 ilaçlı bir kimyasal kütüphane ile HTS metodu kullanılarak TRAIL direncini yenebilecek ilaçlar taranmıştır. Buna göre GBM hücrelerinin TRAIL'a karşı duyarlılığını arttıran yeni bir ilaç sınıfı tanımlanmıştır. Kardiyak glikozitler adı verilen bu ilaç sınıfı üyelerinin. apoptoz yolağı bileşenlerinin üzerindeki etkileri araştırılmıştır. Sonuçlarımız doğrultusunda kardiyak glikozitler ve TRAIL kombinasyonunun GBM hastaları için umut verici bir tedavi yaklaşımı olabileceğini öngörmekteyiz.

ACKNOWLEDGEMENTS

Firstly, I would like to dedicate my thesis to my advisor Dr. Tuğba Bağci-Önder. Apart from her scientific leadership and support, she has been an important role model for me in every aspect of life, defining the true meaning of mentorship. I am more than grateful to her and to be in her "scientific family tree" was amazing.

Secondly, I would like to thank Dr. Özgür Öktem, Dr. Tamer Önder and Dr. Nathan Lack for their support and guidance throughout my masters in Koç University School of Medicine. I owe special thanks to Prof. Dr. Burak Erman and Dr. Özgür Öktem for being in my thesis committee sparing their precious time for me.

I would like to thank the Koç University School of Medicine, Koç University Graduate School of Arts and Sciences and Istanbul Kalkınma Ajansı (ISTKA) for their funding during my master studies.

The members of TBO lab; Ahmet Cingöz, İbrahim Çağrı Kurt, Ezgi Kaya, Kubilay Kaymaz, Elifnur Erdem, Zeynep Kahya and Selena Kazancıoğlu deserve much credit for their help. Also my lovely roommate and colleague Hilal Saraç should take a tremendous amount of recognition for her devoted support that made me enthusiastic each day for work. In addition, I would like to thank all the members of Lack Lab (Hilal Saraç, Zeynep Kaya, Fatma Özgün, Uğur Gatfar and Alper Kurşunel) and TO Lab (Ayyub Ebrahimi, Burcu Özçimen, Deniz Uğurlu, Gülnihal Kavaklıoğlu, Kerem Fidan, Eda Suer and Duygu Payzın), that made working in a shared laboratory a friendly, fun and a loving environment.

Last but definitely not the least, I wish to thank my mother, my brother and my dear childhood friends Demet Şener, Buket Yazıcı, and Barış Cingiz for the endless support they have given. Without them, I could not be what I am today.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

NOMENCLATURE

APAF-1	Apoptotic protease activating factor 1
Bak	Bcl-2 homologous antagonist/killer
Bax	Bcl-2-associated X protein
BBB	Blood–brain barrier
Bid	BH3 interacting-domain death agonist
CDK	Cyclin-dependent kinase
DD	Death Domain
DISC	Death-inducing signaling complex
DSF	Disulfiram
ERK	Extracellular regulated kinase
EGFR	Epidermal growth factor receptor
FADD	Fas-Associated protein with Death Domain
FDA	Food and Drug Administration
FLIP	Flice-like inhibitory protein
GALNT14	N-acetylgalctosaminyltransferase-14
GBM	Glioblastoma multiforme
GSC	Glioma stem cell
HDAC	Histone deacetylase
HTS	High throughput screening
OPG	Osteoprotegerin
PDGF	Platelet-derived growth factor
PI3K	Phosphatidylinositide-3-kinase
RTK	Receptor Tyrosine Kinase
TMZ	Temozolomide
TNF	Tumor necrosis factor
TRAIL	TNF-related apoptosis-inducing ligand
VEGF	Vascular endothelial growth factor
WHO	World Health Organization
XIAP	X-linked inhibitor of apoptosis

Chapter 1

Review of Literature

1. Overview of primary brain tumors

1.1. Glioblastoma multiforme

Primary brain tumors are tumors that originate within the brain tissue and are classified by the tissue type that they arise from. Gliomas, arising from glial tissue, are the most common types of brain tumors effecting 1 in 20,000 individuals annually [1, 2]. According to World Health Organization (WHO) gliomas are classified into astrocytomas, oligodendrogliomas, ependymomas and oligo-astrocytomas (mixed gliomas). Further classification is done by taking the size, level of penetration and the capacity of infiltration of tumor into consideration [3].

Astrocytoma grade IV or glioblastoma multiforme (GBM) is the most aggressive and the most frequent type of primary brain tumors. The term "multiforme" demonstrates the heterogeneity of GBM regarding clinical presentation, pathology, genetic signature and response to the treatment [3]. GBM cases make up 60-70% of all gliomas [1]. Even though only 1% of adult cancers are GBMs, their malignant nature makes them the fourth greatest cause of cancer deaths [4].

In most GBM patients, no specific risk factors can be identified. It can arise *de novo* or from a lower grade tumor [5]. Up to date, only known risk of GBM is exposure to ionizing radiation. GBM epidemiology revealed that 87% of the patients are aged older than 55 years and it is seen more in men compared to women. Only 5% of patients have a familial background of GBM [6]. Despite the advancements in medicine, therapeutics and technology, the median survival of GBM has remained 12-15 months for the past 20 years [7]. Recurrence is another major issue of GBM with a median patient survival of 3-6 months (**Figure 1**) [8].

The hallmarks of GBM can be defined as uncontrolled cell proliferation, diffuse infiltration, tendency for necrosis, angiogenesis, resistance to apoptosis, and genomic instability. Due to heterogeneity of GBM, it is difficult to obtain pathology and gene expression patterns even within a single tumor. Many overactive oncogenes in GBMs were found to be a part of receptor tyrosine kinase (RTK)-Ras-Akt pathway. These include epidermal growth factor receptor (EGFR), platelet-derived growth factor (PDGF), platelet-derived growth factor receptor

(PDGFR) and Ras. Tumor suppressor genes lost in GBM are involved in cell cycle regulation such as p16, p14, p53, Rb, cyclin D, cyclin E, cyclin-dependent kinase (CDK) 4/6 and PTEN [3, 5, 9]

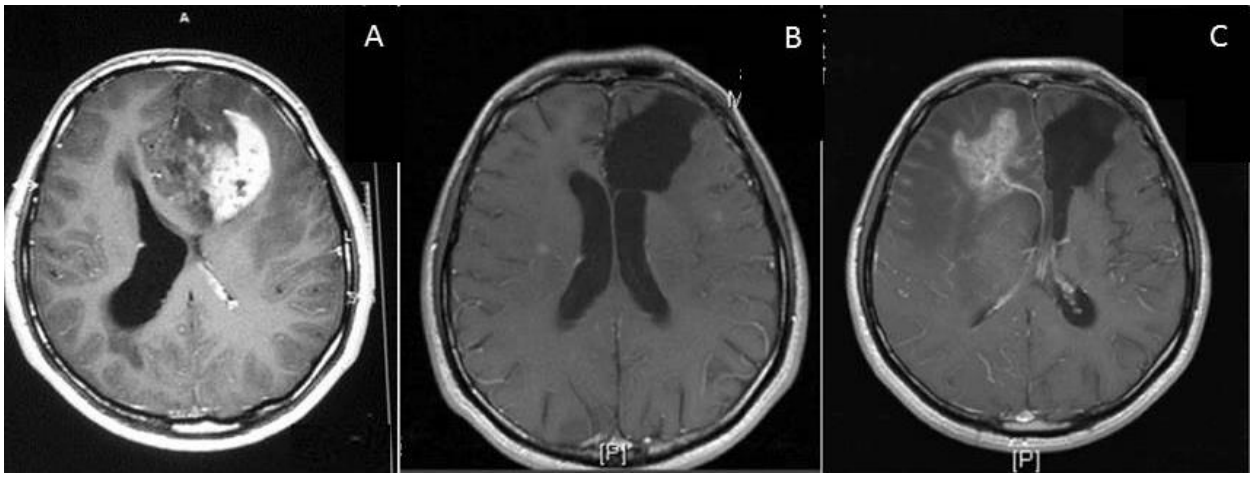


Figure 1. MRI of a GBM patient. ***A.*** Before surgery ***B.*** After surgery ***C.*** Recurrence of tumor 18 months of therapy (MRI scan, kind gift from Prof. Dr. Ihsan Solaroglu)

1.2. Treatment options for Glioblastoma multiforme

Treatment of GBM is extremely difficult due to several factors. First, the tumor cells, despite their relatively rapid cell cycle, are quite resistant to conventional therapies. Also, the brain has a limited capacity to repair itself. In addition, the blood–brain barrier (BBB) permeability makes it difficult to achieve the desired effect chemotherapeutics without dose–limiting systemic side effects [9].

1.2.1. Current treatment options

The aggressive nature and the heterogeneity of GBM make treatment very difficult. Most chemotherapeutic agents or treatment regimens do not increase patient survival significantly.

After diagnosis, the initial step for GBM patients is to undergo surgery either for a biopsy or maximal tumor removal while preserving neurological function. The decision for the surgery is made due to the location of the tumor and size. If possible, during surgery a biodegradable chemotherapy implantation (Carmustine) in the form of impregnated polymer wafers are implanted. These wafers provide release of chemotherapeutic agents to the surrounding tissue for several weeks, which results in statistically significant benefit for patients [10, 11].

Following surgery, radiation therapy and chemotherapy via cytotoxic agents are given. First line cytotoxic agents include temozolomide (TMZ) which is a small alkylating agent that disrupts DNA by forming O^6-methylguanine that miss-pair with thymine during DNA replication. After consequent replications these miss-pairings leads to cell death [3]. TMZ is preferred because it has fewer side effects and has better capacity to cross BBB compared to other chemotherapeutic agents [3, 5]. This agent was found to prolong survival 2-3 months with radiotherapy combination [12]. However, resistance development to these agents is frequent. For this purpose, new treatment options are needed for GBM patients.

Due to the necessity of new GBM treatments, numerous experimental strategies are currently in clinical trials including refined surgery, radiation and chemotherapy approaches. In addition, several novel biologically strategies such as gene therapy, immunotherapy and cytotoxic viral therapy are being tested in clinical trials [5, 10].

1.2.2. Molecular Targeted Therapy

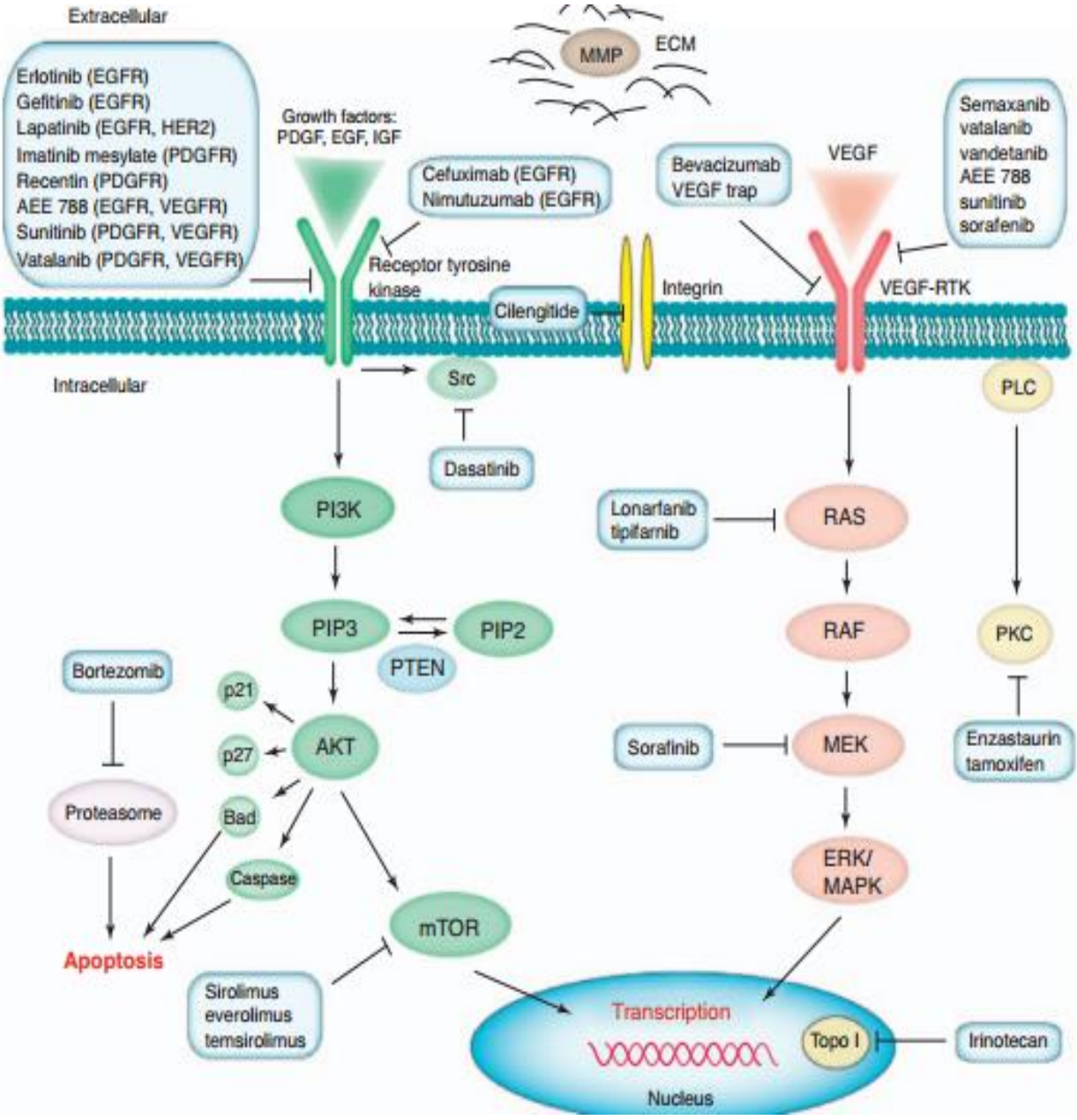
Common molecular alterations among GBM patients such as several growth factors, hormones and cytokines became the focus of research in the last years, which are indicated in **Figure 2.**

1.2.2.1. Inhibition of growth factor ligands and receptors

The high angiogenesis capability of malignant gliomas revealed vascular endothelial growth factor (VEGF) as a key growth factor for blood vessel formation. Bevacizumab (Genentech, USA) is a humanized neutralizing monoclonal antibody that acts as an angiogenesis inhibitor by targeting vascular endothelial growth factor A (VEGF-A). Phase II clinical trials revealed significant antitumor effect and it was approved by the FDA to be used in recurrent GBM patients in 2009 [13]. Vatalanib (Novartis, Switzerland) and AZD2171 (AstraZenaca, USA) are two of the VEGF-R inhibitors that are currently in clinical trials for GBM treatment [14-16]

EGFR was found to be amplified in approximately half of the GBM, thus making it a potential target for molecular targeted therapy. Gefitinib (AstraZeneca, USA) and Erlotinib (Genentech, USA) are EGFR kinase inhibitors that has shown progress in survival rates in clinical trials either alone or in combination with TMZ [17, 18].

PDGF and PDGF receptors (PDGF-R) are important in glioma growth and angiogenesis. A tyrosine kinase inhibitor, Imatinib (Novartis, Switzerland) shows antiglioma activity, however it was not successful as monotheraphy in the clinical trials [11].



.

Figure 2. Schematic overview of current molecularly targeted GBM therapies. Small molecular inhibitors are used to inhibit several oncogenic pathways such as PI3K-Akt (green) and RAS (pink) pathways. EGF, VEGF, PDGF and their receptors can be blocked by small molecules and monoclonal antibodies. Blue boxes include the examples of drugs. [19]

1.2.2.2. Inhibition of Intracellular Effectors

Serine/threonine kinases have important roles in malignant gliomas. Once the growth factor receptor is activated, effector molecules such as RAS, phosphatidylinositide-3-kinase (PI3K) and phospholipase C are recruited to the cell membrane. Majority of gliomas are associated with activation of these effector molecules or negative regulators of these kinases such as PTEN in PI3K pathway. In addition RAF, mitogen-activated protein extracellular regulated kinase (MEK), extracellular regulated kinase (ERK; also termed mitogen-activated protein kinase [MAPK]), AKT, and mammalian target of rapamycin (mTOR) are important intracellular mediators in gliomas [11]. For this reason targeting RAS-RAF-MEK-ERK, PI3K/AKT/mTOR and Protein Kinase C pathways are promising treatment options for GBM.

1.2.2.3. Deacetylase Inhibitors

Epigenetic alterations can lead to defective apoptotic signaling resulting in evasion of apoptosis. However, the reversible nature of epigenetic modifications makes them promising candidates for therapeutic research. Histone deacetylase (HDAC) inhibitors are a class of targeted cancer agents. They induce cell cycle arrest by up-regulating p21 (cyclin-dependent kinase inhibitor 1) and induce apoptosis through intrinsic apoptosis pathways [11, 20]. Treatment with Vorinostat (Aton Pharma, USA) enhanced gliomas cells' sensitivity to chemotherapy and radiation [21].

1.2.2.4. Proteasome Inhibitors

The vital role of ubiquitin-proteasome system in homeostasis by regulating cell cycle proteins to balance cell proliferation and apoptosis makes proteasome inhibitors promising agents in inducing cell cycle growth arrest. Bortezomib (Millennium Pharmaceuticals, USA) is the first proteasome inhibitor tested in humans. Many studies confirmed that it induces cell cycle arrest and apoptosis in gliomas cell lines [22].

1.2.3. Combination therapy

Due to the heterogeneous nature of GBM, monotherapies do not provide the best survival benefits. For this purpose, targeting multiple signaling pathways or different targets in the same pathway is a promising strategy for more efficient treatments. As indicated, there are currently

numerous ongoing clinical trials for GBM, and most agents are not promising as monotherapies. Selected drugs are listed in **Table 1** below. For this purpose, combinations with TMZ, radiation and/or other therapeutics are in search for better efficacies. For example, Rapamycin is not effective as a single agent in GBM, but a current phase III clinical trial shows that Rapamycin with the combination of Avastin is showing promising results in both initial and recurrent GBM patients [23].

Drug	Target	Most Recent GBM Trail; initial or recurrent GBM	Comments
Erlotinib	EGFR	Phase II; initial and recurrent	Minimal efficacy as single agent; modest survival benefit with TMZ and radiation; ongoing trials in combination with other drugs
Gefetinib	EGFR	Phase II; recurrent	Minimal efficacy as monotherapy, combination therapies not effective either.
Lapatinib	EGFR, ErbB2	Phase II; recurrent	No efficacy in a trial with small number of recurrent GBMs; one Phase II trial is ongoing.
Sunitinib	PDGFR, VEGFR, c-Kit	Phase II; recurrent	Phase II trials underway
Sorafenib	Raf, VEGFR, PDGFR	Phase II; initial and recurrent	Minimal efficacy cas monotheraohy; ongoing Phase II trials in combination with other drugs
Dasatinib (Sprycel)	BCR-ABL, Src family kinases	Phase I, II; initial and recurrent	Among seven Phase I/Phase II trials of GBM, one was withdrawn and three were suspended.
Nimotuzumab	EGFR	Phase II, III; initial and recurrent	Well tolerated in patients, modest (17.47 months vs 14.6 months) survival benefit in small subgroup of GBM
Cetuximab (Erbitux)	EGFR	Phase I, II; initial and recurrent	Phase II trials ongoing
AMG 102	Hepatocyte growth factor (HGF)	Phase II; recurrent	Phase II trials ongoing
Imatinib (Gleevec)	PDGFR, c-KIT, BCR-ABL	Phase I, II; recurrent	Minimal efficacy as single agent; further trials of combination therapies are ongoing
Tandutinib (MLN518)	PDGFR, FLT3, c-KIT	Phase II; recurrent	Phase II trials as single agent and in combination with Avastin are underway
Enzastaurin	PKC, PI3K/AKT pathway inhibitor	Phase I, II, III; initial and recurrent	Limited efficacy in recurrent GBM as monotherapy; in a phase III trial with recurrent GBM
Sirolimus (Rapamycin)	mTOR inhibitor	Phase II; initial and recurrent	Not effective as monotheraphy; Phase II trials in combination with EGFR/PI3K pathway inhibitors ongoing
Temsirolimus	mTOR inhibitor,ester analog of sirolimus	Phase I, II; initial and recurrent	Limited efficacy as single agent in recurrent GBM; ongoing trials of combination therapies with EGFR/PI3K pathway inhibitors or Avastin
Everolimus	mTOR inhibitor, derivative of sirolimus	Phase II; initial and recurrent	Multiple Phase II trials of combination therapies ongoing
Veliparib	PARP inhibitor	Phase II; initial and recurrent	Currently Phase II trials ongoing
Iniparib	PARP1 inhibitor	Phase I, II; primary	Currently recruiting for Phase I and Phase II trials
Bortezomib (Velcade)	Proteasome inhibitor	Phase II; initial and recurrent	Phase I trials established the safe doses and showed low response rate in recurrent GBM but favorable tendency in initial GBM with standard RT/TMZ.
Cilengitide	antiangiogenesis	Phase II, III; initial and recurrent	Phase I trials found the drug well tolerated also with TMZ; encouraging results of combining cilengitide with standard TMZ/RT in initial GBM with methylated MGMT promoter, on which a Phase III trial is ongoing

Table 1. Selected drugs for GBM that are currently in clinical trials [19].

2. TNF-related apoptosis-inducing ligand (TRAIL)

2.1. Apoptosis

Apoptosis, programmed cell death, plays a central role in the development and homeostasis of multicellular organisms. Two main characterized apoptosis pathways are defined in mammalian cells: extrinsic and intrinsic pathways. Intrinsic pathway includes the activity of mitochondria and is controlled by Bcl-2 protein family members. Extrinsic pathway is mediated by death receptors which are a subgroup of the tumor necrosis factor (TNF) receptor superfamily (**Figure 3**) [24].

DNA damage, cell cycle checkpoint defects, hypoxia and several other cell stress conditions can trigger intrinsic apoptosis pathway by activation of pro-apoptotic Bcl-2 gene superfamily, which in turn trigger mitochondria to release cytochrome *c* into the cytosol. Following the release, cytochrome *c* binds to Apoptotic protease activating factor 1 (APAF-1), forming the apoptosome complex and activating caspase-9. Active caspase-9 then initiates the activity of executioner caspases; caspase-3, caspase-6 and caspase-7 [25].

TRAIL induces apoptosis through DR4 and DR5 receptor binding, resulting in trimerization of the receptor. Clustering of the receptor's intracellular death domain (DD) triggers the formation of death-inducing signaling complex (DISC). Trimerization of DD results in recruitment of an adaptor molecule called Fas-Associated protein with Death Domain (FADD) which then activates caspase-8 and caspase-10. Following activation of caspase-8 and caspase-10, they lead to the cleavage of caspase-3 which in turn cleaves death substrates [25]

The intrinsic and extrinsic pathways are linked with each other. Specifically, caspase-8 can cleave pro-apoptotic Bcl-2 family member BH3 interacting-domain death agonist (Bid), which results in the translocation of truncated Bid (tBid) in mitochondria. tBid promotes cytochrome *c* release by interactions with Bcl-2-associated X protein (Bax) and Bcl-2 homologous antagonist/killer (Bak) [26] . Therefore, tBid is an important effector of TRAIL signaling.

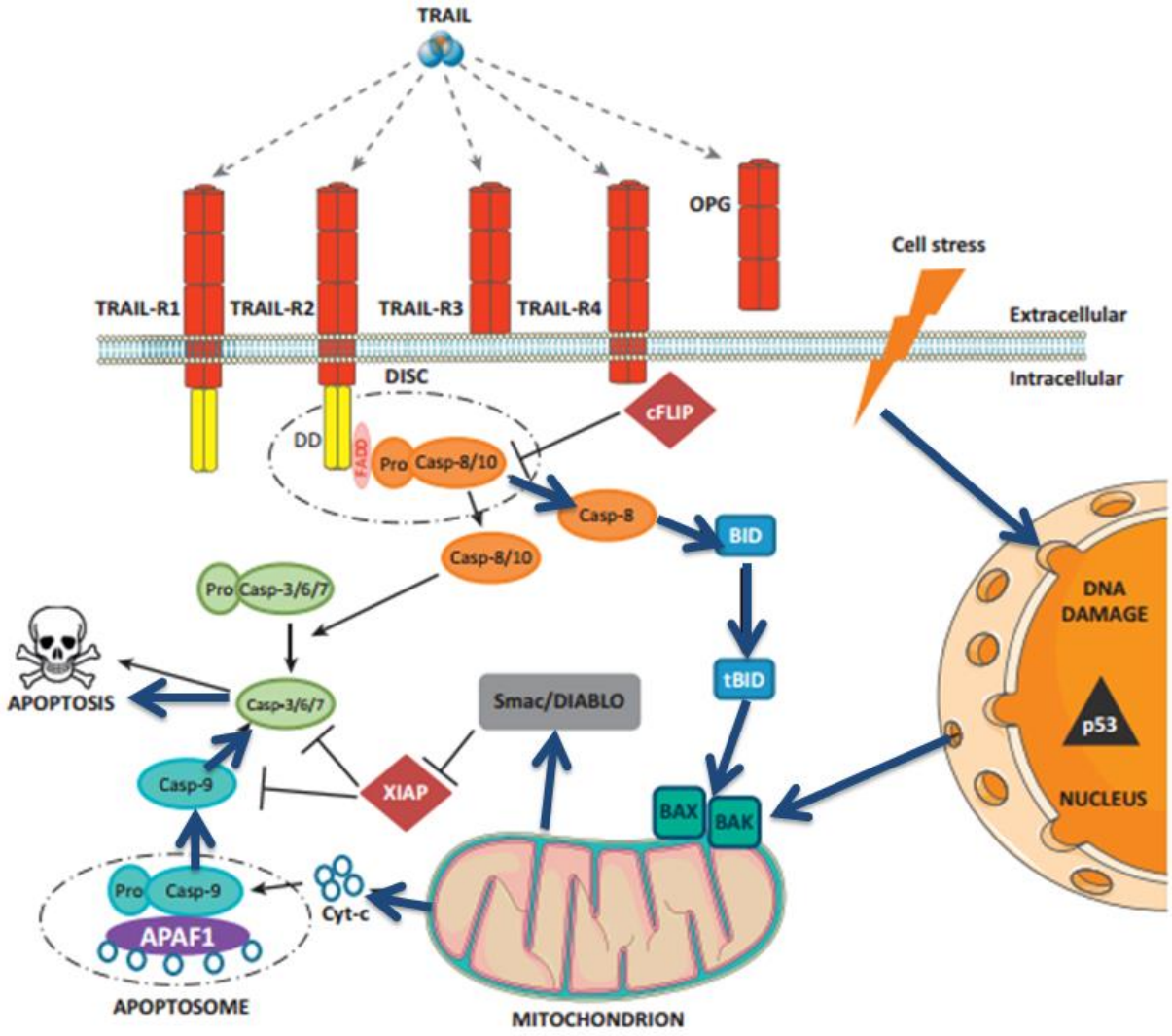


Figure 3. TRAIL induced apoptosis overview. ***The extrinsic apoptosis pathway*** (Black arrows) TRAIL can bind to four membrane-bound receptors and one soluble receptor. Binding of TRAIL to DR4, DR5 leads to recruitment of the adaptor FADD and initiator procaspase-8 and -10 to form the DISC. Procaspase-8 and -10 are cleaved in order to form caspase-8 and -10, which activate effector caspase-3, -6, and -7 committing the cell to apoptosis. At the DISC, cFLIP can inhibit the activation of caspase-8 and thus the apoptotic signaling. ***The intrinsic apoptosis pathway*** (Blue arrows) is initiated in response to cell stress such as chemotherapy and radiotherapy. This results in DNA damage causing the p53 oncogene to activate the proapoptotic Bcl-2 family proteins Bak and Bax. Pro-apoptotic proteins cytochrome *c* and Smac/DIABLO are released from mitochondria. Cytochrome c forms a protein complex, the apoptosome, with APAF-1 and activates caspase-9, which in turn activates effector caspase-3, -6, and -7 resulting in apoptosis. Smac/DIABLO inhibits apoptosis-inhibiting proteins such as XIAP, thus amplifying apoptotic signaling. [27]

2.2. TRAIL

TRAIL ligand (TRAIL/Apo2L) was discovered by two different groups in 1995 and 1996 as a new protein that can induce apoptosis [28, 29]. TRAIL is normally expressed by immune system cells and has a role in homeostasis of certain T cells and in Natural Killer cells. TRAIL is important in T-cell-mediated destruction of virally and oncogenically infected cells [29-31].

TRAIL is normally expressed on both normal and tumor cells as a non-covalent homotrimeric type II transmembrane protein (memTRAIL). A soluble form of TRAIL (sTRAIL) with proapoptotic activity can also be formed by alternative mRNA splicing or proteolytic cleavage of extracellular domain of memTRAIL [32].

2.2.1. TRAIL receptors

TRAIL forms homotrimers and induces apoptosis via interactions with three cross-linked receptor molecules on the cell surface. TRAIL has a complex receptor system with the capacity of binding to 5 receptors in humans: DR4, DR5, DcR1, DcR2 and a soluble receptor called osteoprotegerin (OPG). DR4 and DR5, death receptors, are type I transmembrane proteins with death domains (DD) to induce apoptosis upon TRAIL binding. DcR1 and DcR2 are also called decoy receptors, they lack DD, and they prevent apoptosis. [33-36].

2.2.2. TRAIL selectively induces death on tumor cells, but not on normal cells

A standard solid tumor treatment consists of surgery, radiotherapy and chemotherapy to get rid of cancerous tissue. However, these non-specific treatment options result in loss of healthy cells also. TRAIL has the ability to induce apoptosis in tumor cells but it does not have cytotoxicity on normal cells both *in vitro* [28, 37] and *in vivo* [38-40]. In 2000, an alarming study was performed on TRAIL's toxicity on normal human hepatocytes, however later on it was found to be an artifact caused by particular preparation of TRAIL [41]. To date, TRAIL is used in clinical studies and no significant indications of cytotoxicity on normal cells have been reported. For this reason, TRAIL based therapy is a promising option for cancer treatments.

2.2.3. TRAIL resistance

Although TRAIL has the ability to selectively kill cancer cells, many cancer cells are intrinsically resistant and/or acquire resistance to TRAIL. The mechanism of TRAIL resistance is

still not completely solved, but it is believed to be effected on multiple levels from its receptors to executor caspases within the apoptotic pathway.

2.2.3.1. Resistance caused by anti-apoptotic mechanisms

Reduced caspase expression, increase expression of caspase inhibitors, overexpression of pro-apoptotic Bcl-2 family members and other inhibitors of intrinsic apoptosis pathway are among the major causes of TRAIL resistance.

Flice-like inhibitory protein (FLIP) is a homolog of caspase-8 with mutations in the catalytic domain and it competes with caspases at DISC to prevent binding and activation. FLIP overexpression was found in many TRAIL resistant cancers and downregulation induced TRAIL induced apoptosis [42].

X-linked inhibitor of apoptosis (XIAP) inhibit the activity of caspase 3, 7 and 9 [43], and mediate TRAIL resistance when overexpressed [44].

In intrinsic TRAIL signaling, pro-apoptotic Bid activates Bax and Bak which in turn induce the mitochondrial membrane permeabilization and cytochrome *c* release. Loss of Bax and/or Bak or overexpression of anti-apoptotic Bcl-2, Bcl-xL and Mcl-1, results in TRAIL resistance [44].

2.2.3.2. Resistance caused by defects in death receptor signaling

It was proposed that TRAIL resistance was mediated by the decoy receptors DcR1 and DcR2 and their competition with DR4 and DR5 for TRAIL binding. However, no correlation between expression of death receptors and TRAIL sensitivity among cancer cells were found. On the contrary, it was found that downregulation of DR4 and DR5 by epigenetic changes, such as hypermethylation, deletions or point mutations, is related to TRAIL resistance in cancer [44].

Even though a tumor cell expresses both DR4 and DR5, apoptosis signaling continues through one, regardless of the presence of the other. MacFarlene and colleagues found that in chronic lymphatic leukemia and mantle cell lymphoma, primary tumor cells express both DR4 and DR5, but can only be killed by DR4 agonists [45]. Thus, TRAIL effect may vary in different tumors depending on which death receptor is active.

TRAIL resistance can also be acquired because of inefficient transportation of receptors to the cell membrane. Transportation process involves glycosylation and the protein N-

acetylgalctosaminyltransferase-14 (GALNT14) has a big role. A whole genome microarray profiling project done by Wagner and colleagues among 119 human cancer cell lines revealed GALNT14 as an outstanding molecule in TRAIL resistance. In 61% of the time presence of this enzyme predicted sensitivity to TRAIL and in 88% of absence predicted resistance among all samples [46].

2.3. TRAIL and glioblastoma multiforme

The selective apoptotic ability of TRAIL towards cancer cells made it a candidate for GBM treatment. A study containing 62 primary GBM tumors revealed that DR4 and DR5 were expressed in 75% and 95% of the cases, respectively [47]. However, most GBM tumors are resistant to TRAIL or they acquire resistance over time. Thus, TRAIL as a monotherapy is not the best option for GBM.

TRAIL resistance can be overcome by combination therapies. Combination of TRAIL with chemotherapeutics, radiation or other novel therapeutic drugs in preclinical studies gave positive results on tumor regression [32]. For example, *in vivo* administration of TRAIL and cisplatin, a chemotherapeutic agent, reduced the tumor formation and growth of human glioblastoma xenografts in nude mice [48].

A small molecule inhibitor of histone-deacetylase (HDAC), MS-275, does not have a significant effect on GBM alone. However, when combined with TRAIL, it was shown by Bagci-Onder and colleagues to induce death even in TRAIL resistant GBM cells [49].

TRAIL and Bortezomib is an exciting combination for GBM. Bortezomib works as a proteasome inhibitor and it has different response on normal and cancer cells. It arrests the cell cycle in G2/M phase, but in normal cells can resume division after treatment. The combination has been shown to work very well in GBM cell lines and primary tumors by many investigators *in vitro* [50-54]. However, research done in animal models showed that Bortezomib cannot easily pass the BBB [55], thus further research on Bortezomib and GBM is needed.

Despite these promising approaches, more thorough studies assessing a large panel of TRAIL-sensitizing treatment strategies are needed since acquired TRAIL resistance remains as a problem.

3. High throughput screens

High throughput screening (HTS) is a method used mainly by the pharmaceutical industry for drug discovery. In this method, large numbers of drug-like compounds are tested using robotic methods, data processing and control software, liquid handling devices, and sensitive detectors to identify promising leads for potentially marketable drugs. In the recent years, HTS had made its way in academic research settings with a variety of libraries.

There are numerous types of libraries used by researchers such as chemical, peptide/antibody and genetic inhibitor (siRNA) libraries. Chemical libraries can be composed of natural, synthetic and combinatorial compounds. While generating drug leads, high molecular diversity is important without losing "Drug-like" properties. Natural product structures have high chemical diversity and biochemical specificity, making them more favorable than synthetic and combinatorial compounds [56].

Antibodies are naturally evolved defense mechanisms that recognize and eliminate pathogenic and disease antigens. Their highly specific features and potential to be genetically engineered make them a focus of research for cancer, autoimmune and infection therapies [57]. However, the high cost of producing antibodies with mammalian expression systems is a major challenge.

Advances in genetics allow manipulation of gene expression in living cells. siRNA are 20-25 nucleotide long double-stranded RNA molecules that can selectively silence specific genes [58]. siRNA libraries are used to identify genes and pathways for cancer cell survival and obtain a more specific treatment approach.

After a possible lead is found, detailed biological evaluation is needed; such as the Structure-Activity Relationships of analogous compounds with regard to their *in vitro* and *in vivo* efficacy and safety [59]. Drug repurposing is the application of known drugs to other uses which takes off years that is needed to develop a new drug and provides a drastic economic benefit. In addition, a repurposed drug already comes with the systemic toxicity knowledge, hence reducing cytotoxicity problems [60]. For this reason, many investigators are utilizing drug libraries that contain Food and Drug Administration (FDA) and other agencies' approved chemicals to investigate the hidden potentials of those drugs. The most known example for drug repurposing is sildenafil citrate (Viagra) (Pfizer, USA), which was originally used as a hypertension drug, but

now is a drug for erectile dysfunction therapy. Repurposed drugs contain 30% of FDA approved drugs in recent years. [61]

Drug repurposing by using HTS method has provided many potential candidates for various diseases. For example, a HTS done in 1994 revealed Gefitinib (AstraZeneca, USA) and Erlotinib (Roche, Germany) as specific inhibitor of the epidermal growth factor receptor tyrosine kinase, which were approved by FDA in 2003 and currently used in many cancer treatments [62].

3.1. High throughput screens for glioblastoma multiforme

Investigation of repurposed drugs for GBM can be the solution to the limited success of current GBM treatment options. In 2012, Wang and colleagues performed a high throughput screen for GBM cell growth and invasion, using an annotated chemical library of 1,280 small chemicals and obtained 4 novel inhibitors (6-nitroso-1,2-benzopyrone, S-(p-azidophenacyl) glutathione, phenoxybenzamine hydrochloride and SCH-28080) for human glioblastoma cell lines U87MG and U251MG [63].

Glioma stem cells (GSCs) are taught to have a role in GBM recurrence and resistance to therapy. For this purpose, Hothi and colleagues screened a diverse chemical library of 2,000 chemicals to inhibit GSC proliferation. Of the 78 hits they obtained, they chose an FDA approved drug for alcoholism treatment, disulfiram (DSF) which has relatively low cytotoxicity and capacity to cross BBB [64]. Two different clinical trials (ClinicalTrials.gov Identifier: NCT01907165, NCT01777919) using DSF for GBM patients have been recruiting patients since 2013 [65] .

Selective effect of inducing apoptosis on cancer cells makes TRAIL an encouraging treatment option for GBM patients. Yet, TRAIL resistance is a major issue among GBM tumors that needs to be overcome. Utilizing HTS for investigating TRAIL sensitizers is a fast and cost effective method. A functional drug screen of 1,040 drugs and bioactive compounds *in vitro* in human glioblastoma cell line U87MG combined with TRAIL, revealed Digitoxin, Stropanthidin, Peruvoside, Neriifolin, Digoxin, Ouabain and Lanatoside C which belong to the family of cardiac glycosides [66]. However further studies are needed before the establishment of these drugs into the clinic.

Chapter 2
Materials and Methods

1. Cell culture

Human glioma U87MG, U373, LN229, A172 and human embryonic kidney 293T cells were purchased from American Tissue Type Culture Collection (USA). U87MG TRAIL-resistant cell lines and U87MG Bcl-xL cells were generated by Ahmet Cingoz by selection of U87MG cells under TRAIL treatment for a long time and via virus transduction respectively (See Appendix I). BJ and DH1F Fibroblasts were kind gifts from Dr. Tamer Onder (TURKEY). Primary GBM cell lines KUGBM2 and KUGBM4, as well as patient-derived fibroblasts (KUFibroMNG and KUFibro4) were generated by Dr. Tugba Bagci Onder in collaboration with Dr, Ihsan Solaroglu at Koc University (Appendix II). All cells were cultured in 37^0C humidified incubator with 5% CO_2 in DMEM medium (Gibco, USA) with 10% fetal bovine serum (Invitrogen, USA) and 1% Penicillin-Streptomycin (Gibco, USA). U87MG cells were used between passage numbers 7 and 15 for the consistency of the high throughput drug screening.

2. Production of TRAIL in 293T cells

For TRAIL production, 293T cells were transfected with TRAIL vector encoding a secretable version of TRAIL as described [67]. Transfection was performed with the aid of Fugene Transfection Reagent (Promega, USA).

293T cells were plated to 80% confluency prior to experiment setup. On Day 0, 2.5 $x10^6$ numbers of 293T cells were seeded to 10 cm cell culture plates. In the afternoon of day 1, for each plate 20 ul of Fugene was combined with 180 ul OptiMEM. 5,000 ng of TRAIL vector DNA was added to this mixture and added to 293T cells. After a day of incubation, cells were washed and the medium was replaced with serum-free medium. About 48 hours after transfection, first collection was performed and this procedure was repeated the following day. To concentrate TRAIL, spin filter columns with a 15kD cut-off (Millipore, USA) were used. Spinning was performed at 3,800 rpm at 4^0C for 20 minutes. TRAIL concentration amount was detected by TRAIL Human ELISA Kit, following manufacturer's instructions (Abcam, United Kingdom) as described below. TRAIL was stored at -80^0C.

2.1. TRAIL ELISA:

Abcam's TRAIL Human ELISA Kit contains a monoclonal antibody specific for TRAIL which has been coated onto the wells of the provided microtiter strips. TRAIL detection is performed by comparing the desired sample to known TRAIL concentration standards and control specimens. First, incubation is done with the standards or samples and a biotinylated monoclonal antibody specific for TRAIL. Afterwards the enzyme Streptavidin-HRP, that binds the biotinylated antibody is added. Finally, a substrate solution which acts on the bound enzyme to induce a colored reaction product is added. The intensity of this colored product is directly proportional to the concentration of TRAIL present in the samples. To detect the intensity of the colored product the read was performed at 540 nm by Synergy H1 Reader (Biotek, USA).

3. Chemicals and reagents

The drug library composed of 1,200 FDA (Food and Drug Administration of US) approved drugs were purchased from Prestwick Chemical (FRANCE). The list and structure of the drugs are provided in Appendix III. Bortezomib was purchased from Selleck Chemicals. Individual drugs, namely Niclosamide, Astemizole, Terfenadine, Camptothecine (S,+) Quinacrine dihydrochloride dihydrate, Mitoxantrone dihydrochloride, Amphotericin B, Cyclosporin A, Digitoxigenin, Digoxin, Daunorubicin hydrochloride, Pinaverium bromide, Lanatoside C, Cycloheximide, Azacytidine-5, Digoxigenin, Proscillaridin A, Pyrivinium pamoate, Vorinostat and Topotecan were then separately supplied from Prestwick Chemicals in higher quantities.

4. Drug screen

For the optimal usage of reagents and having more technical replicates at once, 384 well plates (Corning, USA) were chosen for the high throughput drug screen.

The optimal number of cells seeded to a 384 well plate was found by different number cell seeding in the range of 50-10,000 cells/well. 2,000 cells were chosen as optimal for the assays. For the drug screen, U87MG cells were automatically seeded to 384 well plates at the number of 2,000 cells/40 µl/well via Multidrop™ Combi Reagent Dispenser (Thermo-Scientific, USA).

1 day after seeding the cells, a fixed concentration of TRAIL (25 ng/ml chosen after optimization as described in Results section) was applied to cells alone or along with individual drugs at a final concentration of 5 μM. Untreated cells as well as DMSO-treated cells were used as negative controls. Bortezomib is a proteasome inhibitor that is known to sensitize GBM cells to TRAIL [22] and was used as a positive control in the screen.

The Prestwick drug library was supplied as 10 mM stock soluble in DMSO in a total of 15 96-well plates. 1 mM personal stocks were prepared by DMSO dilution in a total of 20 96-well plates by reformatting the plate set-up according to the experimental design. On the day of drug treatment, fresh 50 μM drug plates were prepared with DMEM and added to 384 well plates with an automated pipettor (Matrix™ Multichannel Electronic Pipettes) (Thermo Scientific, USA) for a final concentration of 5μM. Each treatment was done in triplicates (**Figure 4**).

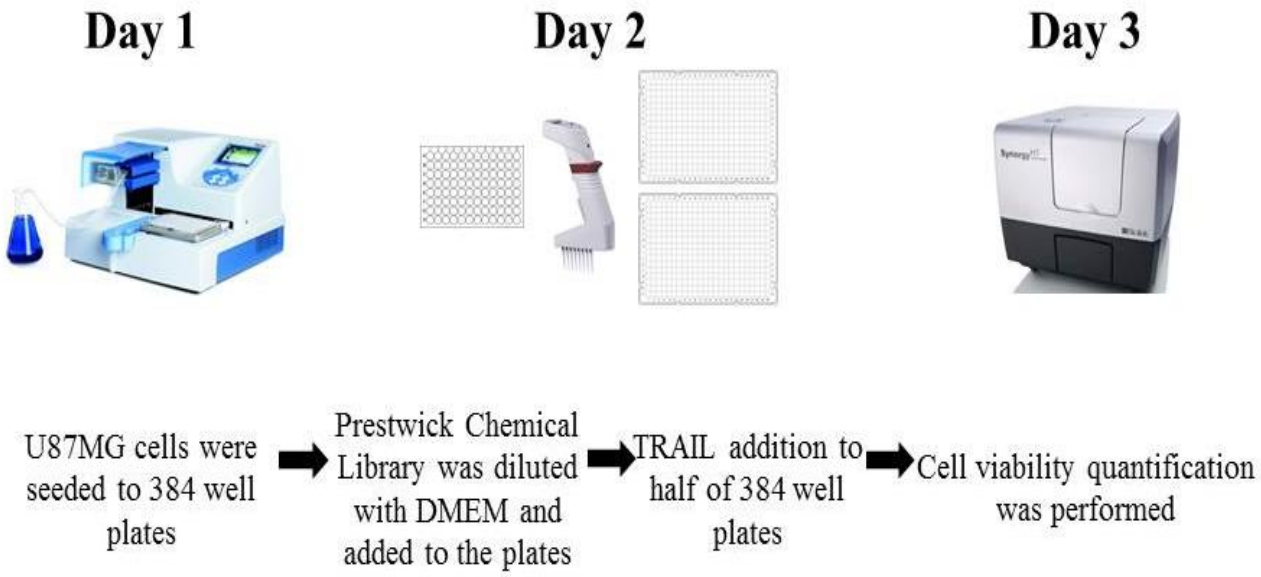


Figure 4. Drug library screen procedure. ***(Day 1)*** U87MG cells were automatically seeded to 384 well plates (2,000 cells/well). ***(Day 2)*** Prestwick Chemical Library stock of 1mM was diluted to 50 μM in DMEM. Drugs, TRAIL, or drugs + TRAIL were added (Final concentration of drug 5 μM, TRAIL 25 ng/ml). ***(Day 3)*** Cell viability was determined by CellTiterGlo assay.

Screen design contained each single agent and combination of agent with TRAIL in triplicates. In addition, each 384 well plate contained control, positive/negative controls and only TRAIL treated cells.

Cell viability was determined after 24 hours of drug treatment as described below.

For the screen of U87MG TRAIL-resistant cells, the same design and method was used.

5. Determination of cell viability

Cell viability was determined via Cell Titer-Glo® (CTG) Luminescent Cell Viability Assay (Promega, USA). CTG determines the number of viable cells via luciferase reaction by measuring ATP amount, which is an indicator of metabolically active cells. CTG reagent lyses cell membranes for ATP release and inhibits endogenous ATPases simultaneously while providing luciferin, luciferase and other reagents for a bioluminescent reaction. CTG assay was slightly modified from the manufacturer's instructions for economizing the HTS.

Each plate was incubated 10 minutes at room temperature before CTG reagent addition. For 384 well plates, 5 µl of CTG reagent was added directly to a well containing 50 µl DMEM. For 96 well plates, medium was removed and 4 µl + 40 µl of DMEM mix was added per well. Following CTG addition, the plate was put into Synergy H1 Reader (Biotek, USA) for 2 minutes of shaking and 8 minutes of incubation at dark before luminometric read. Survival was expressed as the percentage of viable cells in the treated sample compared to untreated control samples.

For the chosen hits, cell viability was further investigated on black 96 well plates (Corning, USA). For U87MG, U373, LN229, 293T and primary GBM cells, 10,000 cells/well and for fibroblast cells 2,500 cells/well were seeded. Drug treatment was performed by aspirating the medium and replacing with medium containing respective drug or the combination of TRAIL with the respective drug. After 24 hours, CTG cell viability determination was performed as explained above.

6. Caspase 3/7 Activity Assay

Caspase 3 and Caspase 7 activation are indicators of late-stage apoptosis. Caspase 3/7 activity was determined by the Caspase-Glo® 3/7 Assay (Promega, USA). The assay provides a proluminescent caspase-3/7 DEVD-aminoluciferin substrate and a proprietary thermostable luciferase in a reagent optimized for caspase-3/7 activity, luciferase activity and cell lysis. With cell lysis, followed by caspase cleavage of the substrates free aminoluciferin, which is consumed by the luciferase, gives out a luminescent signal. The signal is proportional to caspase-3/7 activity.

10,000 cells were seeded into 96-well black bottom plates a day prior to drug treatment. Selected drugs (Digitoxigenin, Digoxin, Digoxigenin, Lanatoside C, Proscillaridin A, and Bortezomib) were added at a final concentration of 500, 50, 50, 50, 25 and 5 nM respectively. For the detection of the combination effect, 25 ng/ml TRAIL was added, and incubated for 4 hours. Afterwards, The Caspase-Glo® 3/7 reagent was added and incubated for half an hour. Measurement was performed at 560 nm by Synergy H1 Reader (Biotek, USA).

7. Drug concentration dependent assays

To characterize the effect of single agent and combination, different concentrations of single agent and combination were tested. For further studies best dose for combination was chosen for each agent as described in detail in Results Section.

8. Statistical analysis

Statistical analyses were performed using Microsoft Excel 2010 and/or GraphPad Prism 4.0. For the drug screen, normalization of raw data to each plate's own control samples was done to remove plate-to-plate variation. The effects of single agent treatment were analyzed to determine the cytotoxicity of drugs on their own. Sensitizing ability of the drug was determined by comparing the decrease in cell viability following combination treatments (i.e. drug in combination with TRAIL) to the reduction in cell viability of single agent and TRAIL alone treatment.

For the selection of hits, a cut off value was determined by taking the average percent cell viability of cells upon treatment with all drugs, and ±3STD values were used as minimum and maximum values. The same procedure was performed for drug + TRAIL combination.

9. Real-time PCR

To determine the mRNA expression, RNA was isolated from drug treated cells. U87MG cells were seeded to 12 well plates at the density of 10^5 cells/well and treated with selected concentration of the respective drug (**Table 5)** for 24 hours. RNA was isolated via Qiagen RNeasy Mini Kit following manufacturer's instructions (Qiagen, Netherlands). RNA quality was determined by Nanodrop measurements. 250 ng cDNA was obtained by reverse transcription of RNA using M-MLV Reverse Transcriptase (Invitrogen, USA). Relative mRNA expression

levels were detected by using LightCycler® 480 SYBR Green I Master (Roche, Germany). The primers used in these experiments are listed in **Table 2**.

Gene	*Sequence*	
BAD	F	5'-CCCAGAGTTTGAGCCGAGTG-3'
	R	5'- CCCATCCCTTCGTCGTCCT- 3'
BAX	F	5'- GGGTGGTTGGGTGAGACTC-3'
	R	5'-AGACACGTAAGGAAAACGCATTA-3'
BCL-2	F	5'-CAGGGCGATGTTGTCCACC-3'
	R	5'-GGGGAGGATTGTGGCCTTC-3'
BCL-xL	F	5'-GGTCGCATTGTGGCCTTTTTC-3'
	R	5'-TGCTGCATTGTTCCCATAGAG-3'
BIM	F	5'- TCCCTGCTGTCTGATCCTC-3'
	R	5'-GGTCTTCGGCTGCTTGGTAA-3'
BID	F	5'-CCTACCCTAGAGACATGGAGAAG-3'
	R	5'-TTTCTGGCTAAGCTCCTCACG-3'
CASP-3	F	5'-CATGGAAGCGAATCAATGGACT-3'
	R	5'-CTGTACCAGACCGAGATGTCA-3'
CASP-7	F	5'-CGGTCCTCGTTTGTACCGTC-3'
	R	5'-CGCCCATACCTGTCACTTTATCA-3'
CASP-8	F	5'-ACACCAGGCAGGGCTCAAAT-3'
	R	5'-GCAGGTTCATGTCATCATCCAGTT-3'
CASP-9	F	5'-CTTCGTTTCTGCGAACTAACAGG-3'
	R	5'-GCACCACTGGGGTAAGGTTT-3'
DR4	F	5′-ACCTTCAAGTTTGTCGTCGTC-3′
	R	5′-AACTC TCCCAAAGGGCTATGT-3′
DR5	F	5′-AAGACCCTTGTGCTCGTTGT-3′
	R	5′-AGGTGGACACAATCCCTCTG-3′
DcR1	F	5'-GCTGCCAGTCCTAGCTTACTCTG-3'
	R	5'-CCCTCTGTGCACGGGTTACA-3'
DcR2	F	5'-TGGCTCCTGGACCCCAAGAT-3'
	R	5'-ATGAGATCCTGCTGGACACTCCTC-3'
FADD	F	5'-GCTGGCTCGTCAGCTCAAA-3'
	R	5'-ACTGTTGCGTTCTCCTTCTCT-3'
GAPDH	F	5'-AGCCACATCGCTCAGACAC-3'
	R	5'-GCCCAATACGACCAAATCC-3'
HRK	F	5'- CCTACTGGCCTTGGCTGTG-3'
	R	5'-TACAAGTTCCGCCTGCCG-3'
Mcl-1	F	5'-TGCTTCGGAAACTGGACATCA-3'
	R	5'-TAGCCACAAAGGCACCAAAAG-3'
OPG	F	5'- GCGCTCGTGTTTCTGGACA -3'
	R	5'- AGTATAGACACTCGTCACTGGTG-3'
PUMA	F	5'-GACCTCAACGCACAGTACGAG-3'
	R	5'-AGGAGTCCCATGATGAGATTGT-3'
XIAP	F	5'-TGTCCTGGCGCGAAAAG-3'
	R	5'-TGCCAGTGTTGATGCTGAAAC-3'

Table 2. List of primers and their sequences used in qRT-PCR. F: forward, R: reverse primer

10.Western blot analysis

Equal numbers of cells ($3x10^6$) were seeded to 10 cm plates prior to single agent or combination treatment for obtaining equal amount of protein extraction. After 6 hours, medium was collected, cells were lifted by trypsinization and cell pellet was obtained with centrifugation at 500*g* for 5 minutes. Pellets were then re-suspended in an appropriate volume of cell lysis buffer containing, 1% NP-40, 150 mM NaCl, 1mM EDTA, 50 mM Tris-HCl (pH 7.8), 1 mM NaF, 0.5 mM PMSF, 1X phosphatase inhibitor cocktail (PhosSTOP, Roche, Germany) and 1X protease inhibitor cocktail (cOmplete Protease Inhibitor Cocktail Tablets, Roche, Germany). Following 20 minutes of incubation on ice, centrifugation at 14,000*g* for 15 min at 4^0C was performed to yield supernatants containing total cell extract.

Proteins were separated by SDS-polyacrylamide gel electrophoresis and electrophoretically transferred onto a PVDF membrane by Trans-Blot® Turbo™ RTA Mini PVDF Transfer Kit (#170-4272, Biorad, USA). The membranes were blocked with 5% nonfat dry milk in TBS-T (20 mM TrisHCl, pH 7.8, 150 mM NaCl, 0.1%, v/v Tween-20) at room temperature for 1 hour. Then the primary antibodies were added and incubated rocking overnight at 4^0C. **Table 3** contains the primary antibodies and the dilutions used. Secondary antibodies conjugated to HRP, anti-rabbit and anti-mouse were used in 1:3,000 (#7074 and #7076 respectively, Cell Signaling, USA). Quantification of protein within membranes was done by using Clarity™ Western ECL Substrate (#170-5061, Biorad, USA). Membranes were exposed using CL-XPosure Film (Thermo-Scientific, USA).

11.Analysis of cell surface DR4 and DR5

For DR4 and DR5 analysis, 200,000 U87MG cells/well were seeded to 6 well plates, treated with Digitoxigenin, Digoxin, Lanatoside C, Digoxigenin, Proscillaridin A and Bortezomib (dosages indicated in **Table 5**) for 48 hours. Cells were then collected with cell scraper and pelleted at 1,500 rpm for 5 minutes. Cell pellets were resuspended in 200 ul PBS containing 1%FBS and stained with corresponding PE-conjugated antibodies (DR4:anti human CD261 (DR4) PE; DR5: anti human CD262 (DR5) PE, eBioscience) on ice for 30 minutes in dark. After 2 rounds of washing, cells were resuspended in 300 ul of 1%FBS/PBS and subjected to Flow Cytometry. Flow cytometry was performed according manufacturer's instructions on BD Accuri

instrument (BD Bioscience), where 10,000 events were collected from live cells on FL-2 channel. Quantification was performed using instrument settings.

Antibody	*Catalog Number*	*Brand*	*Dilution*
α-Tubulin	T9026	Sigma-Aldrich	1:5000
Bax	#5023	Cell Signaling	1:1000
β-actin	#4967	Cell Signaling	1:3000
BID	#2002	Cell Signaling	1:1000
Caspase-9	#9508	Cell Signaling	1:1000
c-FLIP	ADI-AAP-440-E	Enzo Life Sciences	1:1000
Cleaved Caspase-3	#9664	Cell Signaling	1:1000
DR4	1139	ProSci	1:500
DR5	2019	ProSci	1:500
PARP	#9542	Cell Signaling	1:1000
XIAP	610716	BD Life Sciences	1:500

Table 3. List of primary antibodies used.

Chapter 3

Results

1. TRAIL selectively affects cancer cells, but not normal cells

TRAIL's ability to selectively induce apoptosis in tumor cells *in vitro* and *in vivo* without significant effect on normal cells was previously explained [38]. The cytotoxic effect of TRAIL was tested on two patient-derived primary fibroblasts (KUFibroMNG and KUFibro4) and two different fibroblast cell lines (BJ and DH1F). In all cases, TRAIL did not have a significant cytotoxic effect even at a high dosage of 200 ng/ml (**Figure 5**).

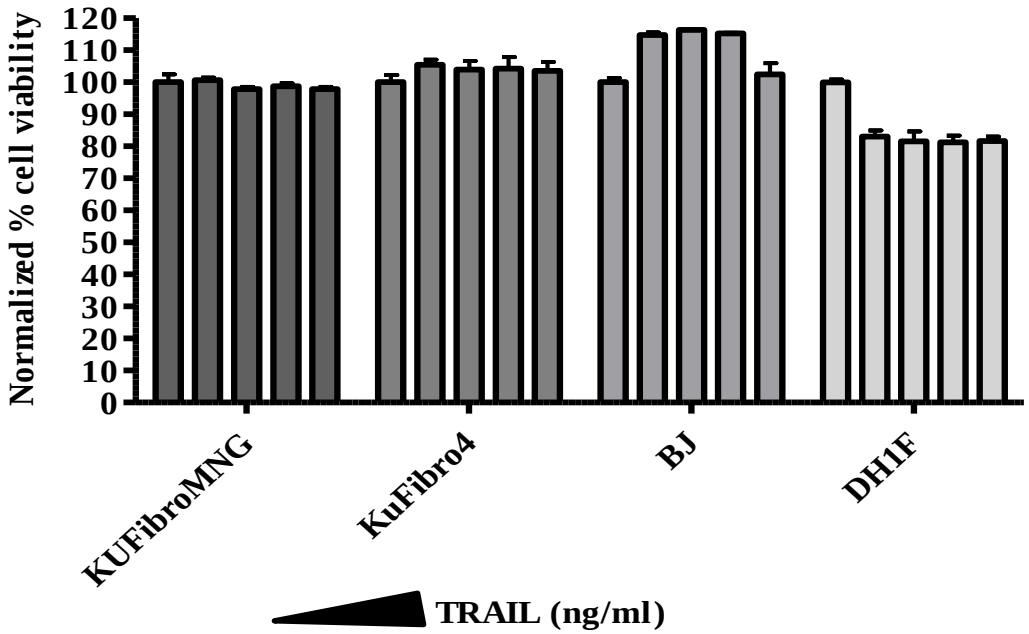


Figure 5. TRAIL does not have a cytotoxic effect on normal cells. 4 different fibroblast cell lines were exposed to different concentrations of TRAIL (25, 50, 100, 200 ng/ml respectively). After 24 hours cell viability was tested using CTG.

2. TRAIL response varies among different GBM cell lines

As previously described, TRAIL response varies among different GBM cell lines [49]. For this purpose, the cytotoxic effects of TRAIL were tested on 4 different GBM cell lines. Cell proliferation assays validated the previous knowledge: A172 is very sensitive, U87MG is mid-

sensitive, U373 and LN229 cell lines are very resistant to TRAIL (**Figure 6a**). Treatment with TRAIL 50 ng/ml for 6 hours induced cell death with characteristic apoptotic features such as cell shrinkage and apoptotic body formations observed by bright-field microscopy in TRAIL sensitive cell lines, but not in TRAIL resistant cell lines (**Figure 6b**). PARP is a nuclear poly (ADP-ribose) polymerase and is involved in DNA repair in response to environmental stress. Cleavage of PARP facilitates cellular disassembly and serves as a marker of cells undergoing apoptosis [68]. Comparing 4 different cell lines, the TRAIL sensitive cell line A172 displayed more cPARP after 6 hours of 50 ng/ml TRAIL treatment (a-tubulin was used as loading control) (**Figure 6c**).

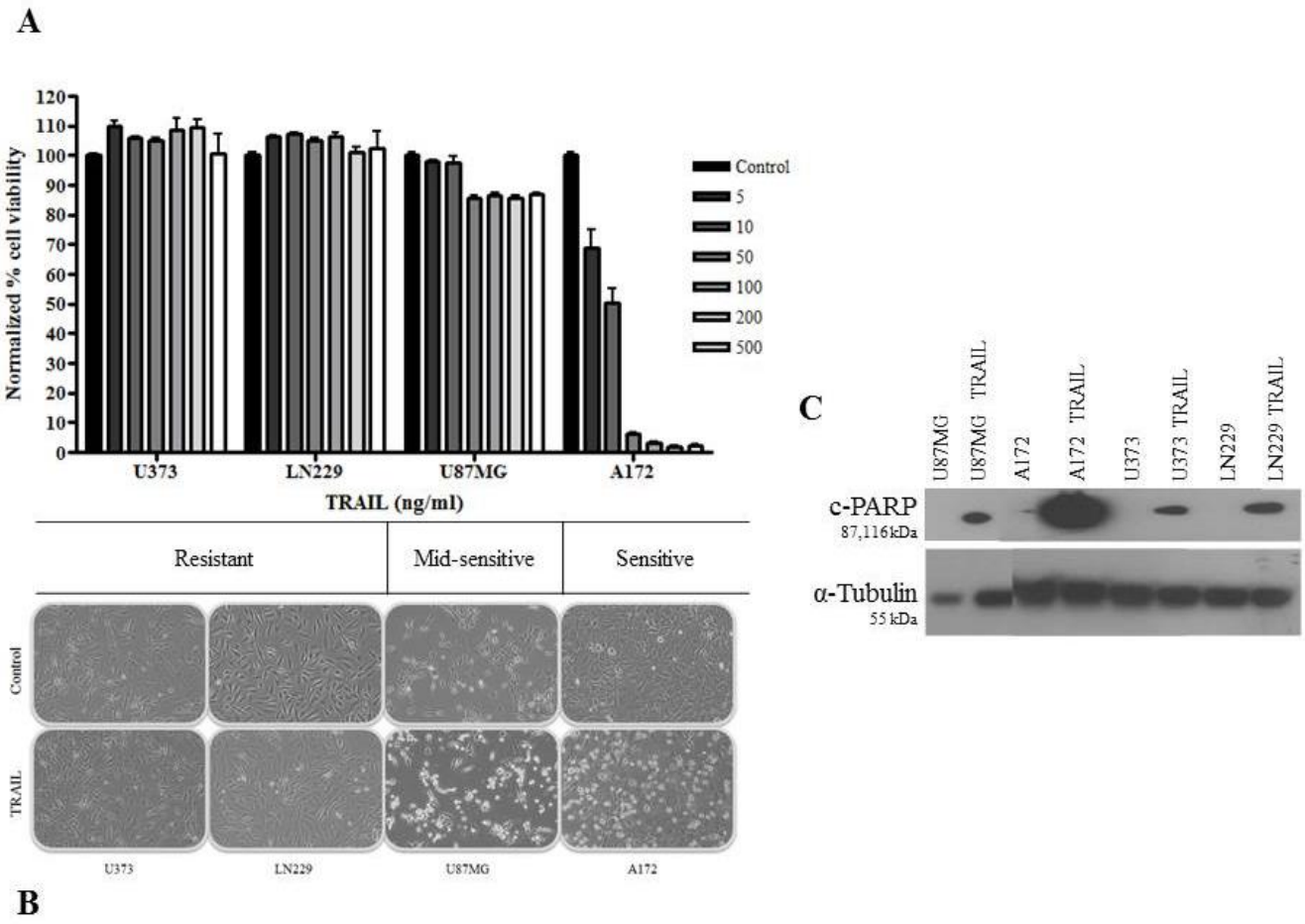


Figure 6. TRAIL response among different GBM cell lines. ***A.*** 4 different GBM cell lines were exposed to different concentrations of TRAIL (5, 10, 50, 100, 200, 500 ng/ml respectively). After 24 hours, cell viability was tested using CTG. ***B.*** Microscopy images of GBM cell lines after 6 hours of 50 ng/ml TRAIL treatment. ***C.*** Western blot images displaying cleaved PARP expression of 4 different cell lines treated with TRAIL (50 ng/ml) for 6 hours.

3. TRAIL sensitization with drug combinations is possible

To validate the effect of drug combinations on TRAIL sensitization, known TRAIL sensitizers MS-275 and Bortezomib were used. Both drugs did not cause any significant changes in cell viability when applied alone. However the combination with 50 ng/ml TRAIL reduced cell viability of 5 μM MS-275 treated cells by 70% and 50 nM Bortezomib treated cells by 99% (**Figure 7**). Bortezomib + TRAIL combination showed more drastic changes in cell viability than MS-275 + TRAIL combination. For this reason Bortezomib is further studied and was chosen to be the positive control in drug screen.

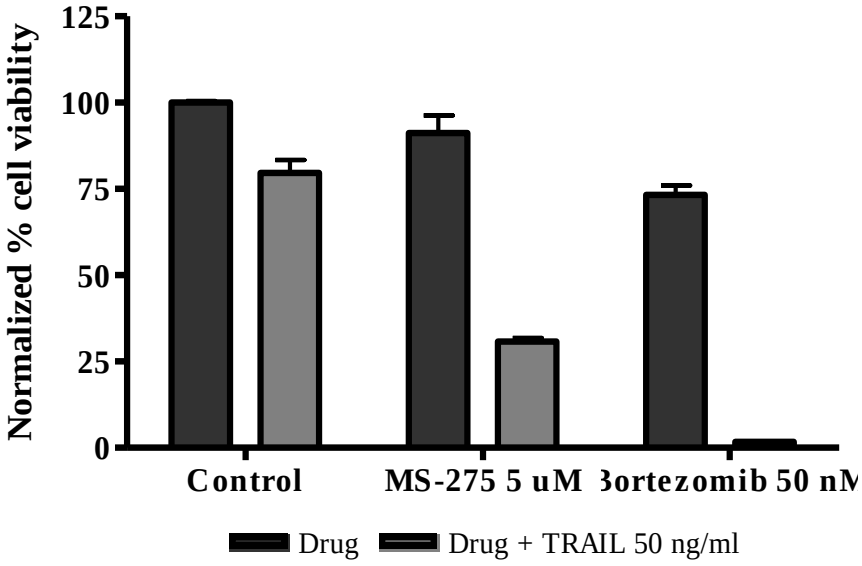


Figure 7. TRAIL sensitization with drug combinations. 5μM MS-275 and 50 nM Bortezomib was added as single agents and in combination with 50 ng/ml TRAIL to U87MG cells. Cell viability was determined after 24 hours with CTG assay.

Different concentrations of Bortezomib (10^{-6} to 10 μM) were tested on U87MG cells to test the effect of cell viability (**Figure 8a**). Bortezomib treatment up to 0.5 μM dosages did not affect the cell viability, whereas the combination with TRAIL revealed drastic alterations: 50 nM of Bortezomib with TRAIL combination as low as 5 ng/ml caused more than 90% cell death (**Figure 8b**). With the guidance of these results, 50 nM of Bortezomib was used for the drug screen. For gene and protein expression level studies, 5 nM of Bortezomib was chosen.

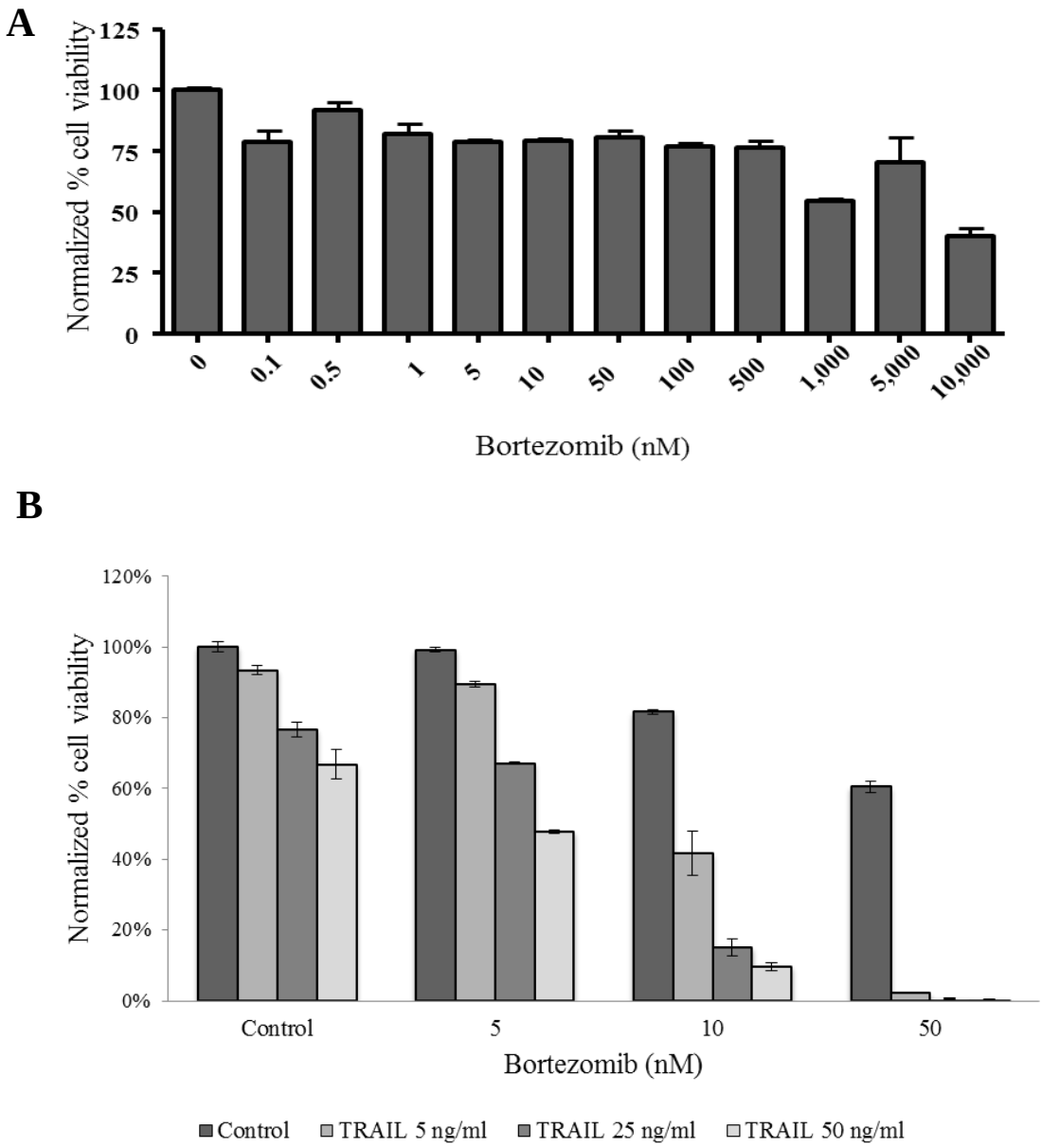


Figure 8. The effect of Bortezomib on U87MG cells. ***A.*** Bortezomib ranging from 10^{-6} to 10 μM was applied to U87MG cells and cell viability was determined by CTG after 24 hours. ***B.*** 5, 10 and 50 nM of Bortezomib was combined with 5, 25 and 50 ng/ml of TRAIL. Cell Viability was detected by CTG after 24 hours.

U373 and LN229 cell lines are TRAIL resistant cell lines and are not affected by 50 ng/ml TRAIL under normal conditions. However, the combination of 50 nM Bortezomib and 50 ng/ml TRAIL decreased cell viability of these TRAIL-resistant cells significantly, indicating the potential of TRAIL sensitizers in TRAIL-resistant cell lines (**Figure 9**).

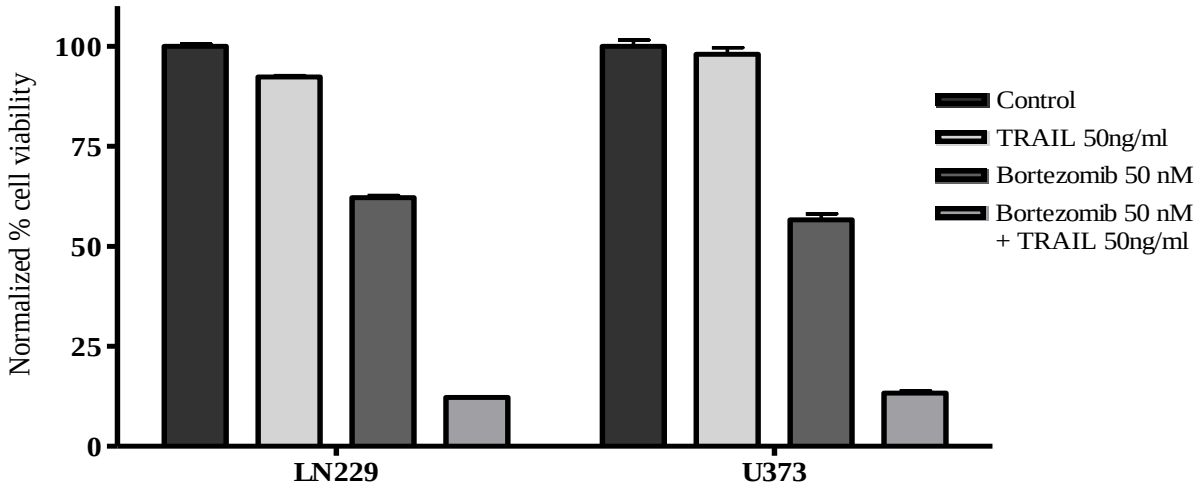


Figure 9. Bortezomib and TRAIL combination's effect on TRAIL resistant cells. U373 and LN229 cells were treated with 50 nM Bortezomib, 50 ng/ml TRAIL and their combination. After 24 hours cell viability was detected by CTG assay.

4. Cell number and TRAIL concentration optimizations prior to high throughput drug screen

The optimal number of U87MG cells used for the HTS assay to a 384 well plate was found by different number cell seeding in the range of 50 - 10,000 cells. 2,000 cells were found to be the best confluency for the assay. For the drug screen, cells were automatically seeded to 384 well black plates at the number of 2,000 cells/40 µl/well.

As indicated before, TRAIL resistance varies among and within different cell lines. For this purpose the resistance of U87MG cells was detected prior to drug screen. With the negative 15-25 % effect on cell viability (**Figure 10**), TRAIL 25 ng/ml was used for the screen.

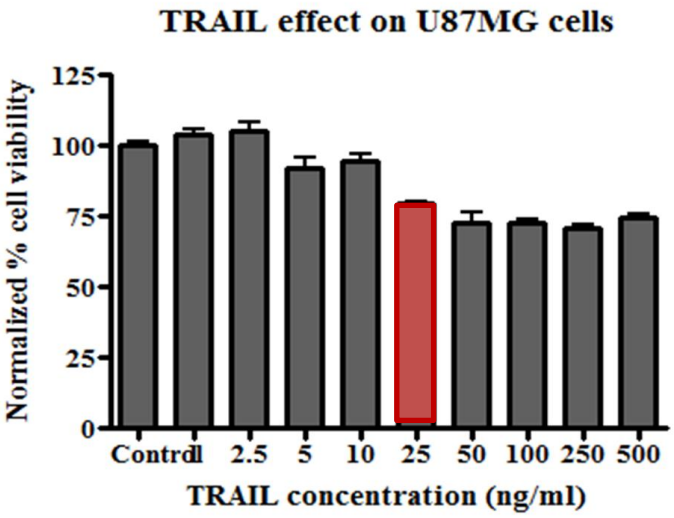


Figure 10. U87MG TRAIL response. TRAIL dose dependent assay was performed prior to drug screen to detect the optimum dosage. After 24 hours of treatment cell viability was detected by CTG assay. The chosen dosage for the primary screen, 25 ng/ml, is indicated in red.

5. High throughput drug screen reveals potential TRAIL sensitizers in U87MG cells

Initially, the screen was designed to be performed in 5 batches with cells of passage numbers between 9 and 12. However, TRAIL effect showed high variance (STDEV: 15%) among different sets of experiments. To eliminate the problem of TRAIL response variance, a second screen was designed to be done in a single batch, in which all plates were seeded at the same time on Day 0 and reagent addition was done in Day 1, 14 hours after seeding.

The cut off value for the screen was determined to be "Average ± (3STDEV)". However, due to the high variance of TRAIL in the first screen, the lower cut off value of drug + TRAIL effect was hard to estimate. For this purpose, normalization to TRAIL only effect was performed, resulting in a cut off value of 28%. Every compound that resulted in the normalized percent cell viability under the lower cut off value were considered as a "hit". The results from the primary and secondary screen are presented as scatter plots in **Figure 11b** and **11c.** Notably, at the concentration of 5 μM some of the drugs had high cytotoxicity as single agents.

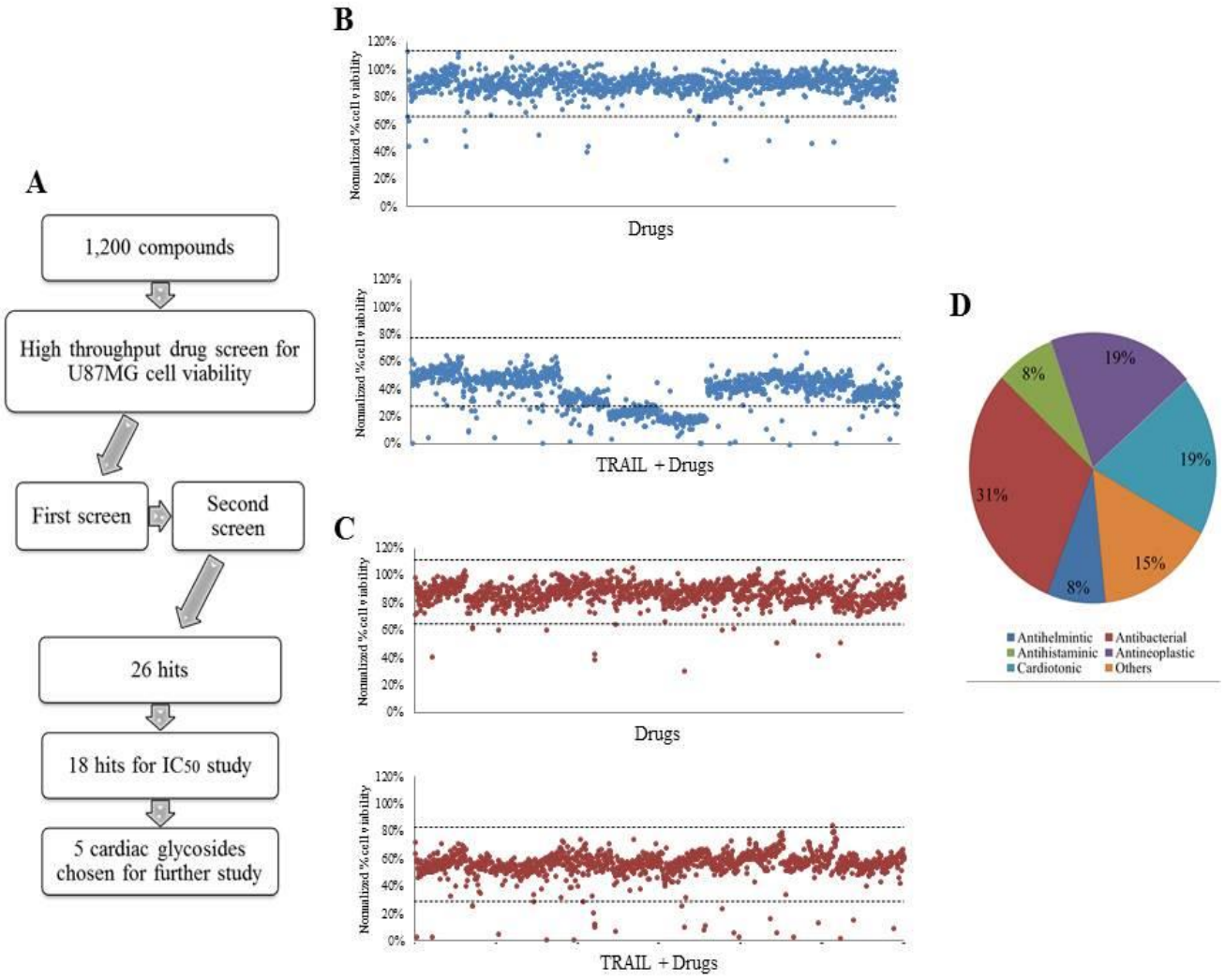


Figure 11. Drug screens. ***A.*** A general scheme of different steps of the HTS. ***B.*** Scatter plot of first screen, drug and drug + TRAIL effect. Results are presented as percent cell viability normalized to untreated control. ***C.*** Scatter plot of the second screen, drug and drug + TRAIL effect. Results are presented as percent cell viability normalized to untreated control. ***D.***Statistical pie representing the different classes of drug hits. Data are presented as percentage of drugs from the same class compared to total drug hit number.

From the first screen, 26 hits were chosen from the drug + TRAIL combination. 14 of those hits caused a cell viability value lower than drug-only cut off value (66%), indicating the need for adjustment of the dosage for these drugs.

The second screen validated the same 26 hits, with little variance of TRAIL alone effect (STDEV: 3%) (**Figure 12**). Hits were found to be clustered around several pharmacological

classes (antibacterials, antineoplastics and cardiotonics) suggesting that these classes should be further explored for GBM therapy development (**Figure 11d**). The summary of drugs identified as TRAIL sensitizing agents in GBM are indicated in **Table 4.** Some previously known agents such as Quinacrine dihydrochloride, Mitoxantrone dihydrochloride, Vorinostat, Campthothecine (S,+), Daunorubicin hydrochloride, Topotecan, Digoxin, Doxorubicin hydrochloride, Proscillaridin A, which had been studied in preclinical experiments, evaluated in clinical trials or used in clinical as chemotherapeutic agents for tumors including GBM were identified in the screen, thereby validating the efficiency of our screening approach.

Since all the drugs were obtained in DMSO solutions, the screen contained only DMSO treated cells to observe the effect of DMSO alone on viability. No effect was observed in only DMSO or DMSO + TRAIL treated cells (**Figure 12b**).

Further experimental designs were based on the results of the second screen.

6. Screen performed on U87MGTRAIL-resistant subpopulations reveal the same hits

A third screen containing U87MG TRAIL-resistant subpopulations (U87MG R50) was performed. As indicated in Appendix I, these cells were generated by selection under 50 ng/ml TRAIL for 30 days. These cells are considered to be a subpopulation within U87MG cell lines with very low TRAIL sensitivity, and can be selected and maintained as a separate cell line. In order to assess the TRAIL-sensitizing efficacy of the drugs the screen was conducted similarly with the same conditions (TRAIL dose (25 ng/ml) that was used as in the 1^{st} and 2^{nd} screens). In normal U87MG cells 25 ng/ml TRAIL decreases cell viability up to 30%, however TRAIL-R50 are only affected by 5-10% decrease.

As indicated in **Figure 13b** screen performed on U87MG-R50 resulted in 16 hits. All of the 16 hits are among the top 26 hits of the previous screens performed on U87MG cells. These results are promising in that the chosen hits can still affect cell viability even in TRAIL-resistant subpopulations.

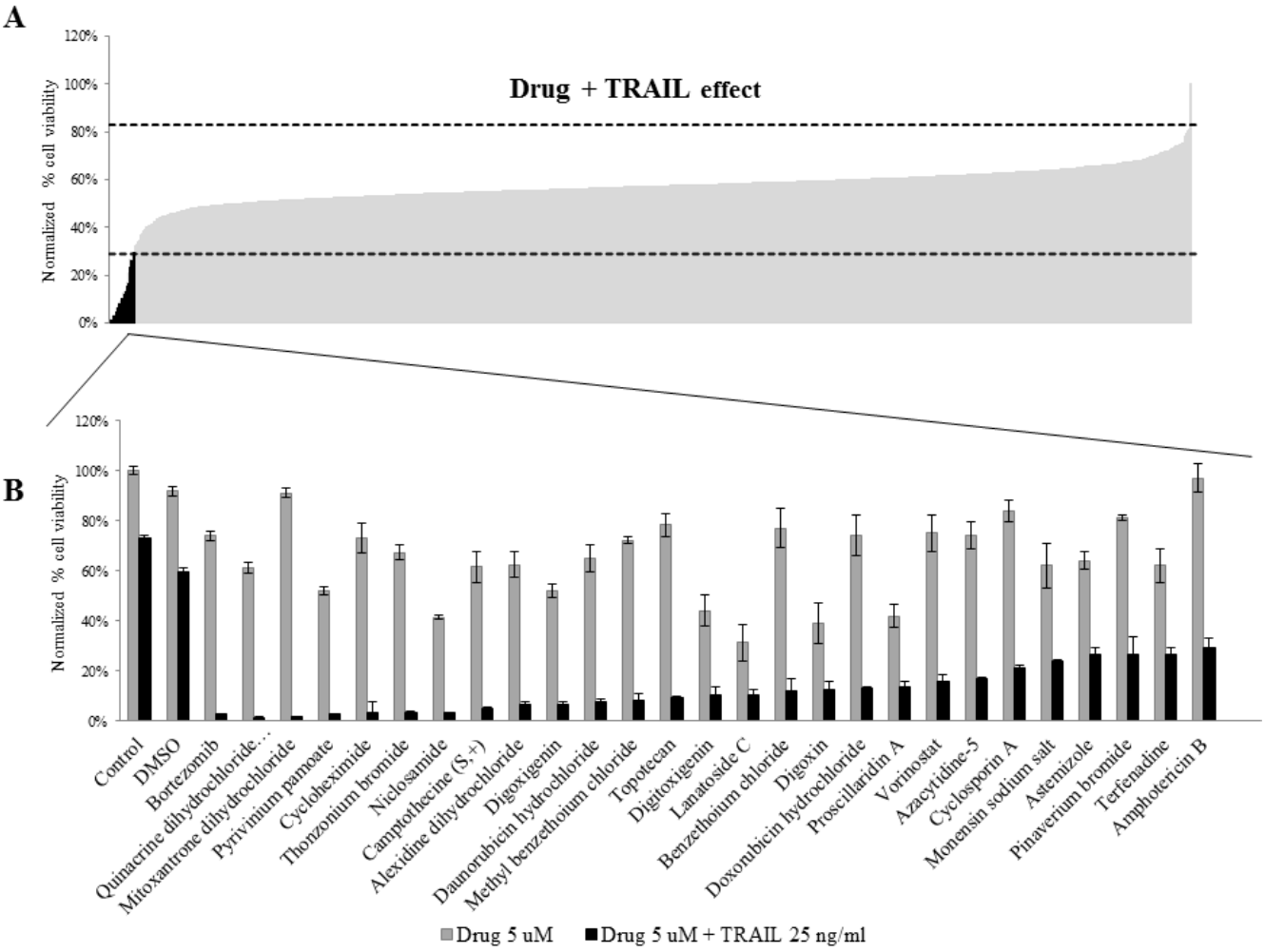


Figure 12. Secondary screen of U87MG validated top 26 hits of primary screen. ***A.*** Normalization to each plate's untreated control samples was performed to eliminate variance among different plates. Results of drug + TRAIL effect were ordered from lowest to highest. Cut off values 29% and 83% were determined by "average ±3 STDEV". ***B.*** 26 hits were determined via cut off value of 28% cell viability after drug and TRAIL addition. The hits are ordered from highest to lowest effect of drug + TRAIL combination. Error bars represent "mean ± NSEM".

Drug Name	*Therapeutic effect*	*Activity*	*2nd screen*		*TRAIL sensitizer?*	*TRAIL sensitizer in GBM?*
			D	*D+T*		
Amphotericin B	antibacterial	$Na^+ K^+$ ATPase membrane pump inhibiton [69]	84%	21%	No	No
Cycloheximide		Translation inhibition [70]	73%	3%	Yes [71]	Yes[72]
Daunorubicin hydrochloride		DNA topoisomerase II inhibitor [73, 74]	65%	8%	Yes [75]	No
Methyl benzethonium chloride		Mitochondrial membrane depolarization [76]	72%	8%	No	No
Benzethonium chloride		Mitochondrial membrane depolarization [76]	77%	12%	No	No
Monensin sodium salt		Na^+ ionophore, blocks glycoprotein secretion [77]	62%	24%	Yes[78]	Yes[78]
Doxorubicin hydrochloride		DNA topoisomerase II inhibitor [79]	74%	13%	Yes [80, 81]	Yes[80]
Alexidine dihydrochloride		PTPMT1 inhibitor [82]	62%	6%	No	No
Niclosamide	antihelmintic	Transcription inhibition [83], Wnt/Frizzled-1 signaling inhibitor [84]	41%	3%	No	No
Quinacrine dihydrochloride		Phospholipase A2 inhibitor [85]	61%	1%	Yes [86]	No
Astemizole	antihistaminic	hERG potassium channel blocker [87]	64%	26%	No	No
Terfenadine		hERG potassium channel blocker [87]	62%	27%	No	No
Vorinostat	antineoplastic	Histone de acetylase inhibitor [88]	75%	16%	Yes[89-91]	No
Topotecan		DNA topoisomerase I inhibitor [92]	78%	9%	Yes[93, 94]	Yes[95]
Azacytidine-5		DNA methyltransferase inhibitor [96]	74%	17%	Yes[97]	No
Mitoxantrone dihydrochloride		DNA intercalator, DNA topoisomerase II stabilizer [98]	91%	2%	Yes[99, 100]	No
Camptothecine (S,+)		DNA topoisomerase I inhibitor [101]	61%	5%	No	No
Pinaverium bromide	antispastic	Calcium channel inhibitor [102]	81%	27%	No	No
Digitoxigenin	cardiotonic	$Na^+ K^+$ ATPase membrane pump [103]and DNA topoisomerase I /II inhibitor [104]	39%	12%	No	No
Digoxin		$Na^+ K^+$ ATPase membrane pump [103]and DNA topoisomerase I /II inhibitor [104]	74%	13%	Yes[66]	Yes[66]
Lanatoside C		$Na^+ K^+$ ATPase membrane pump inibitor [105]	31%	10%	Yes[105]	Yes[66]
Digoxigenin		$Na^+ K^+$ ATPase membrane pump inibitor	52%	7%	No	No
Proscillaridin A		$Na^+ K^+$ ATPase membrane pump [106], DNA topoisomerase I /II inhibitor [104]	42%	14%	No	No
Cyclosporin A	immuno-suppressant	Canonical NFAT pathway inhibitor [107]	44%	10%	No	No
Pyrvinium pamoate	metabolism	WNT signaling inhibitor [108], Mitochondrial ETC I and II inhibitor [109]	52%	3%	No	No
Thonzonium bromide	antiseptic	vacuolar ATPase inhibitor [110]	67%	3%	No	No
		Cut off value for % cell viability	*64%*	*29%*		

Table 4. Summary of drugs identified as TRAIL sensitizing agents and their previously known activity. Numbers indicated are percent cell viability results normalized to untreated control samples. D represents the effect of Drug alone; D + T represent the effect of Drug + TRAIL.

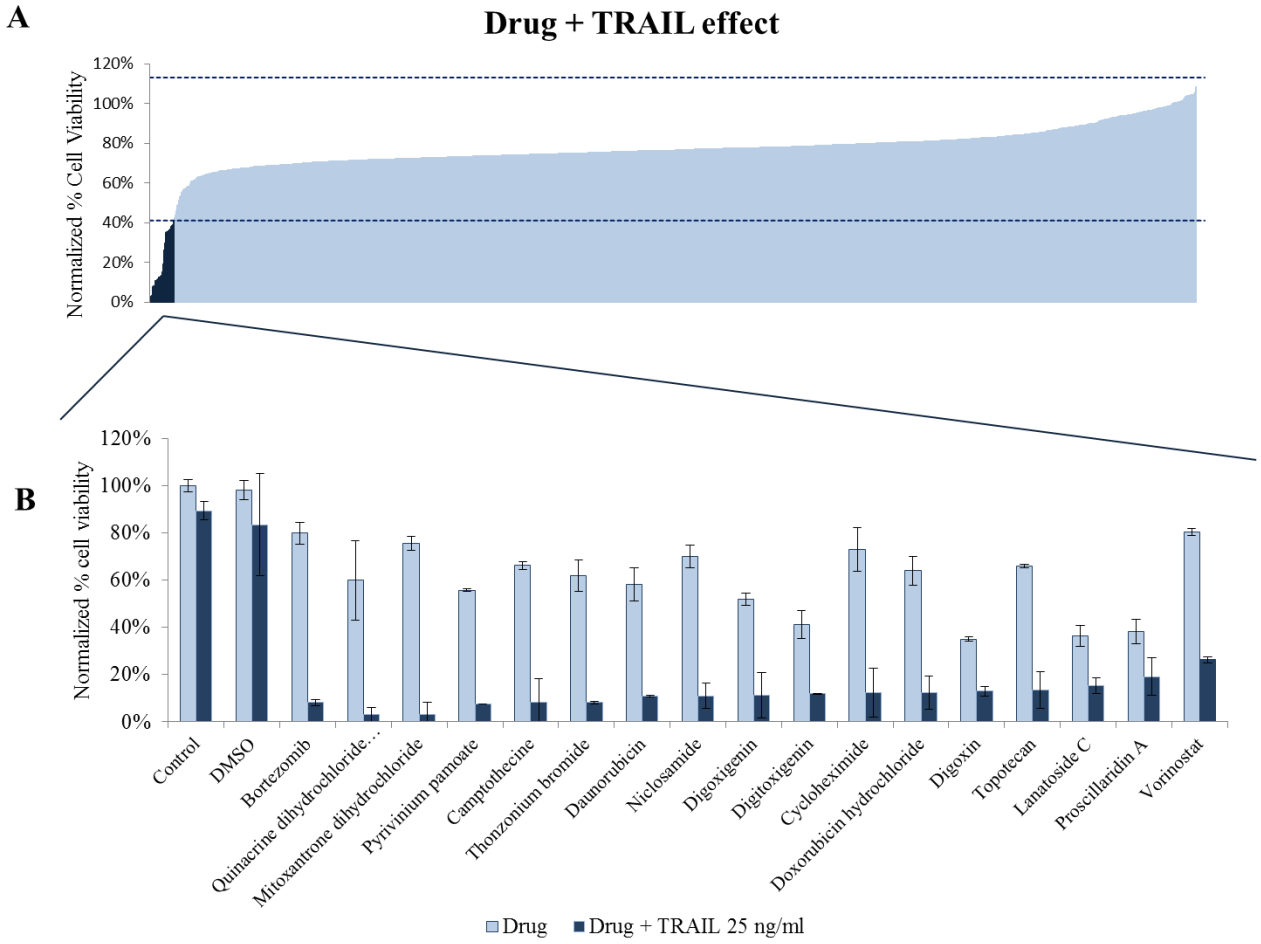


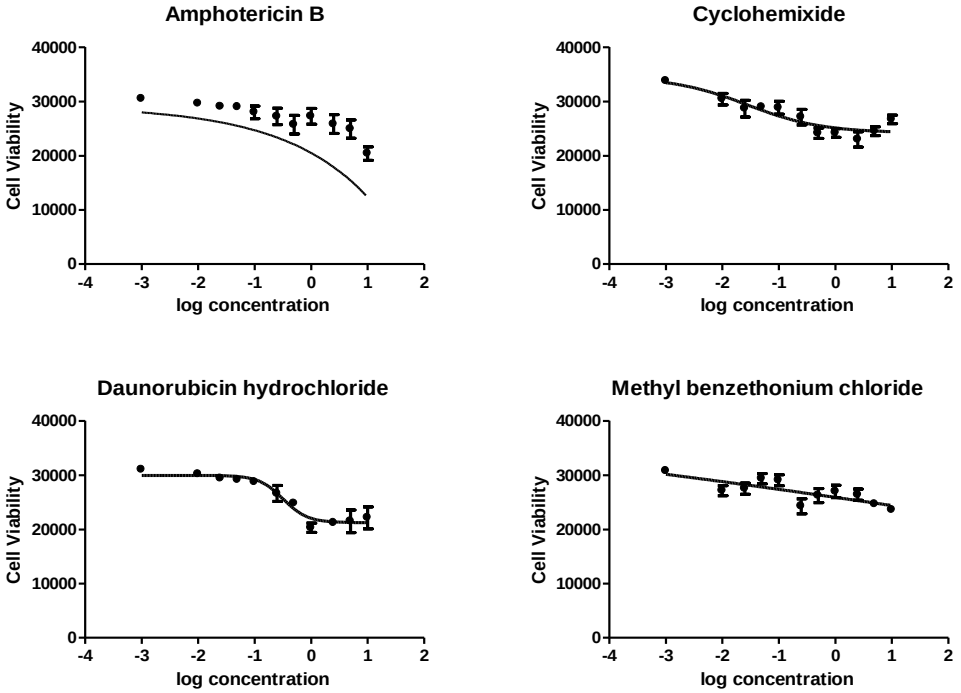
Figure 13. Screen of U87MG-R50 (selected TRAIL resistant cell line). ***A.*** Normalization to each plate's untreated control samples was done to eliminate variance among different plates. Results of drug + TRAIL effect were ordered from lowest to highest. Cut off values 41% and 113% were determined by "average ±3 STDEV". ***B.*** 16 drugs scored as hits by the cut off value of 41% cell viability after drug and TRAIL addition. The hits are ordered from highest to lowest effect of drug + TRAIL combination. Error bars represent "mean ± NSEM".

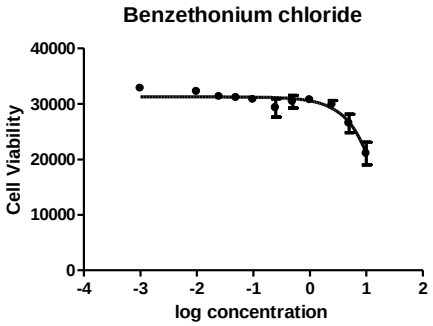
7. Validation of selected hits for TRAIL sensitization

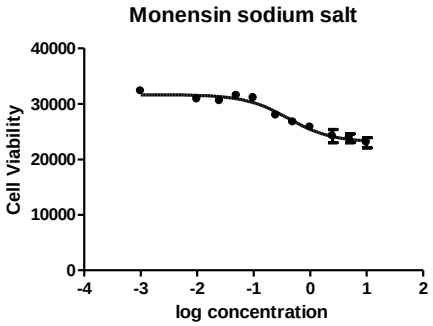
The concentration of each drug was 5μM for the screen and 26 out of 1,200 drugs scored as hits at this concentration. In order to further to assess the dose-dependent effects of these drugs, a range of different concentrations of the 26 hits was re-analyzed (0.01 – 10 μM). Niclosamide, Digitoxigenin, Digoxin, Lanatoside C, Proscillaridin A, Pyrivinium Pamoate and Vorinostat showed cytotoxicity as single agents at μM concentrations as indicated in their respective figures (**Figure 14**).

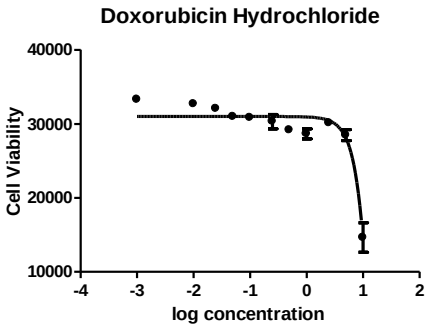
Selected 20 hits were further studied with TRAIL. 50, 500 and 5,000 nM of drugs were combined with 5, 25 and 50 ng/ml TRAIL for determination of the optimum dosage combination. Results can be seen in **Figure 15**. Chosen doses for the best combinatorial effects are indicated in **Table 5**.

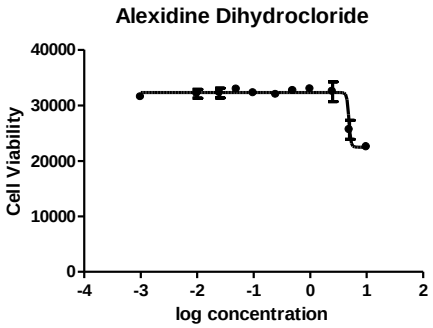
As will be shown later, 12 of the hits (Digitoxigenin, Digoxin, Lanatoside C, Digoxigenin, Proscillaridin A, Pyrivinium Pamoate, Topotecan, Vorinostat, Mitoxantrone, Campthothecine, Cycloheximide and Niclosamide) were further studied for gene expression and 5 of them (Digitoxigenin, Digoxin, Lanatoside C, Digoxigenin, Proscillaridin A) for protein expression experiments.

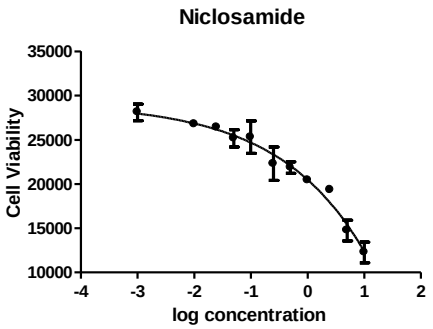


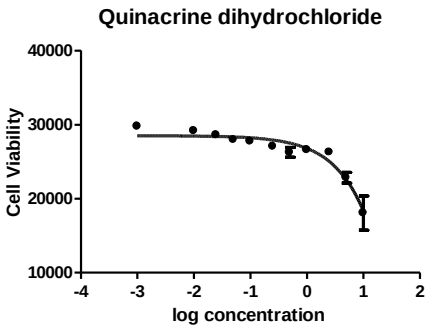

Benzethonium chloride
Cell Viability
log concentration

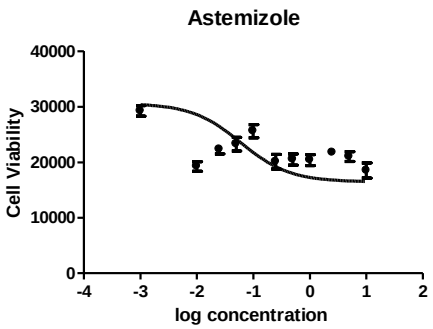

Monensin sodium salt
Cell Viability
log concentration

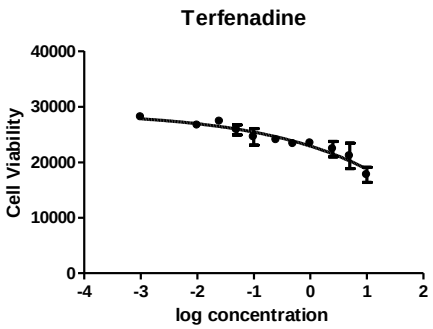

Doxorubicin Hydrochloride
Cell Viability
log concentration

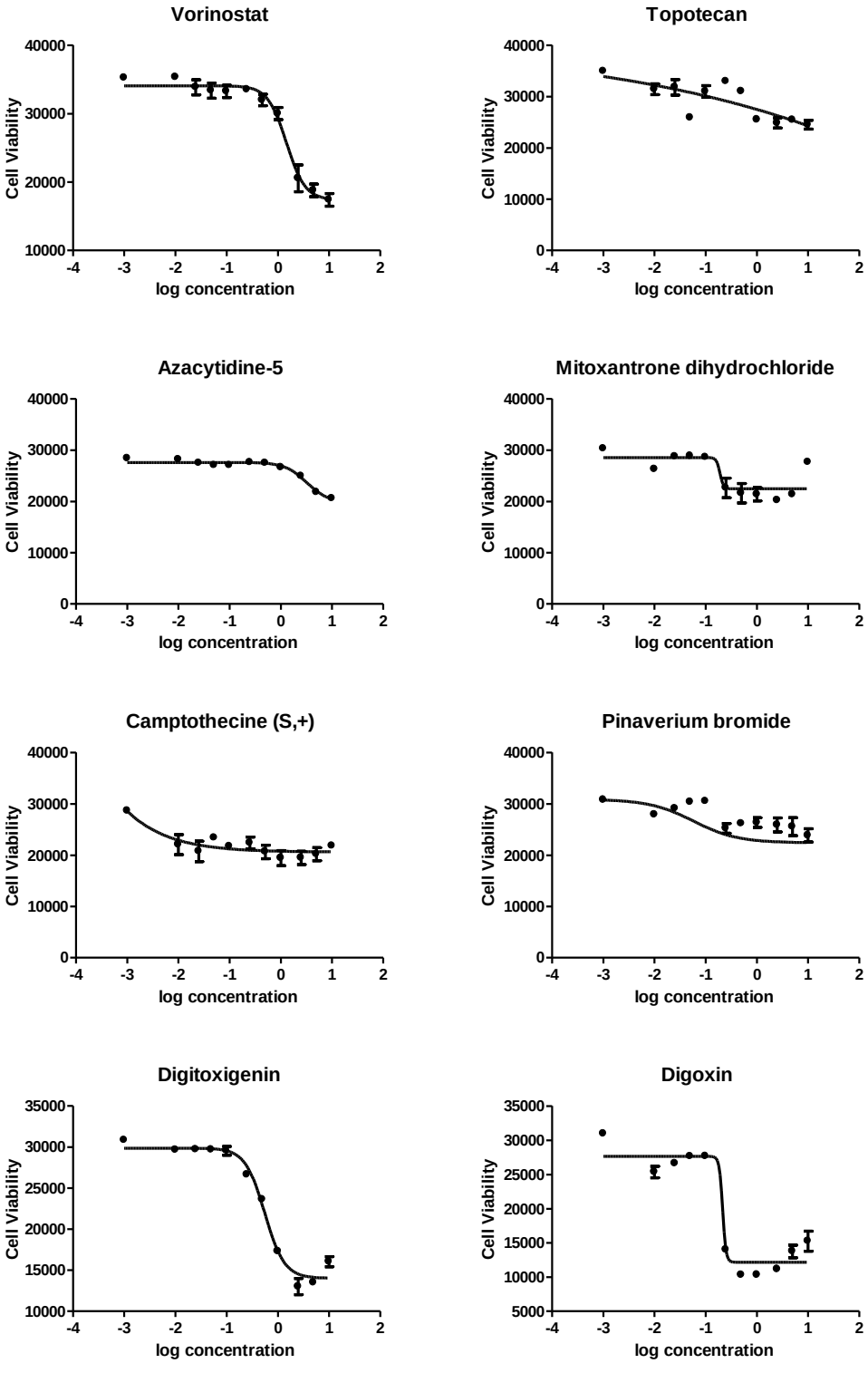

Alexidine Dihydrocloride
Cell Viability
log concentration


Niclosamide
Cell Viability
log concentration


Quinacrine dihydrochloride
Cell Viability
log concentration


Astemizole
Cell Viability
log concentration


Terfenadine
Cell Viability
log concentration


Vorinostat
Topotecan
Azacytidine-5
Mitoxantrone dihydrochloride
Camptothecine (S,+)
Pinaverium bromide
Digitoxigenin
Digoxin
Cell Viability
log concentration

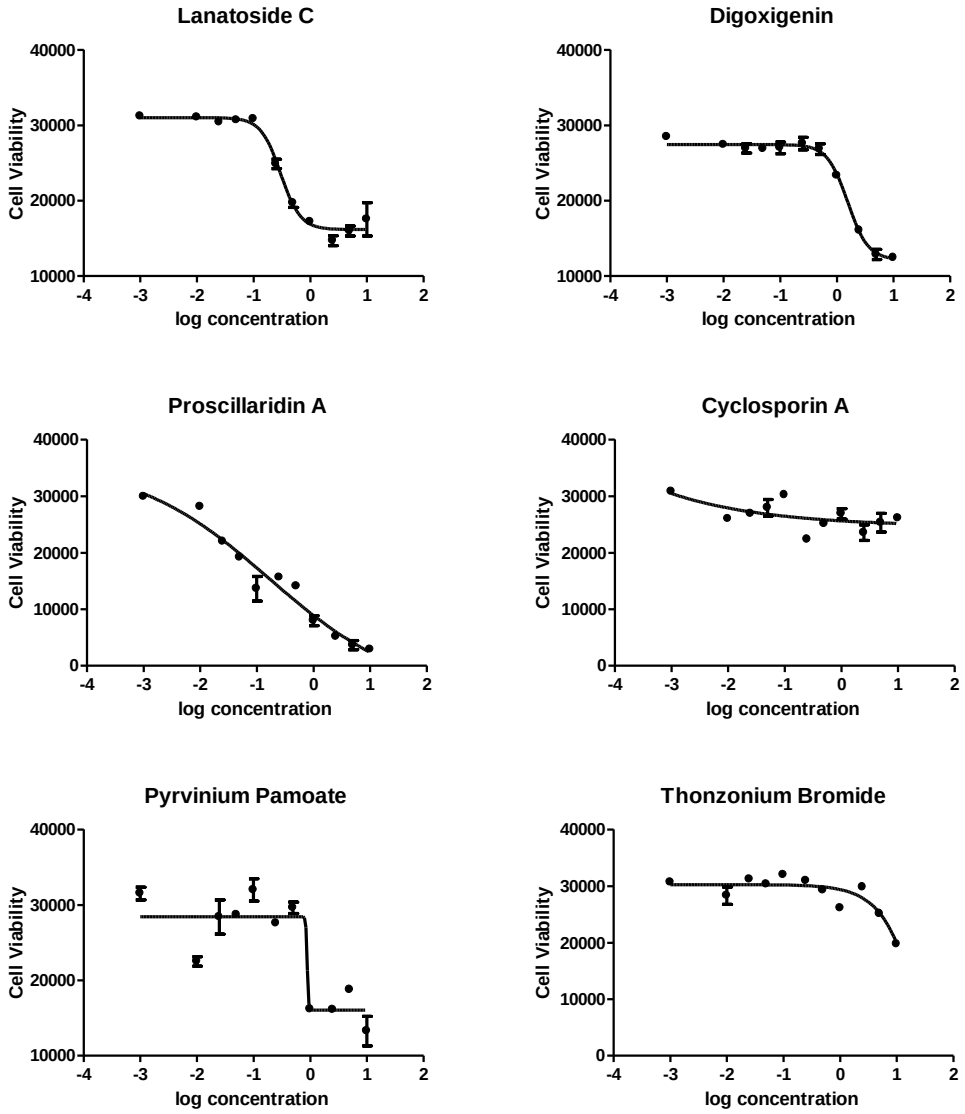
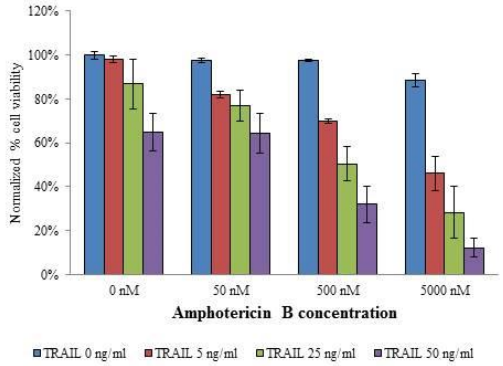
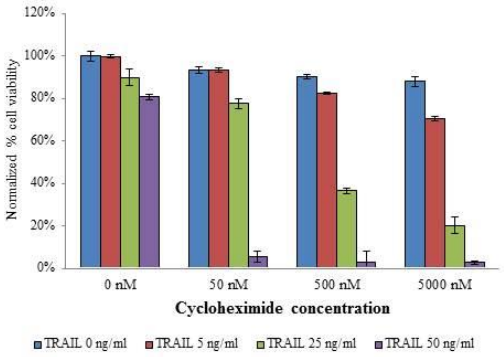
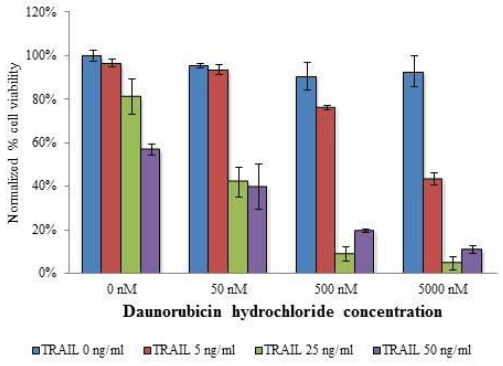
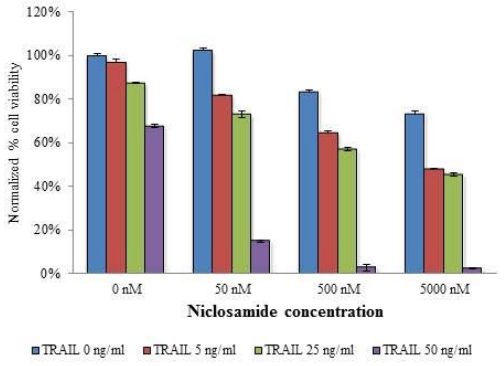


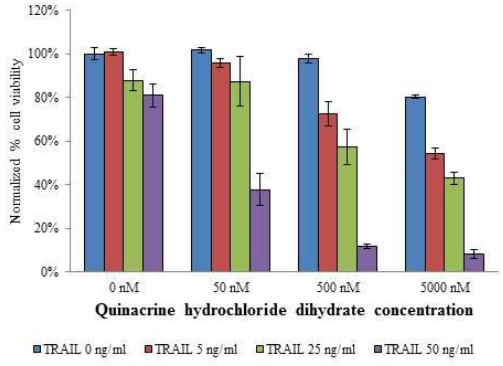
Figure 14. Dose-dependent effects of the 26 hits on cell viability. Cells were treated with respective drugs with concentrations ranging from 0.01 to 10 μM. Cell viability was assessed with CTG after 24 hours. Graphs were prepared by GraphPad Prism 4.0.

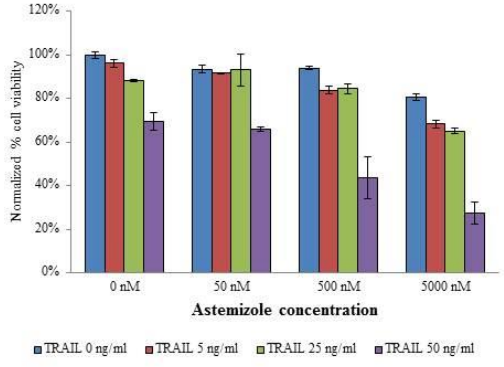

Normalized % cell viability
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Amphotericin B concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml

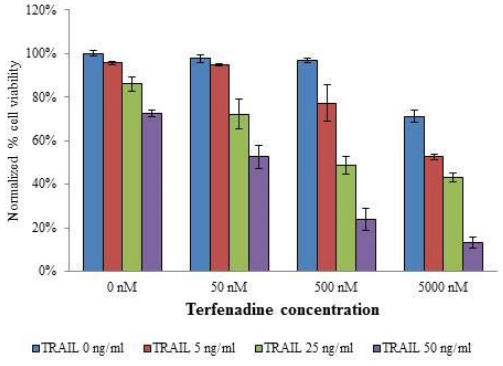

Normalized % cell viability
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Cycloheximide concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml

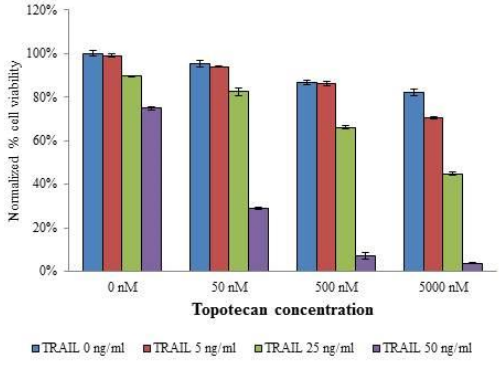

Normalized % cell viability
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Daunorubicin hydrochloride concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml

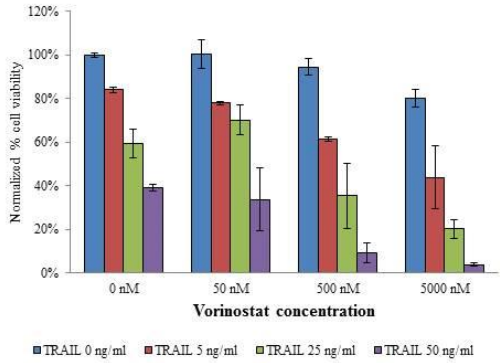

Normalized % cell viability
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Niclosamide concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml

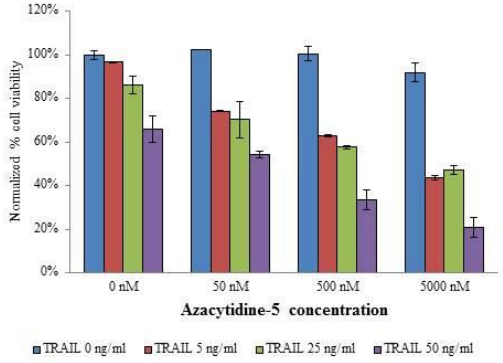

Normalized % cell viability
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Quinacrine hydrochloride dihydrate concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml

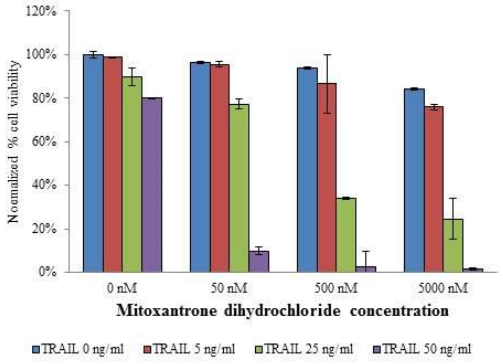

Normalized % cell viability
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Astemizole concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml

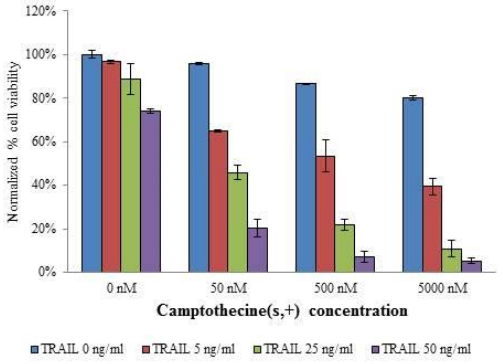

Normalized % cell viability
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Terfenadine concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml

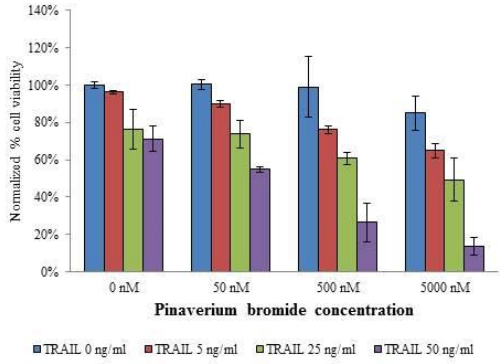

Normalized % cell viability
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Topotecan concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml

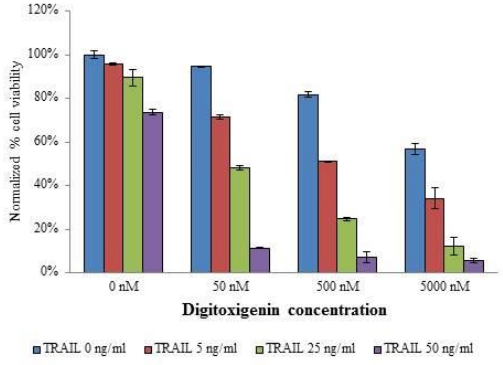

Normalized % cell viability
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Vorinostat concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml

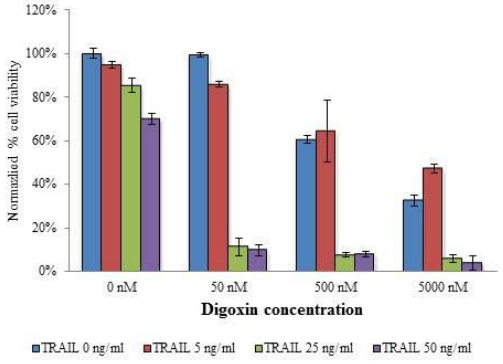

Normalized % cell viability
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Azacytidine-5 concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml

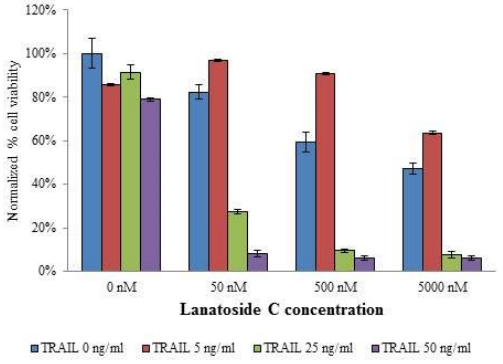

Normalized % cell viability
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Mitoxantrone dihydrochloride concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml


Normalized % cell viability
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Camptothecine(s,+) concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml


Normalized % cell viability
140%
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Pinaverium bromide concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml


Normalized % cell viability
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Digitoxigenin concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml


Normalized % cell viability
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Digoxin concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml


Normalized % cell viability
120%
100%
80%
60%
40%
20%
0%
0 nM
50 nM
500 nM
5000 nM
Lanatoside C concentration
TRAIL 0 ng/ml
TRAIL 5 ng/ml
TRAIL 25 ng/ml
TRAIL 50 ng/ml

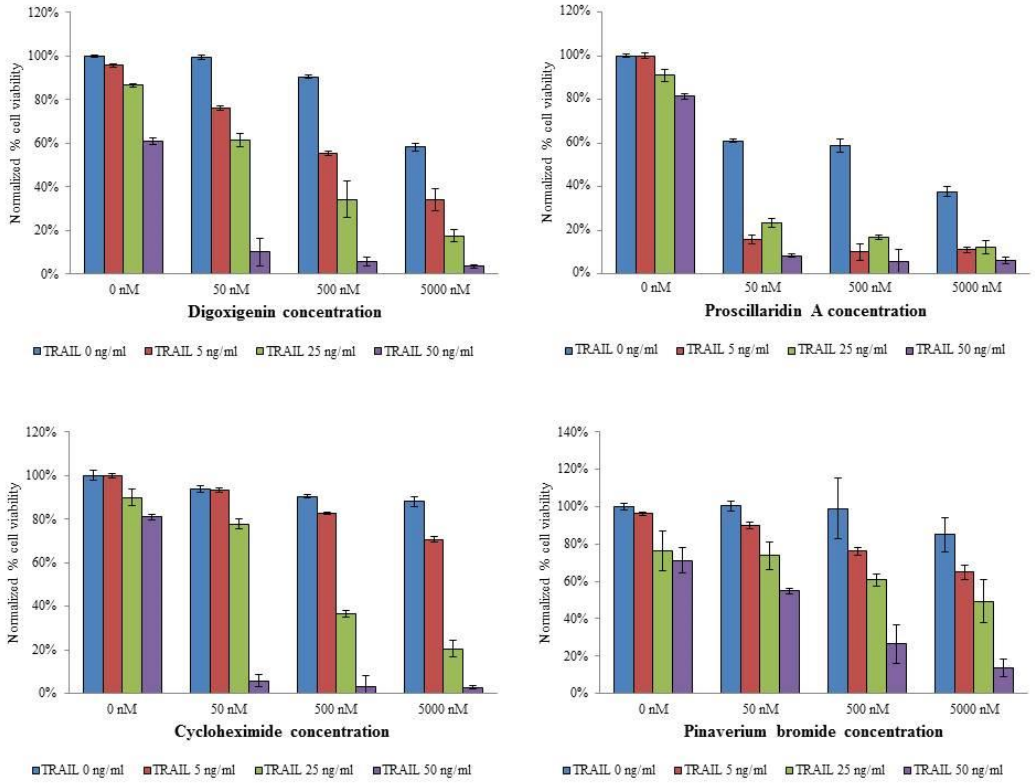


Figure 15. Graph of Drug + TRAIL combinations for selected hits. 50, 500 and 5,000 nM of drugs were combined with 5, 25 and 50 ng/ml TRAIL for determination of the optimum dosage combination. Reagents were added and cell viability was determined 24 hours later by CTG.

Drug name	Drug concentration (nM)	TRAIL concentration (ng/ml)
Amphotericin B	5000	50
Cycloheximide	50	50
Methyl benzethonium chloride	500	25
Niclosamide	500	50
Quinacrine dihydrochloride dihydrate	500	50
Astemizole	5000	50
Terfenadine	5000	50
Vorinostat	5000	50
Topotecan	500	50
Azacytidine-5	5000	50
Mitoxantrone dihydrochloride	500	50
Camptothecine (S,+)	500	50
Pinaverium bromide	5000	50
Digitoxigenin	500	50
Digoxin	50	25
Lanatoside C	50	50
Digoxigenin	50	50
Proscillaridin A	25	50
Cyclosporin A	5000	50
Pyrvinium pamoate	500	50

Table 5. Drug and TRAIL concentrations of selected hits used for further studies.

8. Gene expression changes with drug-treated U87MG cells

In order to understand the mechanism of the effects of selected TRAIL-sensitizing drugs on GBM cells, the expression changes of apoptosis-related genes upon drug treatment were assessed. As previously reported, most TRAIL-sensitizing drugs work partially by changing the TRAIL-receptor expression levels [45-49]. RNA samples were collected after 24 hours of drug treatment and quantitative RT-PCR was performed for each drug treated sample. Normalization was done against housekeeping gene GAPDH.

To this end, we examined the mRNA levels of TRAIL receptors, namely, DR4, DR5, DcR1, DcR2 and OPG. As shown in **Figure 16**, DR4 levels increased 3 fold of control with Proscillaridin A and Pyrivinium Pamoate treatments. With Topotecan and Campthothecine treatment DR4 levels increased 17 fold of control, whereas with Mitoxantrone dihydrochloride this fold change was as high as 59. DR4 levels were unchanged with MS-275, Digitoxigenin,

Lanatoside C, Digoxigenin, Cycloheximide and Niclosamide administration. In addition DR4 levels decreased compared to control in Vorinostat treated sample.

Similarly DR5 expression was altered to 3 folds with Proscillaridin A, Pyrivinium Pamoate and Mitoxantrone dihydrochloride treated cells. The levels of DR5 remained relatively unchanged to control with the rest of the drugs.

Decoy receptors DcR1, DcR2 and the soluble receptor osteoprotegerin (OPG) also showed alterations in expression levels among different drugs. Digitoxigenin, Pyrivinium Pamoate, Mitoxantrone, Campthothecine and Cycloheximide increased, whereas Bortezomib and Topotecan the fold change expression decreased. Bortezomib and Campthothecine elevated the expression levels of DcR2 3 and 4 fold changes respectively. Digoxigenin, Topotecan and Vorinostat had decreased levels of DcR2 compared to control. Digitoxigenin and Proscillaridin A showed 2 folds of change in OPG expression levels compared to control, whereas Bortezomib, Pyrivinium Pamoate and Vorinostat had decreased levels.

The next most upstream component of TRAIL apoptotic signaling is the DISC formation, where FADD and initiator pro-caspase-8 and -10 are recruited. FADD expression changes with different drug treatments were examined and no significant alterations were observed, except the decrease in Vorinostat treated sample (**Figure 17a**).

For apoptosis to take place the activation of initiator and effector caspases is needed. For this reason gene expression changes in caspases-8, -9, -3 and -7 was studied. For caspase-8, only Topotecan treated sample indicated a fold change over control decrease, while the rest of the samples expressed relatively same as control sample (**Figure 17a**). Caspase-9 expression levels were decreased in Digitoxigenin and Proscillaridin A treated samples, while the rest did not express significant fold changes compared to control. No drastic changes were observed for caspase-3 and -7, except Topotecan treated samples showed decreased expression levels of both caspase-3 and -7. In addition, Vorinostat indicated a 2 fold increase in caspase-3 expression levels (**Figure 17b**).

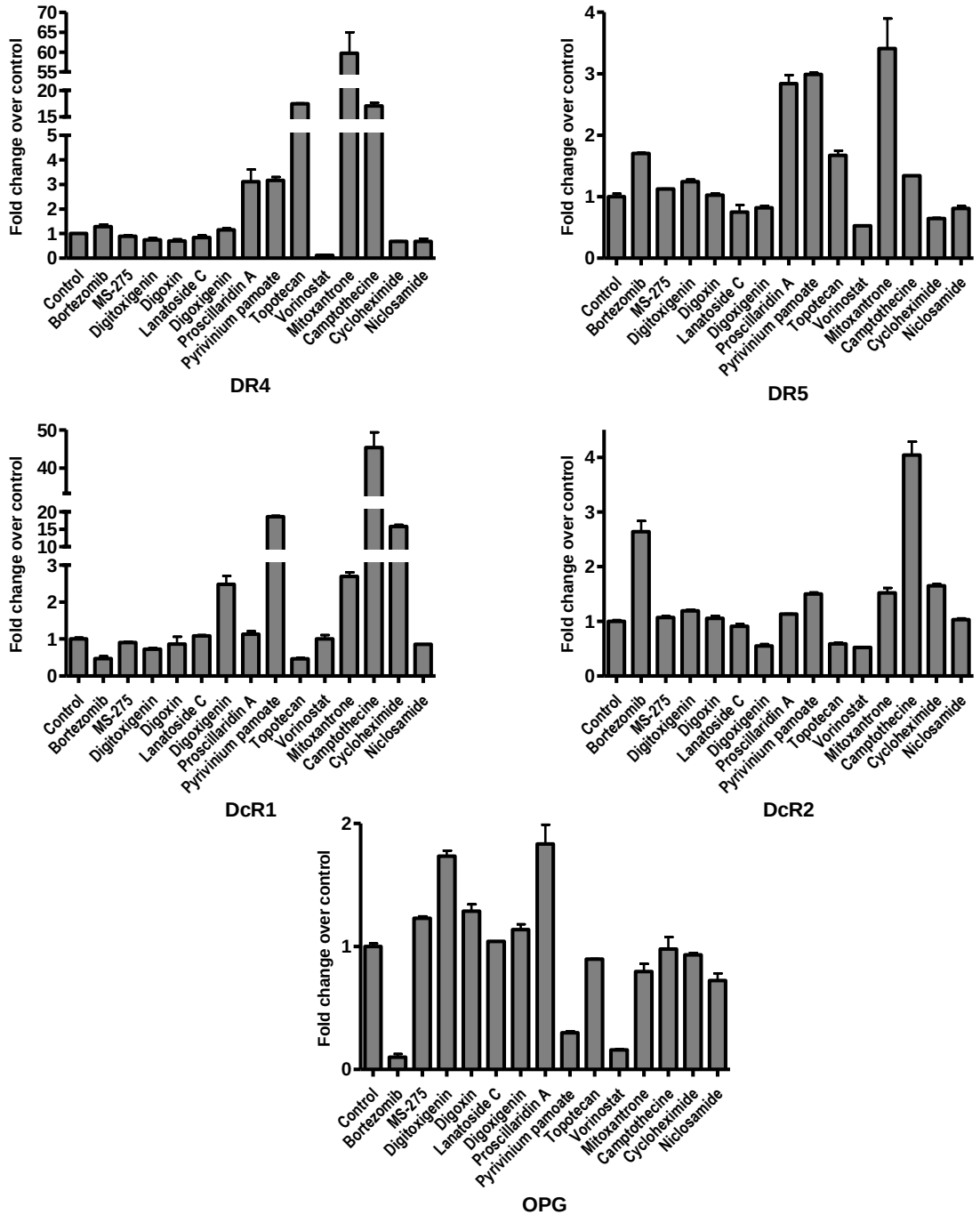
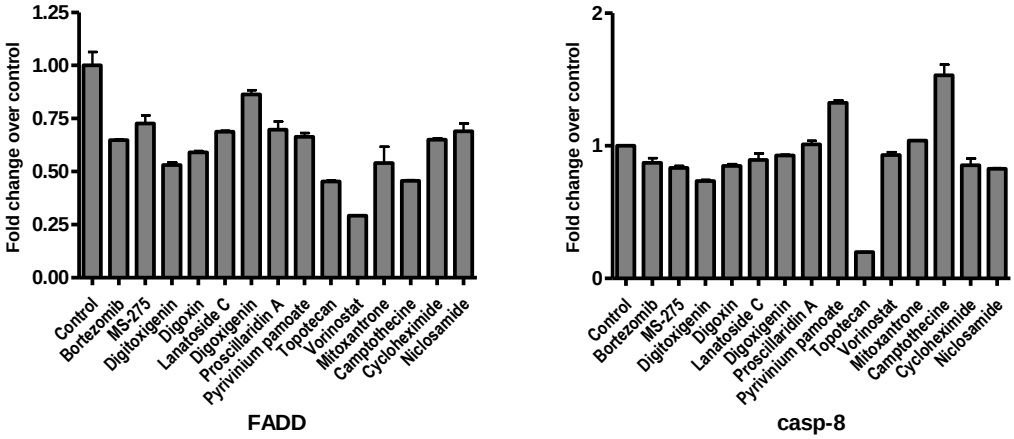
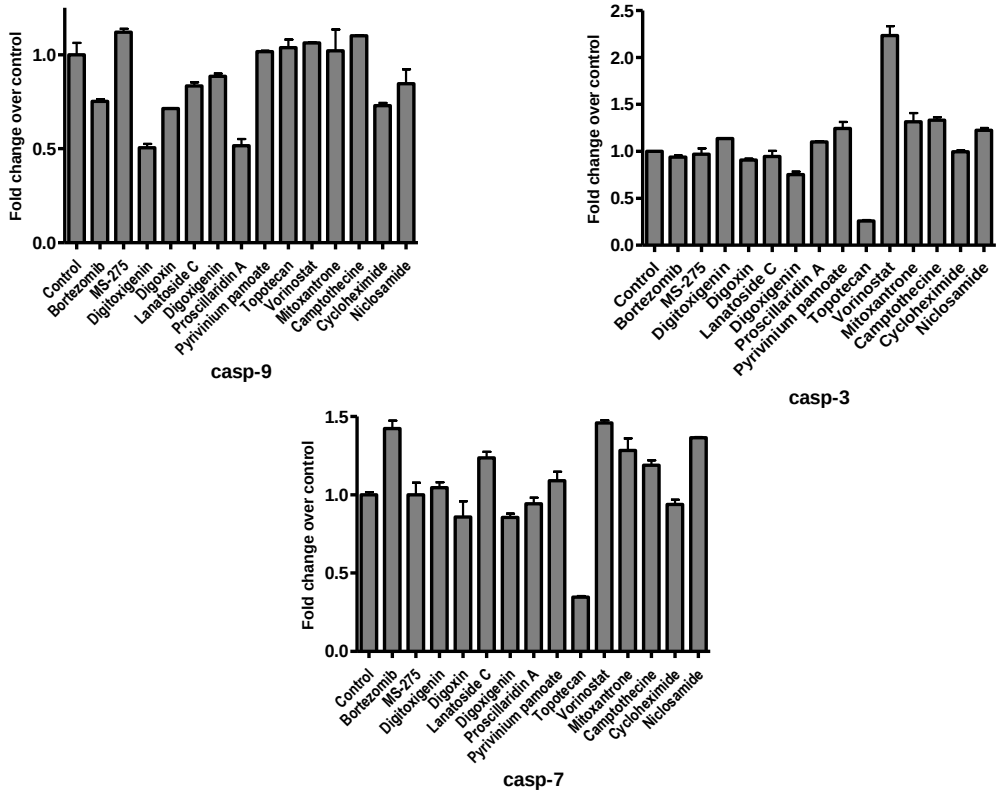


Figure 16. TRAIL receptors DR4, DR5, DcR1, DcR2 and OPG expression levels with selected drug treatments.



A



B

Figure 17. FADD and caspases gene expression changes with drug treatment. ***A.*** FADD and caspase 8 gene expression levels ***B.*** Caspases-9, -3 and -7 gene expression levels.

Then, whether the pro-apoptotic Bcl-2 family members are regulated by the TRAIL-sensitizing drugs or not was checked. Namely, Bid, Bax, Bad, Puma and Hrk expression was measured (**Figure 18**). Specifically, Bid expression was elevated in all samples except Vorinostat and Mitoxantrone. In Digoxin and Lanatoside C treated samples Bid expression levels were 14 and 20 fold increased respectively compared to control. In response to apoptotic signaling Bid interacts with Bax, leading Bax insertion to the mitochondrial membrane [27]. Proscillaridin A, Topotecan, Mitoxantrone and Camptothecine treated samples expressed elevated levels of BAX expression, whereas Digitoxigenin and Digoxin treated samples have shown decreased levels. Phosphorylated Bad, Puma and HRK inactivate anti-apoptotic Bcl-2 and Bcl-xL proteins, thus permitting Bax to trigger apoptosis [111]. Bad expression remained relatively same in every sample; it was only decreased in Vorinostat treated sample. For Puma expression, it was observed that Proscillaridin A, Pyrivinium Pamoate, Topotecan, Mitoxantrone and Camptothecine expressed elevated levels, whereas Bortezomib, MS-275, Digitoxigenin, Vorinostat and Cycloheximide shown decreased levels of fold change to control. Lastly for HRK, all samples had similar expression levels of HRK as the control sample, except 5 fold of control increase was observed with Proscillaridin A treated sample.

Finally, anti-apoptotic Bcl-2 family members, Bcl-2 Bcl-xL, Mcl-1, and inhibitor of apoptosis XIAP which are negative regulators of apoptosis were examined (**Figure 19**). In Vorinostat treated samples pro-apoptotic Bid and Bad had decreased expression levels. Expectedly, Vorinostat treated samples have shown increased Bcl-2 and XIAP expression levels. In contrast Topotecan treated sample had elevated Bax expression levels whereas Bcl-2, Bcl-xL and XIAP expression levels were lower than control sample.

Taken together, these results suggest marked changes in apoptosis-related gene expression as a result of TRAIL-sensitizing drug treatments.

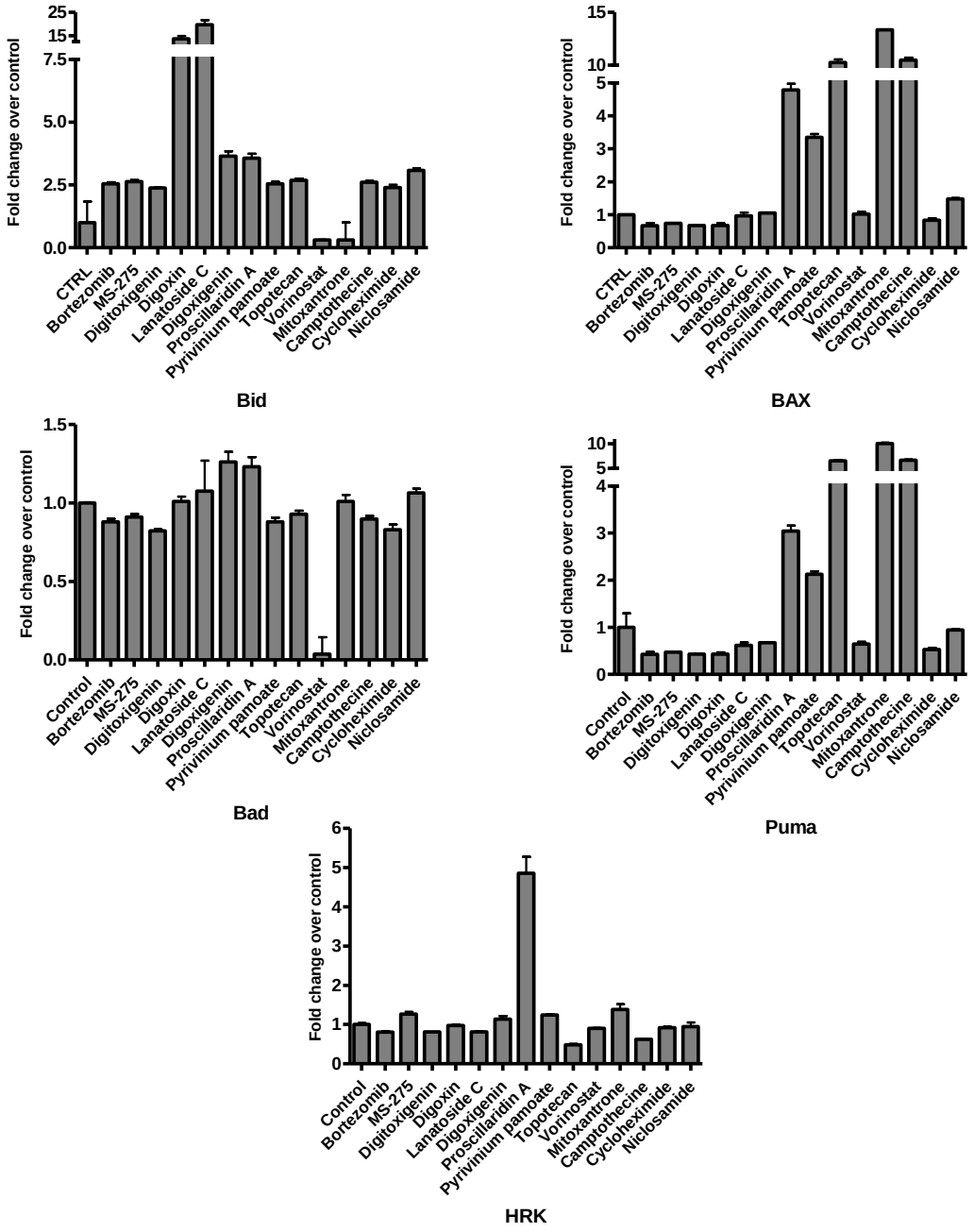


Figure 18. Gene expression levels of pro-apoptotic Bcl-2 family members on drug treated samples.

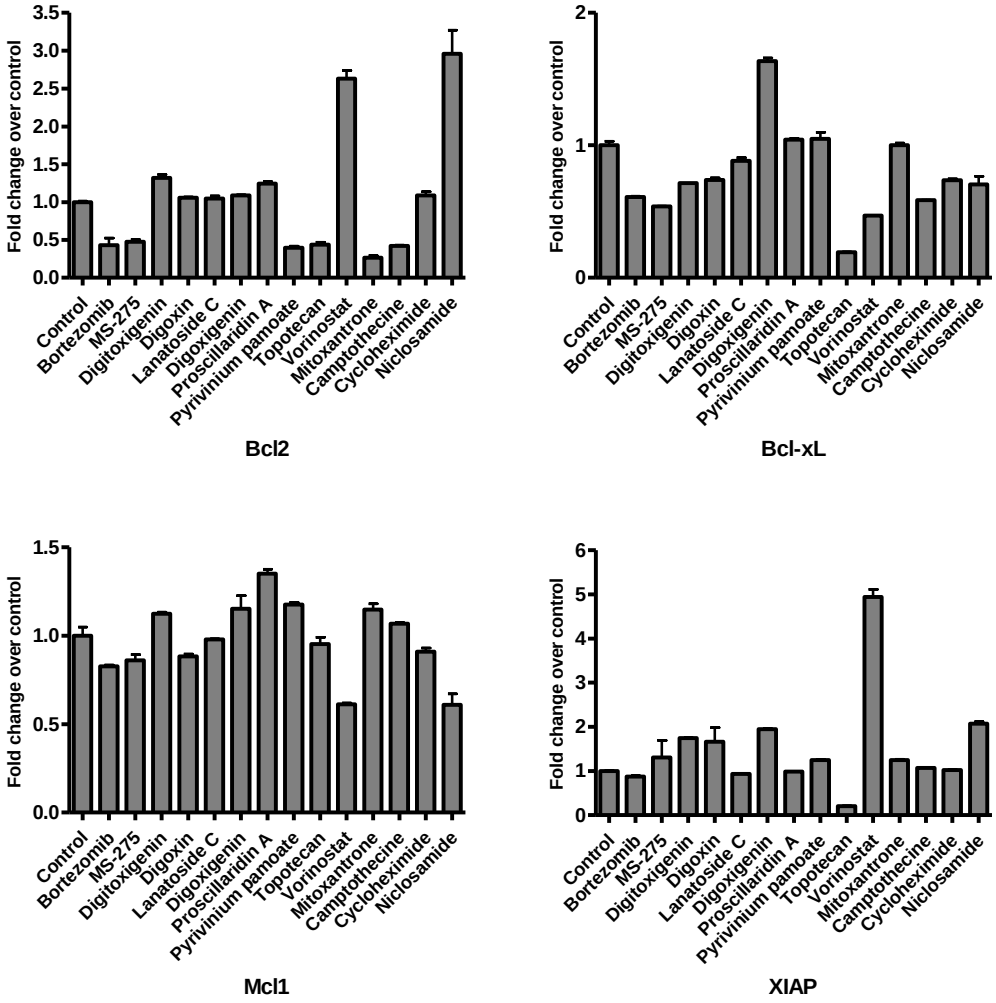


Figure 19. Gene expression levels of anti-apoptotic Bcl-2 family members on drug treated samples.

9. Cardiac glycosides were chosen for further study

Cardiac glycosides, which are normally used for the treatment of heart disorders work by inhibiting Na^+/K^+-ATPase membrane pump. Over the years, epidemiological studies indicated lower mortality rates among cancer patients who use cardiac glycosides triggered an interest on this group of drugs for cancer treatments [112]. In the light of this knowledge and as these constituted the second largest class of drug hits in the screen we chose to focus on this class of drugs.

9.1. Protein expression changes in U87MG cells treated with cardiac glycosides

To further elucidate the molecular events underlying the observed enhancement of apoptosis by the combination of TRAIL and cardiac glycosides in U87MG the expression of TRAIL receptors and apoptotic proteins were examined (**Figure 20**). The chosen cardiac glycosides for this study included Digitoxigenin, Digoxin, Lanatoside C, Digoxigenin, and Proscillaridin A. Bortezomib was used as positive control. 6 hours after the treatment with the respective drug and its combination with TRAIL, protein collection was performed.

Overall, TRAIL receptors DR4 and DR5 expression levels did not change with drug treatment for 6 hours. The short time period of 6 hours was not enough for execution of apoptosis; hence receptor expression levels were still not affected.

Caspase family proteins have a central role in apoptosis. For the activation of caspases cleavage of the pro-enzyme is necessary. Caspase-3 interacts with caspase-8 and -9, meaning with intrinsic and extrinsic apoptosis pathways respectively [113]. Western blotting performed on the samples revealed that caspase-3 cleavage did not happen with TRAIL alone or drug alone, but the combination of drugs and TRAIL led to caspase-3 cleavage. In addition, caspase-9 expression was observed in Digitoxigenin + TRAIL, Lanatoside C + TRAIL, Digoxigenin, Digoxigenin + TRAIL, Proscillaridin A, Proscillaridin A + TRAIL samples. In accordance with these results, PARP cleavage, which is an indicator of cells undergoing apoptosis [68] was only observed in the combinations with TRAIL, but not the single agent treatments.

BID is a pro-apoptotic Bcl-2 protein containing only the BH3 domain. In apoptosis, BID interacts with another Bcl-2 family protein, Bax, leading to the insertion of Bax into the outer mitochondrial membrane. Following Bax, cytochrome *c* and other pro-apoptotic factors are

released, such as SMAC/DIABLO from the mitochondria into cytoplasm, resulting in activation of caspases [111]. Enhanced BID expression was seen in Digitoxigenin, Digoxin, Digoxin + TRAIL, Lanatoside C and Lanatoside C + TRAIL. Unexpectedly, Digitoxigenin + TRAIL, Proscillaridin + TRAIL and Bortezomib + TRAIL showed less BID expression compared to their single agent treated cells. No significant Bax expression differences were seen among samples.

The inhibitor of apoptosis protein (IAP) family includes c-IAP1, c-IAP2, XIAP, survivin, livin, and NAIP. In general, the IAP proteins inhibit the activity of several caspases, including caspase-3, caspase-7, and caspase-9 [43]. XIAP expression was seen in all samples except TRAIL only treated cells and Digitoxigenin + TRAIL.

Cellular FLIP (FLICE inhibitory protein) is a regulator of apoptosis that interacts with FADD and pro-caspase-8 [114]. The levels of FLIP were relatively same in all the samples, their combination with TRAIL and control.

Taken together, these results suggest that, while not affecting the levels of receptors or some of the pro-apoptotic and anti-apoptotic proteins markedly, (as shown by modest to no changes in DR4, DR5, Bax, XIAP, FLIP expressions), cardiac glycosides were sufficient to induce apoptosis when combined with TRAIL (as shown by ccasp-3 and PARP expression).

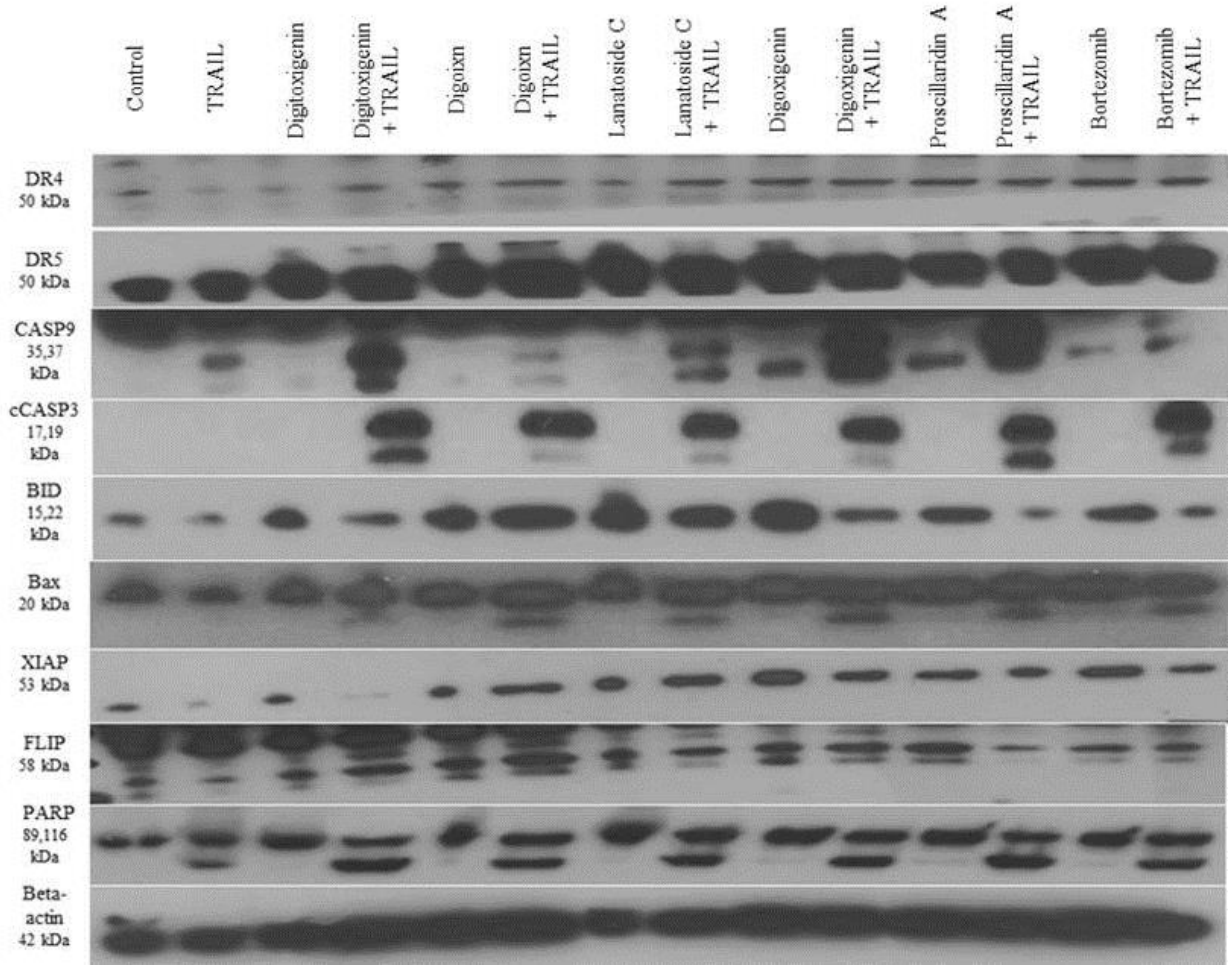


Figure 20. TRAIL sensitization with cardiac glycosides. U87MG cells were exposed to cardiac glycosides and Bortezomib with or without TRAIL (25 ng/ml) for 6 hours. Protein isolation and Western Blotting was performed as explained in Materials and Methods.

9.2. Caspase 3/7 activity assay U87MG cells treated with cardiac glycosides

Caspase 3/7 activity was detected by Caspase-Glo® 3/7 Assay as explained in Materials and Methods. No significant increase was detected compared to untreated control cells in 4 hours.

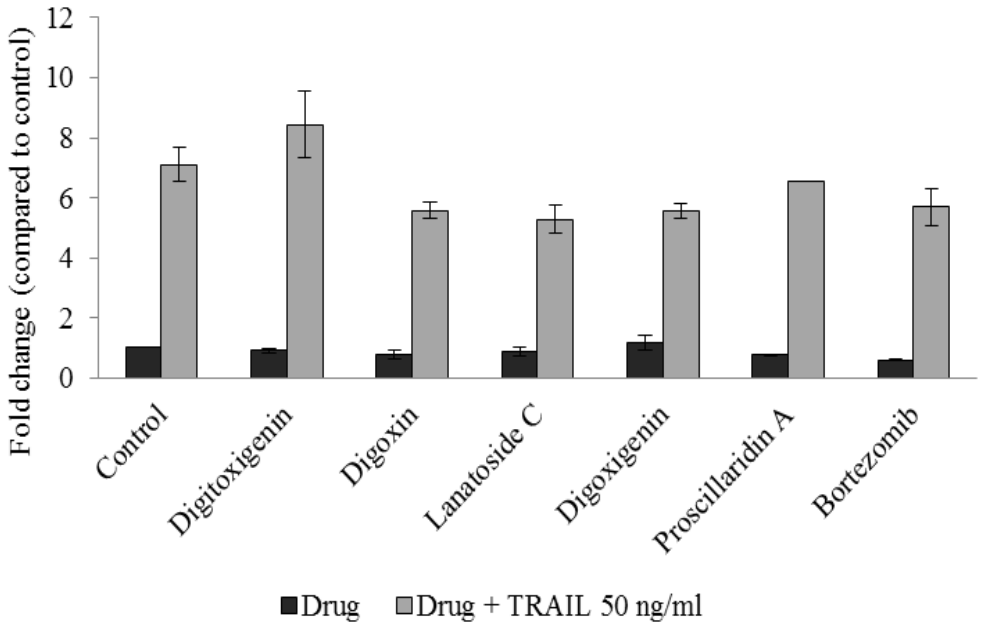


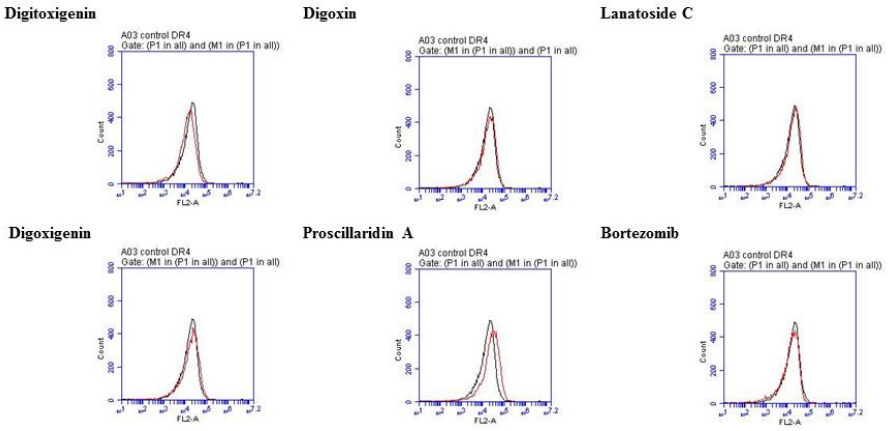
Figure 21. Caspase 3/7 activity of U87MG cells treated with cardiac glycosides and the combination with TRAIL after 4 hours. Activity was detected by Caspase-Glo 3/7 Assay.

9.3. DR4/DR5 expression after drug treatment were detected by flow cytometry

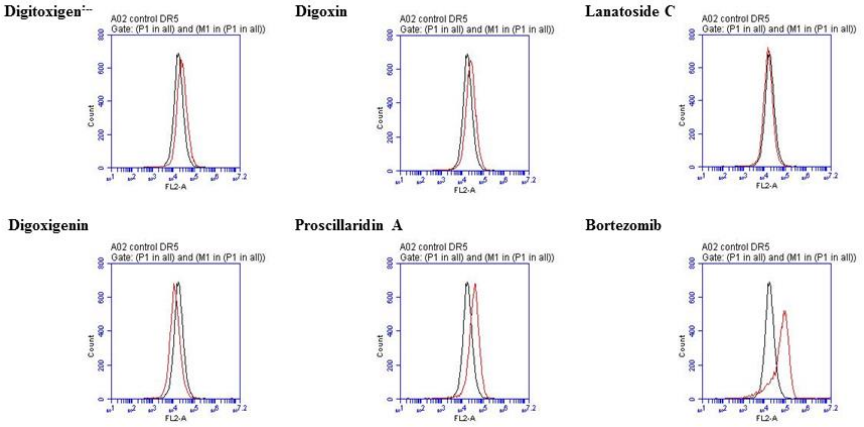
Surface expressions of receptors are determinants of the initiation of signaling events at the cell surface. While the expression levels of receptors might not be altered, their localization to cell surface and availability for signaling might be different with drug treatments. In order to assess the cell surface levels of TRAIL-receptors, flow cytometry using antibodies targeting the extracellular domains of DR4 and DR5 was performed.

Accordingly, DR4 levels were not altered with the drug treatments, except Proscillaridin A. It was seen that Bortezomib enhances DR5 levels drastically. One of the cardiac glycosides, Proscillaridin A increased the surface levels of DR5 significantly. These results suggest that Proscillaridin A has the capacity to alter death receptor expression levels on U87MG cells (**Figure 22**).

A. DR4



B. DR5



C.

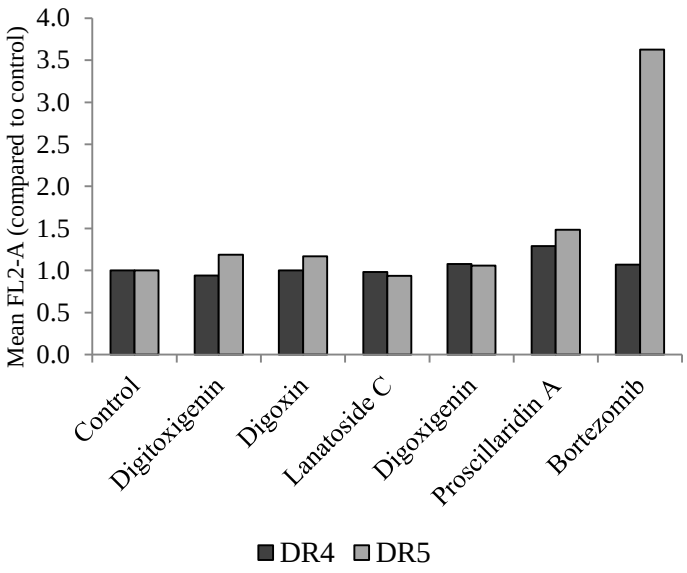


Figure 22. DR4/DR5 expression after drug treatment. ***A.*** DR4 expression levels ***B.*** DR5 expression levels. ***C.*** Relative DR4 and DR5 expression values compared to control.

9.4. Cell viability assays on TRAIL resistant cells

All three screens revealed 5 cardiac glycosides as potential TRAIL sensitizers, namely Digoxin, Digitoxigenin, Lanatoside C, Proscillaridin A and Digoxigenin. Cardiac glycosides are a large family of naturally derived compounds that have the capacity to inhibit Na^+/K^+-ATPase. They are used in the clinic for many years for heart failure and atrial arrhythmia treatments. In addition, their antiproliferative and apoptotic effects in several cancer cell lines are known for many years [112, 115], which make them perfect candidates for TRAIL sensitizing agents for GBM.

Chosen doses that were indicated in table 5 were also tested on TRAIL-resistant cell lines U373 and LN229, and U87MG TRAIL-resistant subpopulations and Bcl-xL overexpressing U87MG cells (**Figure 23**). While Digitoxigenin slightly affected the viability of U87-R50 cells, they did not suffice to induce apoptosis in TRAIL-resistant cells U373 and LN229 nor Bcl-XL expressing U87MG cells. However, Proscillaridin A was the most effective cardiac glycoside and reduced cell viability down to 10-25% when applied with TRAIL.

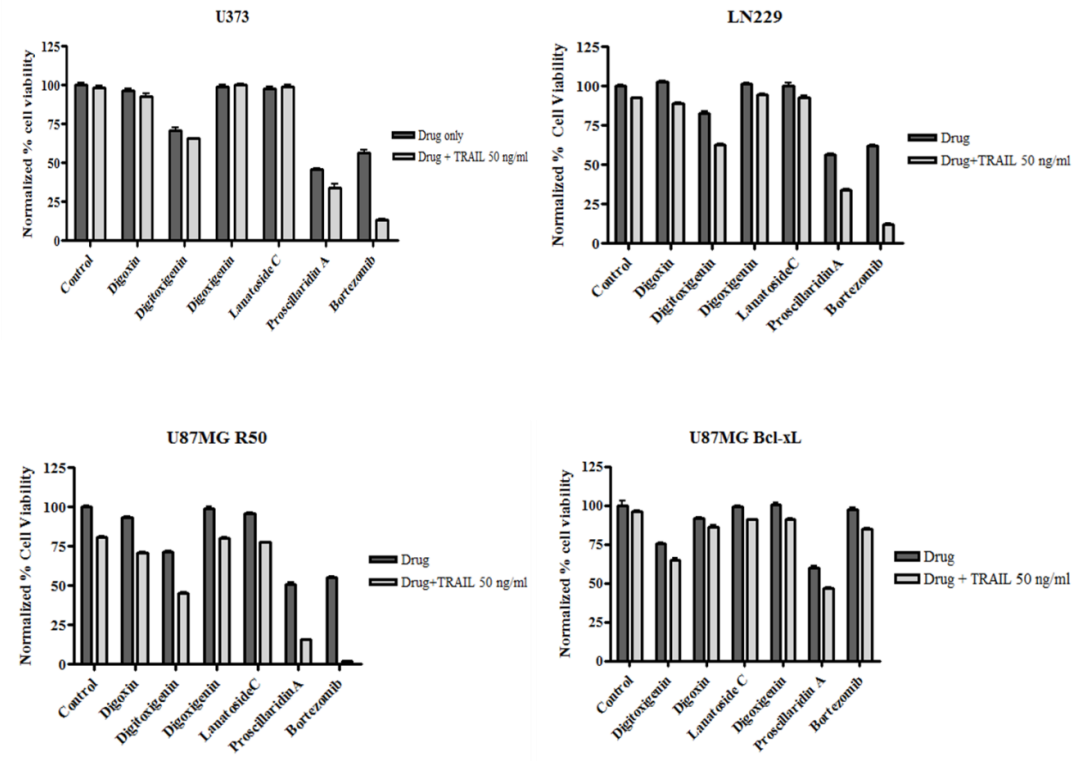


Figure 23. Cardiac glycosides and Bortezomib effect on TRAIL resistant cell lines, U373, LN229, TRAIL resistant subpopulation U87MG-R50 cells and Bcl-xL overexpressing U87MG cells. Cell viability was tested 24 hours after addition of reagents with CTG.

10. Primary cell culture

The use of primary cells, maintained for only short periods of time *in vitro*, might serve as a representative of the main functional component of the tissue *in vivo* from which they are derived. Hence, primary GBM cultures that are derived directly from patient samples were cultured to observe the TRAIL sensitizer agents affects.

Two of the patient derived GBM cells, called KUGBM2 and KUGBM3 (as indicated in Appendix II) were tested with different TRAIL concentrations. Both cells were observed to be TRAIL resistant (**Figure 24a**). When treated with chosen 5 cardiac glycosides, Bortezomib and Quinacrine dihydrochloride, Mitoxantrone dihydrochloride and Niclosamide in the dosages indicated in Table 5, both cell lines showed similar responses. Digitoxigenin, Digoxin and Proscillaridin A decreased cell viability significantly in combination with TRAIL (**Figure 24b, c**).

In addition, same treatment was performed to primary cell culture fibroblasts namely, KUFibroMG and KUFibro 4 (**Figure 25**). Digitoxigenin, Digoxin and Proscillaridin A treatment and their combinations with TRAIL also showed cytotoxicity on fibroblasts, indicating the necessity of dose adjustments.

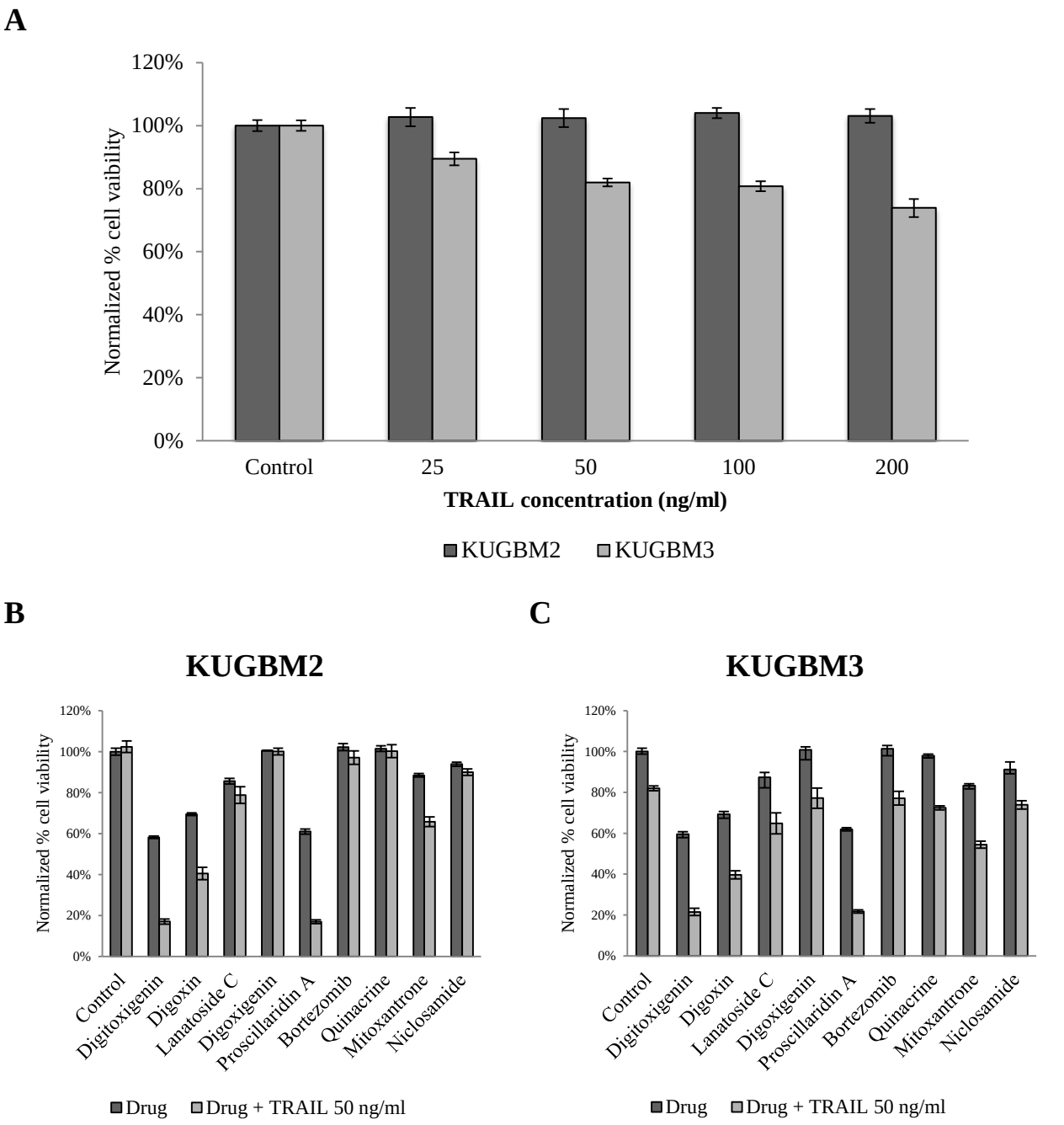


Figure 24. Primary cell cultures KUGBM2 and KUGBM3. ***A.*** Both KUGBM2 and KUGBM3 are TRAIL resistant primary GBM cell lines. ***B & C.*** Cardiac glycosides Digitoxigenin, Digoxin and Proscillaridin A decreases cell viability as single agents and in combination to TRAIL.

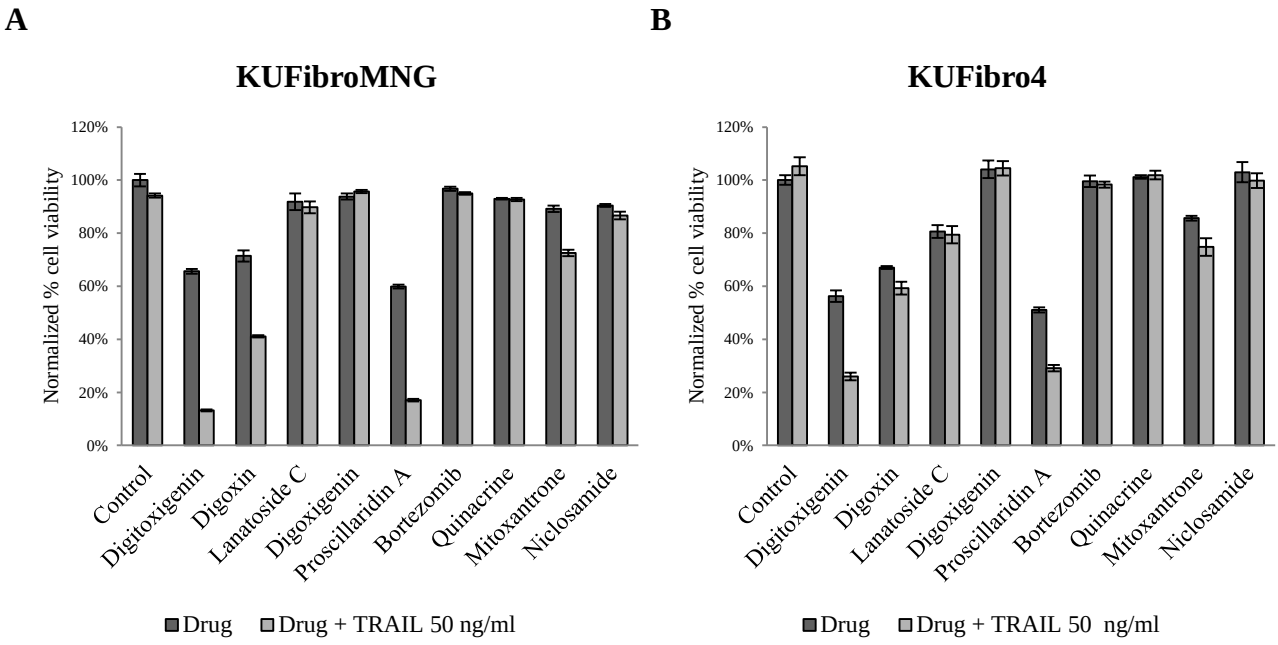


Figure 25. Primary cell culture derived Fibroblasts. ***A.*** KUFibroMNG cell viability ***B***. KuFibro4 cell viability. Both fibroblast cells were treated with chosen 10 drugs. Cell viability was assessed with CTG after 24 hours.

Chapter 4
Discussion

Glioblastoma multiforme (GBM) is a complex disease with a mean survival rate of 12-15 months [7]. The current treatment route includes surgery, radiation therapy and chemotherapy; however resistance to these developments is frequent. Thus, new treatment options are needed for GBM.

In this study, a chemical library of 1,200 FDA approved drugs, with various therapeutics effects were screened for identifying novel TRAIL sensitizing drugs for GBM. Particularly, we sought to identify TRAIL sensitizing drugs from a library with already approved chemicals for clinical usage, since existing drugs have characterized pharmacokinetics. High throughput drug screens are a newly established area for drug screens with the possibility of screening vast amount of reagents in a short period of time. However, determination of hits requires laborous work and optimizations are very important. For this purpose, designing the assays with positive/negative controls is vital for eliminating false positive and false negative hits. In this experimental assay, several optimizations were performed regarding cell seeding number, drug library and assay plate design as explained before. On the basis of this design, we successfully identified “hits”.

26 initial hits belonged to distinct pharmacological classes including antibacterials, antineoplastics, antihelmintic and cardiotonics. Antibacterials and cardiac glycosides were the major classes of hits; these classes may be worth looking into anticancer effects in more detail. 11 of the 26 hits were previously validated as TRAIL sensitizers by other groups. From the 11 TRAIL sensitizing drugs, 6 of them, Cycloheximide, Monensin sodium salt, Doxorubicin hydrochloride, Topotecan, Digoxin and Lanatoside C were already identified as TRAIL sensitizers in GBM [66, 72, 78, 80, 95, 105].

Temozolomide (TMZ), an alkylating agent that is currently used for GBM, was also included in the drug library. However, it was not a hit as a single agent or in combination with TRAIL in any of the screens. This result validated the necessity of other therapeutic agents for GBM treatment.

Epigenetic alterations are a promising approach in GBM treatment as they can lead to defective apoptotic signaling resulting evasion of apoptosis. Histone deacetylase (HDAC)

inhibitors are a class of targeted cancer agents. MS-275, an HDAC inhibitor was previously found to have a significant effect on GBM cell viability when combined with TRAIL by Bagci-Onder [49]. Out of 1,200 drugs the only HDAC inhibitor in the library, Vorinostat, was found to be a hit, indicating the promising aspect of epigenetic alterations for GBM. Vorinostat was identified as a TRAIL sensitizing agent in various cancers including leukemia and lung cancer [89-91]. It was also used in GBM as a single agent by other investigators [116-118]. With the previous knowledge and our findings, HDAC inhibitors can be considered as potential candidates for GBM.

Cardiac glycosides contain a large family of naturally derived compounds. Plants that contain cardiac glycosides were utilized for more than 1,500 years as arrow poisons, diuretics and heart tonics. Since 1775, cardiac glycosides are being used for the treatment of congestive heart failure and arrhythmia [119]. The most known cardiac glycosides are digoxin, digitoxin, oubain, bufalin and oleandrin.

Cardiac glycosides have structural diversity, but they consist of a common motif: a glycone, a steroid and a variable lactone ring (**Figure 26**). Glucose, galactose, mannose, rhamnose and digitalose are among the sugars that are attached to natural cardiac glycosides. Addition of these sugars to the steroid backbone alters the pharmacodynamics and pharmacokinetic profile of each glycoside [120].

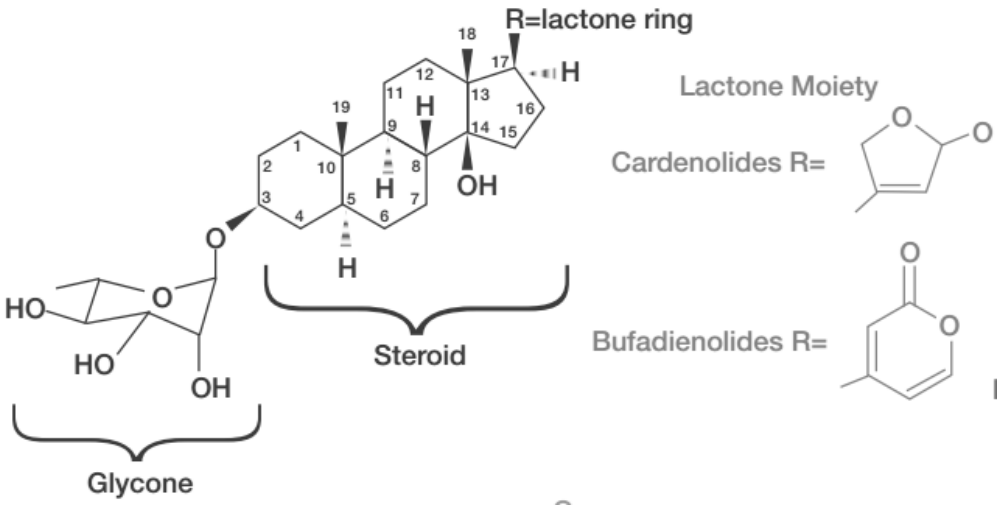


Figure 26. General chemical structure of a cardiac glycoside [115]

The mechanism of action for cardiac glycosides includes the inhibition of Na^+/K^+ ATPase membrane pump [112]. Plasma membrane ATPases are active transporters of cations that maintain steep concentration gradients. The Na^+/K^+-ATPase moves three Na^+ ions out of, and two K^+ ions into the cell for each ATP that is hydrolyzed. When inhibited by cardiac glycosides, Na^+ concentration in the cytoplasm increases whereas K^+ concentration decreases, the end result is increase in intracellular calcium concentration, which in the end causes cardiac contraction [115].

With the increase in epidemiological knowledge of lifespan increase in cancer patients who were also using cardiac glycosides, cardiac glycosides turned out to be interesting for investigators as potential cancer therapeutics. When treated with cardiac glycosides, cancer cell proliferation inhibition, apoptosis induction and chemotherapy sensitization have been reported in various cancer types [112, 115, 121, 122].

The mechanism of cardiac glycosides' antiproliferative effects can be explained by Na^+/K^+-ATPase triggering a complex signaling system including protein tyrosine kinase Src, EGFR transactivation, followed by Ras and MAPK activation, called Na^+/K^+-ATPase signalasome complex (**Figure 27**) [123]. Other mechanisms of cardiac glycoside induced apoptosis such as inhibition of NF-kB [124, 125], upregulation of death receptors [126, 127], and induction of reactive oxygen species [128] have been suggested.

Although these compounds have been used for many years, their effect on TRAIL sensitization for GBM is not well characterized. As the cardiac glycosides were the major class of drugs among our hits, and because of their previously reported anticancer potentials, we chose to further investigate their effects on GBM. Thus, from the 26 hits, we focused on the 5 members of cardiac glycosides: Digitoxigenin, Digoxin, Lanatoside C, Digoxigenin and Proscillaridin A (**Table 6**).

Digoxin is a purified cardiac glycoside extracted from the foxgrove plant and Digoxigenin is the aglycon form of Digoxin. Digitoxigenin is the aglycon of Digitoxin, which is another cardiac glycoside with similar structure and effect to Digoxin [103, 129]. Lanatoside C is composed of four monosaccharides and Digoxigenin. It is interesting that out of 1,200 drugs these 4 with similar structures and effects were obtained as hits.

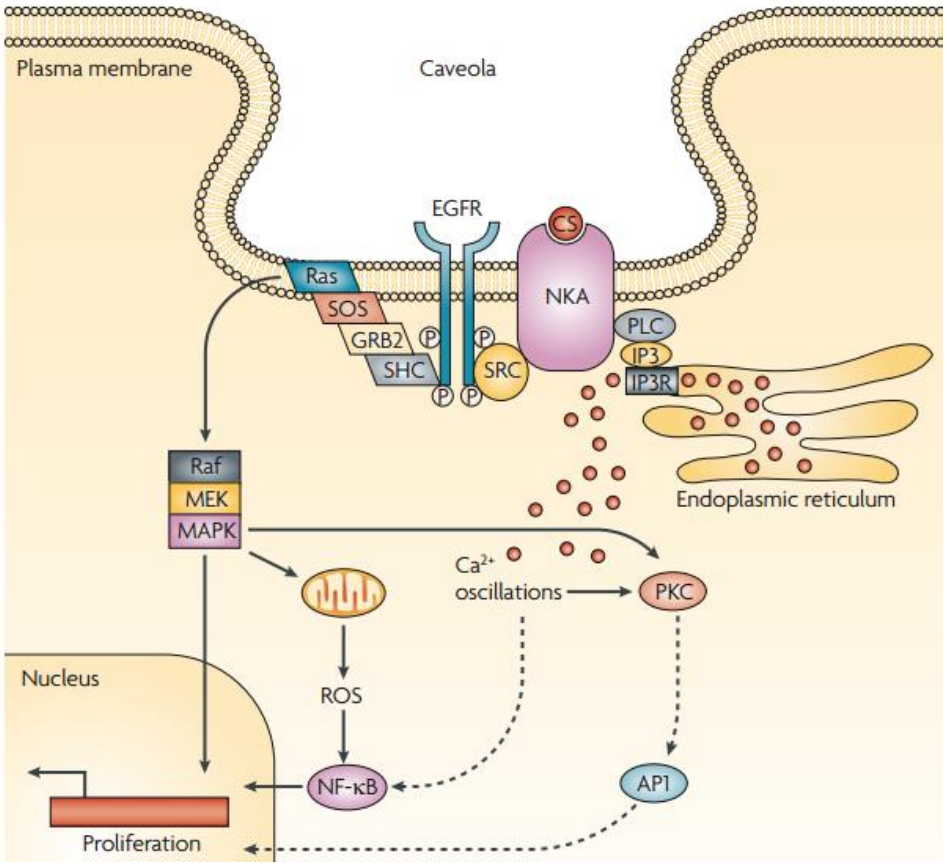


Figure 27 . Na^+/K^+-ATPase signaling cascade. Upon binding of cardiac glycosides Src (a tyrosine kinase) is activated, which in turn activates EGFR. Activated EGFR recruits the adaptors SHC, GRB2 and SOS. MAPK or PLC and IP3 cascades are activated as a result. Intracellular calcium levels are enhanced which modulates a diverse range of cellular events such as cell proliferation, differentiation and apoptosis [112].

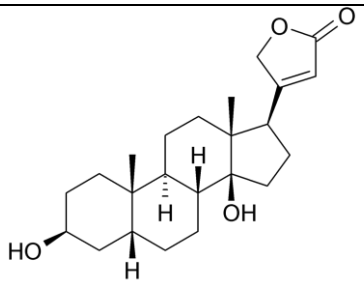
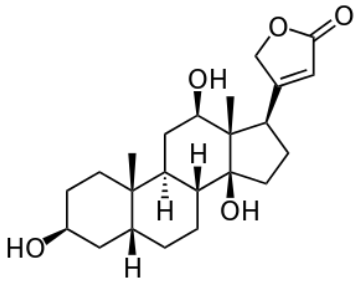
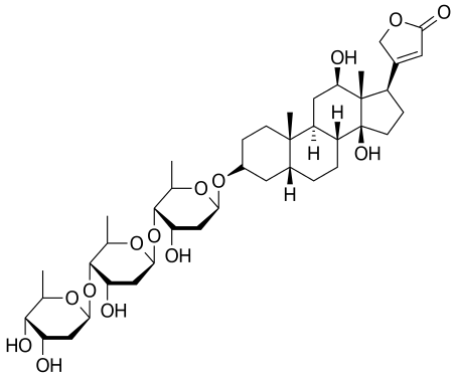
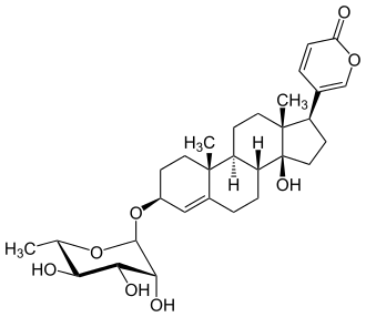
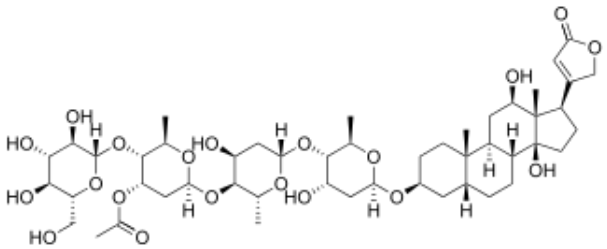
Drug name	*Chemical structure*
Digitoxigenin	
Digoxigenin	
Digoxin	
Proscillaridin A	
Lanatoside C	

Table 6. Chemical structures of cardiac glycosides [112, 115].

Following the screen, cell viability assays with different concentrations of drug alone and combination to TRAIL was executed. Our results indicated that the 5 cardiac glycosides as single agents did not have the capacity to alter cell viability more than 20% in U87MG cells. However, when combined with TRAIL 25 ng/ml, percent cell viability decreased dramatically.

Later on, we performed quantitative RT-PCR for each drug to examine the TRAIL receptor, caspase, pro- and anti-apoptotic Bcl-2 family member gene expression levels.

Digitoxigenin, Digoxin and Digoxigenin revealed similar results: No significant change was observed in TRAIL receptors DR4, DR5, DcR1, DcR2 and OPG expression levels or in caspases-8,-9,-3 and -7. From the pro-apoptotic Bcl-2 family members (Bid, BAX, Bad, Puma and HRK) they all had elevated expression of Bid, which has a pivotal role in execution of apoptosis. They did not show significant changes in anti-apoptotic Bcl-2 family members Bcl-2, Bcl-xL, Mcl-1 and XIAP. Looking at the protein expression levels, caspase-9 and cleaved caspase-3 expression was observed only in combinations with TRAIL, indicating the success of their TRAIL sensitization. However, they were not effective in TRAIL resistant cells, U373, LN229, U87MGR50 and U87MG-Bcl-xL. For the patient derived GBM cells, KUGBM2 and KUGBM3, Digitoxigenin and Digoxin had the capacity to affect cell viability as single agents and with combination with TRAIL. Digoxigenin did not alter cell viability in these cells. As a downside, Digitoxigenin and Digoxin also negatively affected the cell viability of patient derived fibroblasts, KUFibroMNG and KUFibro2.

Lanatoside C was previously identified as a TRAIL sensitizer in GBM [105]. They combined 250 nM of Lanatoside C with 50 ng/ml TRAIL. We observed that Lanatoside C can work with a lower dose of 50 nM and 50 ng/ml TRAIL. We also validated their findings of caspase independency in Lanatoside C treated samples. In addition, we are in agreement with their findings of DR4 and DR5 levels detection by flow cytometry. After Lanatoside C treatment DR4 was not altered and a slight increase in cell-surface expression of DR5 was detected.

Proscillaridin A was not previously worked on GBM, thus it is a novel GBM TRAIL sensitizing drug discovered by our group. Proscillaridin A was found to have cytotoxic effects as a single agent, thus its dosage was decreased to 25 nM in experiments. With the combination of TRAIL, it has the ability to decrease cell viability significantly. Looking at the gene expression levels, Proscillaridin A had elevated 3 folds of control DR4 and DR5, 2 folds to control OPG expression levels. In addition, pro-apoptotic Bcl2 family members' expression was elevated: Bid,

BAX and Puma (3 folds of control), HRK (5 folds of control). Anti-apoptotic Bcl-2 family factors Bcl-2, Bcl-xL, Mcl-1 and XIAP were not altered with Proscillaridin A treatment. Proscillaridin A was also effective with TRAIL resistant cell lines and primary GBM cell lines. However toxicity was observed on patient derived fibroblasts, indicating the need to adjust the cytotoxicity of this drug for treatment.

In summary, we optimized a high throughput screen from an annotated compound library with an attempt to provide useful therapeutic leads for GBM treatment. As a result, we identified 26 TRAIL sensitizing drugs for GBM, which can also be potential TRAIL sensitizers in other cancer types. From these hits, cardiac glycosides, Digitoxigenin, Digoxin, Lanatoside C, Digoxigenin and Proscillaridin A was studied in detail with cell proliferation, gene and protein expression levels experiments.

It should be noted that although these compounds indicated favorable effects in GBM cells *in vitro* both in cell lines and primary cell culture, the effect *in vivo* needs to be studied in detail before these drugs can move to clinical trials. Their pharmacokinetic and pharmacodynamics activities and effectiveness of crossing the blood brain barrier need to be studied in advanced detail.

Appendix I

Generation of U87MG TRAIL resistant cell lines and U87MG Bcl-xL overexpression cell lines

1. Generation of TRAIL-resistant U87MG cells

U87MG cells were seeded on 6-well plate and treated with 50 ng/mL TRAIL 1 day later. On subsequent days cells were re-treated with same dose of TRAIL every three days. This process was continued on for 30 days. After the 30 days, TRAIL was removed and cells were analyzed.

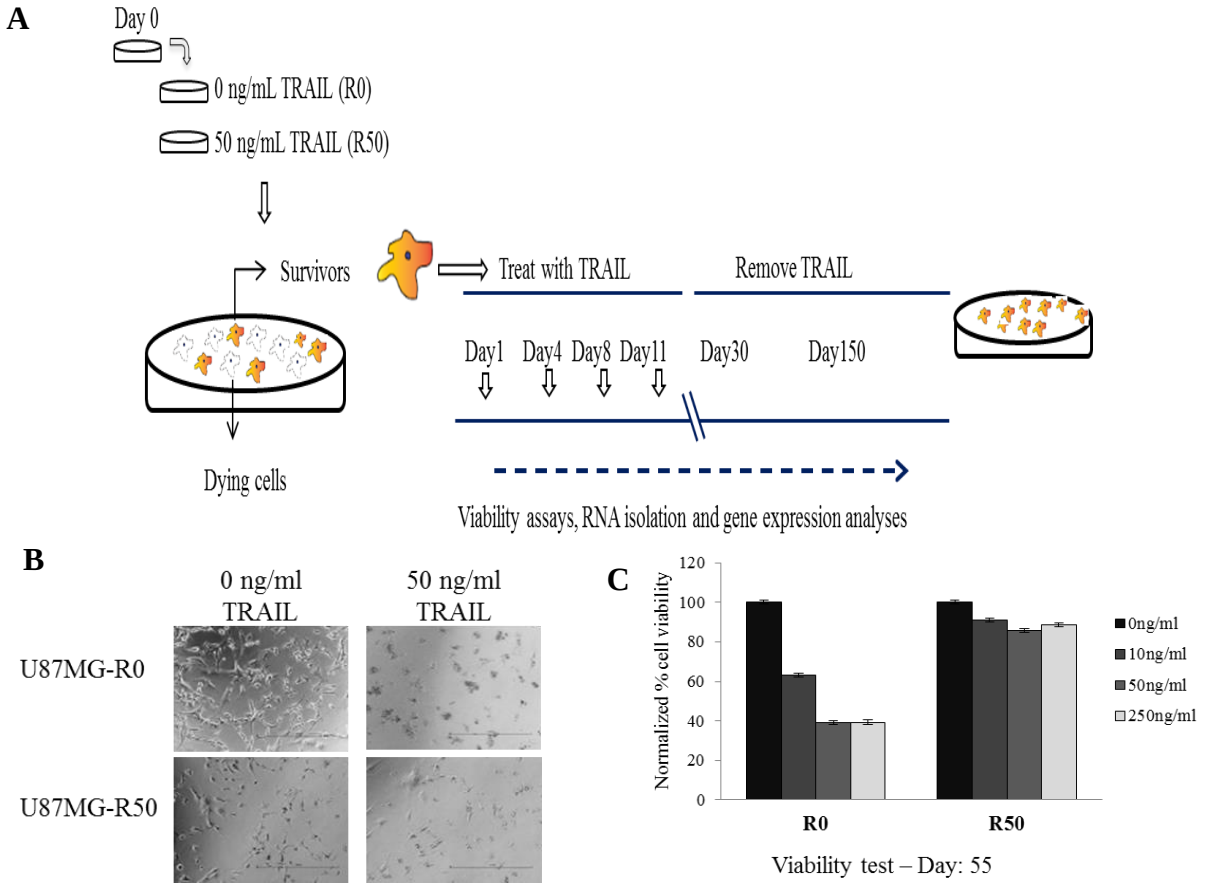
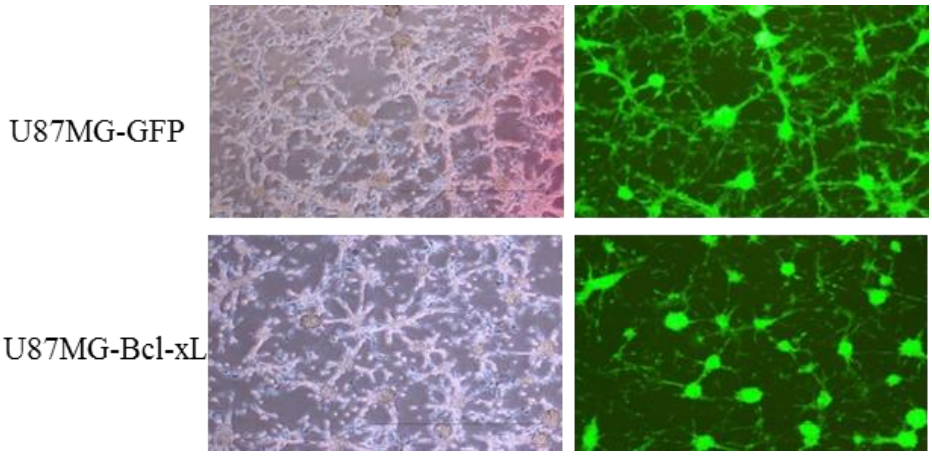


Figure 28. Generation of U87MG R50 ***A.*** Schematic representation of experimental setup for TRAIL-resistant cell subpopulation picking after TRAIL treatment (50 ng/mL) in U87MG cells. ***B.*** Phase microscopy (4x magnification) images. ***C.*** Viability analyses of control (R0) and TRAIL-resistant subpopulations (U87MG-R50) of U87MG cells treated with different TRAIL doses (0, 10, 50, 250 ng/mL) for 24 hours.

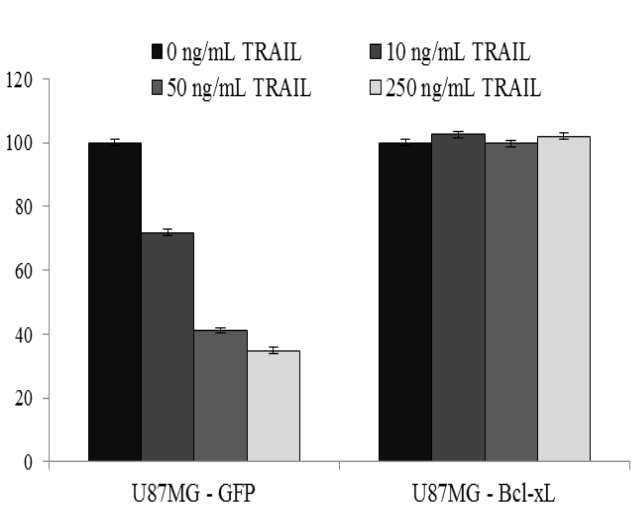
2. Generation of U87MG Bcl-xL overexpressed cells

U87MG cells were seeded on 12-well plate and Bcl-xL overexpression viruses were added 1 day later. GFP viruses were used as a control. 12-16 hours later medium were removed and replaced with fresh virus free medium. GFP expression was observed by fluorescent microscopy.

A



B



C

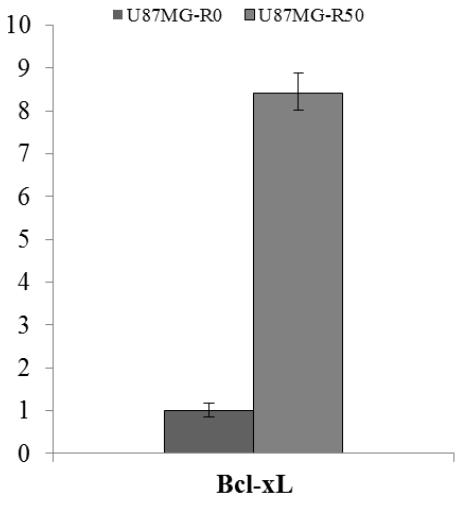


Figure 29. Generation of U87MG Bcl-xL overexpressed cells ***A.*** Representative phase and fluorescent microscopy snapshots (4x magnification) of U87MG-GFP/Bcl-xL overexpressed cells. ***B.*** Viability analyses of control (U87MG-GFP) and Bcl-xL overexpressed U87MG cells treated with different TRAIL doses (0, 10, 50, 250 ng/mL) for 24 hours. ***C.*** Quantitative Real Time-PCR analysis of Bcl-xL gene in U87MG-R50 TRAIL-resistant subpopulation of GBM cell line U87MG.

Appendix II

Generation of Primary GBM and Fibroblast Cell Cultures

3. Generation of Primary GBM cell lines

In accordance with the Ethical Council Documents and Guidelines, primary GBM cell lines were established from patients who underwent surgical resection. At the time of surgery, freshly removed tumor tissue was placed in DMEM and kept on ice during delivery. When reached to the cell culture room from the operating room, tumor tissue was manually dissociated with p200 pipettor and dissociated cells were pelleted with centrifugation at 1500 rpm for 5 min. Resulting pellet was resuspended in DMEM +10%FBS and 1% Pen-Strep and seeded to 10 cm dishes. Cells were allowed to attach for 3 days, after which the medium was replaced. Continuous passaging was performed with Trypsin, and cells at P2 were frozen and kept at liquid nitrogen. Representative pictures of KUGBM2 and KUGBM3 cell lines can be seen in **Figure 30**.

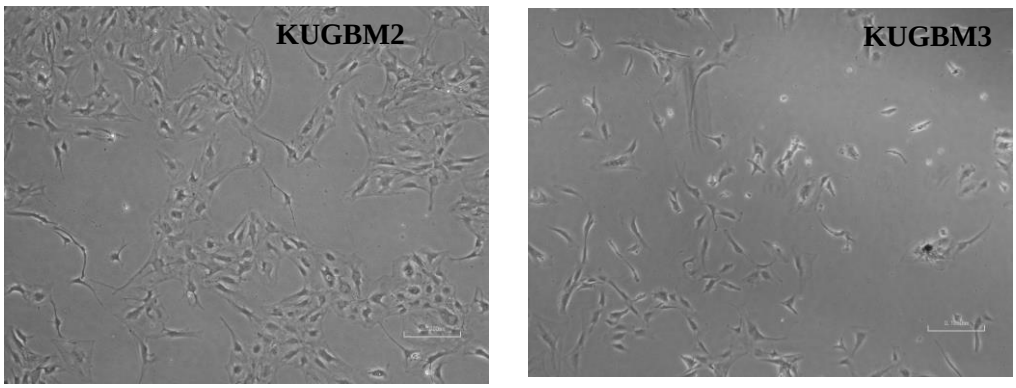


Figure 30. Representative phase snapshots (4x magnification) of primary GBM cell lines.

4. Generation of Primary GBM patient-derived fibroblasts

In accordance with the Ethical Council Documents and Guidelines, GBM-derived fibroblasts were established from patients who underwent surgical resection. At the time of surgery, a 1mm2 skin tissue was dissected out around the incision site, placed in DMEM and kept on ice during delivery. When reached to the cell culture room from the operating room, skin tissue was manually minced with scalpel and small chunk of tissues were placed under coverslips in 6-well

plates. Resulting tissues were grown in DMEM +10%FBS and 1% Pen-Strep for 1-2 weeks before single fibroblasts started to emerge from the tissues. When individual fibroblasts reached confluency, these cells were passaged and placed in 10 cm dishes and grown further. Cells at P2 were frozen and kept at liquid nitrogen. Representative pictures of KUFibroMNG and KUFibro4 cell lines can be seen in **Figure 31**.

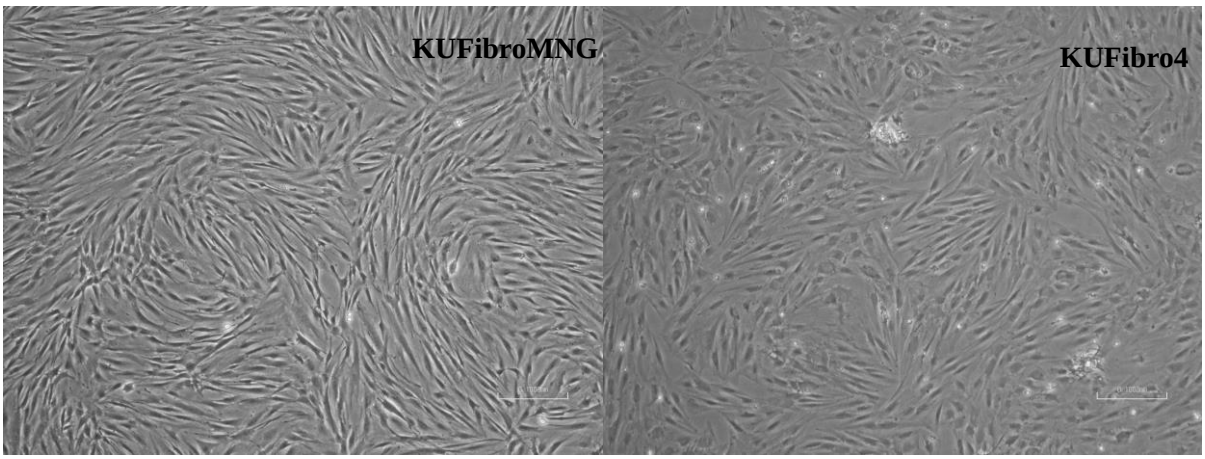


Figure 31. Representative phase snapshots (4x magnification) of primary patient-derived fibroblast cells.

Appendix III – Prestwick Chemical Library

Prestwick Name	Chemical name	Chemical structure	MW (g/mol)	Therapeutic class
01A02	Azaguanine-8	C4H4N6O	152.12	Oncology
01A03	Allantoin	C4H6N4O3	158.12	Dermatology
01A04	Acetazolamide	C4H6N4O3S2	222.25	Metabolism
01A05	Metformin hydrochloride	C4H12ClN5	165.63	Endocrinology
01A06	Atracurium besylate	C65H82N2O18S2	1243.51	Neuromuscular
01A07	Isoflupredone acetate	C23H29FO6	420.48	Endocrinology
01A08	Amiloride hydrochloride dihydrate	C6H13Cl2N7O3	302.12	Metabolism
01A09	Amprolium hydrochloride	C14H20Cl2N4	315.25	Infectiology
01A10	Hydrochlorothiazide	C7H8ClN3O4S2	297.74	Metabolism
01A11	Sulfaguanidine	C7H10N4O2S	214.25	Infectiology, Metabolism
01B02	Meticrane	C10H13NO4S2	275.35	Metabolism
01B03	Benzonatate	C30H53NO11	603.76	Neuromuscular
01B04	Hydroflumethiazide	C8H8F3N3O4S2	331.29	Metabolism
01B05	Sulfacetamide sodic hydrate	C8H11N2NaO4S	254.24	Infectiology, Metabolism
01B06	Heptaminol hydrochloride	C8H20ClNO	181.71	Cardiovascular
01B07	Sulfathiazole	C9H9N3O2S2	255.32	Infectiology, Metabolism
01B08	Levodopa	C9H11NO4	197.19	Central Nervous System
01B09	Idoxuridine	C9H11IN2O5	354.10	Infectiology
01B10	Captopril	C9H15NO3S	217.29	Cardiovascular
01B11	Minoxidil	C9H15N5O	209.25	Cardiovascular
01C02	Sulfaphenazole	C15H14N4O2S	314.37	Infectiology, Metabolism
01C03	Panthenol (D)	C9H19NO4	205.26	Metabolism
01C04	Sulfadiazine	C10H10N4O2S	250.28	Infectiology, Metabolism
01C05	Norethynodrel	C20H26O2	298.43	Endocrinology
01C06	Thiamphenicol	C12H15Cl2NO5S	356.23	Infectiology, Metabolism
01C07	Cimetidine	C10H16N6S	252.34	Gastroenterology
01C08	Doxylamine succinate	C21H28N2O5	388.47	Central Nervous System
01C09	Ethambutol dihydrochloride	C10H26Cl2N2O2	277.24	Infectiology
01C10	Antipyrine	C11H12N2O	188.23	Central Nervous System
01C11	Antipyrine, 4-hydroxy	C11H12N2O2	204.23	Metabolism
01D02	Chloramphenicol	C11H12Cl2N2O5	323.13	Infectiology, Metabolism
01D03	Epirizole	C11H14N4O2	234.26	Central Nervous System
01D04	Diprophylline	C10H14N4O4	254.25	Cardiovascular, Respiratory
01D05	Triamterene	C12H11N7	253.27	Metabolism
01D06	Dapsone	C12H12N2O2S	248.31	Infectiology
01D07	Troleandomycin	C41H67NO15	813.99	Infectiology
01D08	Pyrimethamine	C12H13ClN4	248.72	Infectiology
01D09	Hexamethonium dibromide dihydrate	C12H34Br2N2O2	398.22	Cardiovascular
01D10	Diflunisal	C13H8F2O3	250.20	Central Nervous System
01D11	Niclosamide	C13H8Cl2N2O4	327.13	Infectiology
01E02	Procaine hydrochloride	C13H21ClN2O2	272.78	Neuromuscular
01E03	Moxisylyte hydrochoride	C16H26ClNO3	315.84	Cardiovascular
01E04	Betazole hydrochloride	C5H11Cl2N3	184.07	Gastroenterology
01E05	Isoxicam	C14H13N3O5S	335.34	Central Nervous System

01E06	Naproxen	C14H14O3	230.27	Central Nervous System
01E07	Naphazoline hydrochloride	C14H15ClN2	246.74	Cardiovascular
01E08	Ticlopidine hydrochloride	C14H15Cl2NS	300.25	Hematology
01E09	Dicyclomine hydrochloride	C19H36ClNO2	345.96	Gastroenterology
01E10	Amyleine hydrochloride	C14H22ClNO2	271.79	Neuromuscular
01E11	Lidocaine hydrochloride	C14H23ClN2O	270.81	Cardiovascular
01F02	Trichlorfon	C4H8Cl3O4P	257.44	Infectiology
01F03	Carbamazepine	C15H12N2O	236.28	Central Nervous System
01F04	Triflupromazine hydrochloride	C18H20ClF3N2S	388.89	Central Nervous System
01F05	Mefenamic acid	C15H15NO2	241.29	Central Nervous System
01F06	Acetohexamide	C15H20N2O4S	324.40	Endocrinology
01F07	Sulpiride	C15H23N3O4S	341.43	Central Nervous System
01F08	Benoxinate hydrochloride	C17H29ClN2O3	344.89	Neuromuscular
01F09	Oxethazaine	C28H41N3O3	467.66	Neuromuscular
01F10	Pheniramine maleate	C20H24N2O4	356.43	Allergology
01F11	Tolazoline hydrochloride	C10H13ClN2	196.68	Cardiovascular
01G02	Morantel tartrate	C16H22N2O6S	370.43	Infectiology
01G03	Homatropine hydrobromide (R,S)	C16H22BrNO3	356.26	Diagnostic, Ophthalmology
01G04	Nifedipine	C17H18N2O6	346.34	Cardiovascular
01G05	Chlorpromazine hydrochloride	C17H20Cl2N2S	355.33	Cardiovascular
01G06	Diphenhydramine hydrochloride	C17H22ClNO	291.82	Allergology
01G07	Minaprine dihydrochloride	C17H24Cl2N4O	371.31	Central Nervous System
01G08	Miconazole	C18H14Cl4N2O	416.14	Infectiology, Metabolism
01G09	Isoxsuprine hydrochloride	C18H24ClNO3	337.85	Cardiovascular
01G10	Acebutolol hydrochloride	C18H29ClN2O4	372.90	Cardiovascular
01G11	Tolnaftate	C19H17NOS	307.42	Infectiology
01H02	Todralazine hydrochloride	C11H13ClN4O2	268.70	Cardiovascular
01H03	Imipramine hydrochloride	C19H25ClN2	316.88	Central Nervous System
01H04	Sulindac	C20H17FO3S	356.42	Central Nervous System
01H05	Amitryptiline hydrochloride	C20H24ClN	313.87	Central Nervous System
01H06	Adiphenine hydrochloride	C20H26ClNO2	347.89	Neuromuscular
01H07	Dibucaine	C20H29N3O2	343.47	Neuromuscular
01H08	Prednisone	C21H26O5	358.44	Endocrinology
01H09	Thioridazine hydrochloride	C21H27ClN2S2	407.04	Central Nervous System
01H10	Diphemanil methylsulfate	C21H27NO4S	389.52	Gastroenterology
01H11	Trimethobenzamide hydrochloride	C21H29ClN2O5	424.93	Central Nervous System
02A02	Metronidazole	C6H9N3O3	171.16	Infectiology, Metabolism
02A03	Fulvestrant	C32H47F5O3S	606.79	Endocrinology, Oncology
02A04	Edrophonium chloride	C10H16ClNO	201.70	Diagnostic, Neuromuscular
02A05	Moroxidine hydrochloride	C6H14ClN5O	207.66	Infectiology
02A06	Baclofen (R,S)	C10H12ClNO2	213.67	Central Nervous System
02A07	Acyclovir	C8H11N5O3	225.21	Metabolism
02A08	Diazoxide	C8H7ClN2O2S	230.67	Cardiovascular
02A09	Amidopyrine	C13H17N3O	231.30	Central Nervous System
02A10	Busulfan	C6H14O6S2	246.30	Oncology
02A11	Pindolol	C14H20N2O2	248.33	Cardiovascular
02B02	Khellin	C14H12O5	260.25	Cardiovascular
02B03	Zimelidine dihydrochloride monohydrate	C16H21BrCl2N2O	408.17	Central Nervous System

02B04	Azacyclonol	C18H21NO	267.37	Central Nervous System
02B05	Azathioprine	C9H7N7O2S	277.27	Oncology
02B06	Lynestrenol	C20H28O	284.45	Endocrinology
02B07	Guanabenz acetate	C10H12Cl2N4O2	291.14	Central Nervous System
02B08	Disulfiram	C10H20N2S4	296.54	Metabolism
02B09	Acetylsalicylsalicylic acid	C16H12O6	300.27	Central Nervous System
02B10	Mianserine hydrochloride	C18H21ClN2	300.83	Cardiovascular
02B11	Nocodazole	C14H11N3O3S	301.33	Oncology
02C02	R(-) Apomorphine hydrochloride hemihydrate	C34H38Cl2N2O5	625.60	Central Nervous System
02C03	Amoxapine	C17H16ClN3O	313.79	Central Nervous System
02C04	Cyproheptadine hydrochloride	C21H22ClN	323.87	Allergology, Central Nervous System
02C05	Famotidine	C8H15N7O2S3	337.45	Gastroenterology
02C06	Danazol	C22H27NO2	337.47	Endocrinology
02C07	Nicorandil	C8H9N3O4	211.18	Cardiovascular
02C08	Pioglitazone	C19H20N2O3S	356.45	Endocrinology
02C09	Nomifensine maleate	C20H22N2O4	354.41	Central Nervous System
02C10	Dizocilpine maleate	C20H19NO4	337.38	Central Nervous System
02C11	Oxandrolone	C19H30O3	306.45	Endocrinology
02D02	Naloxone hydrochloride	C19H22ClNO4	363.84	Central Nervous System
02D03	Metolazone	C16H16ClN3O3S	365.84	Cardiovascular
02D04	Ciprofloxacin hydrochloride monohydrate	C17H21ClFN3O4	385.83	Infectiology, Metabolism
02D05	Ampicillin trihydrate	C16H25N3O7S	403.46	Infectiology, Metabolism
02D06	Haloperidol	C21H23ClFNO2	375.87	Central Nervous System
02D07	Naltrexone hydrochloride dihydrate	C20H28ClNO6	413.90	Central Nervous System
02D08	Chlorpheniramine maleate	C20H23ClN2O4	390.87	Allergology, Central Nervous System
02D09	Nalbuphine hydrochloride	C21H28ClNO4	393.91	Central Nervous System
02D10	Picotamide monohydrate	C21H22N4O4	394.43	Hematology
02D11	Triamcinolone	C21H27FO6	394.44	Endocrinology
02E02	Bromocryptine mesylate	C33H44BrN5O8S	750.72	Central Nervous System
02E03	Amfepramone hydrochloride	C13H20ClNO	241.76	Central Nervous System
02E04	Dehydrocholic acid	C24H34O5	402.54	Gastroenterology
02E05	Tioconazole	C16H13Cl3N2OS	387.72	Infectiology
02E06	Perphenazine	C21H26ClN3OS	403.98	Central Nervous System
02E07	Mefloquine hydrochloride	C17H17ClF6N2O	414.78	Infectiology
02E08	Isoconazole	C18H14Cl4N2O	416.14	Infectiology
02E09	Spironolactone	C24H32O4S	416.58	Endocrinology
02E10	Pirenzepine dihydrochloride	C19H23Cl2N5O2	424.33	Gastroenterology
02E11	Dexamethasone acetate	C24H31FO6	434.51	Endocrinology
02F02	Glipizide	C21H27N5O4S	445.54	Endocrinology
02F03	Loxapine succinate	C22H24ClN3O5	445.91	Central Nervous System
02F04	Hydroxyzine dihydrochloride	C21H29Cl3N2O2	447.84	Allergology
02F05	Diltiazem hydrochloride	C22H27ClN2O4S	450.99	Cardiovascular
02F06	Methotrexate	C20H22N8O5	454.45	Oncology
02F07	Astemizole	C28H31FN4O	458.58	Allergology
02F08	Clindamycin hydrochloride	C18H34Cl2N2O5S	461.45	Infectiology

02F09	Terfenadine	C32H41NO2	471.69	Allergology
02F10	Cefotaxime sodium salt	C16H16N5NaO7S2	477.45	Infectiology
02F11	Tetracycline hydrochloride	C22H25ClN2O8	480.91	Infectiology
02G02	Verapamil hydrochloride	C27H39ClN2O4	491.08	Cardiovascular
02G03	Dipyridamole	C24H40N8O4	504.64	Cardiovascular
02G04	Chlorhexidine	C22H30Cl2N10	505.46	Infectiology
02G05	Loperamide hydrochloride	C29H34Cl2N2O2	513.51	Gastroenterology
02G06	Chlortetracycline hydrochloride	C22H24Cl2N2O8	515.35	Infectiology
02G07	Tamoxifen citrate	C32H37NO8	563.65	Endocrinology, Oncology
02G08	Nicergoline	C24H26BrN3O3	484.40	Cardiovascular
02G09	Canrenoic acid potassium salt	C22H29KO4	396.58	Endocrinology
02G10	Thioproperazine dimesylate	C24H38N4O8S4	638.85	Central Nervous System
02G11	Dihydroergotamine tartrate	C70H80N10O16	1317.48	Central Nervous System
02H02	Erythromycin	C37H67NO13	733.95	Infectiology
02H03	Chloroxine	C9H5Cl2NO	214.05	Dermatology
02H04	Didanosine	C10H12N4O3	236.23	Infectiology
02H05	Josamycin	C42H69NO15	828.02	Infectiology
02H06	Paclitaxel	C47H51NO14	853.93	Oncology
02H07	Ivermectin	C48H74O14	875.12	Infectiology
02H08	Gallamine triethiodide	C30H60I3N3O3	891.54	Neuromuscular
02H09	Neomycin sulfate	C23H48N6O17S	712.73	Infectiology
02H10	Dihydrostreptomycin sulfate	C42H88N14O36S3	1461.43	Infectiology
02H11	Gentamicine sulfate	C60H125N15O25S	1488.81	Infectiology
03A02	Isoniazid	C6H7N3O	137.14	Infectiology
03A03	Pentylenetetrazole	C6H10N4	138.17	Central Nervous System
03A04	Chlorzoxazone	C7H4ClNO2	169.57	Central Nervous System
03A05	Ornidazole	C7H10ClN3O3	219.63	Infectiology
03A06	Ethosuximide	C7H11NO2	141.17	Central Nervous System
03A07	Mafenide hydrochloride	C7H11ClN2O2S	222.69	Infectiology
03A08	Riluzole hydrochloride	C8H6ClF3N2OS	270.66	Central Nervous System
03A09	Nitrofurantoin	C8H6N4O5	238.16	Infectiology
03A10	Hydralazine hydrochloride	C8H9ClN4	196.64	Cardiovascular
03A11	Phenelzine sulfate	C8H14N2O4S	234.28	Central Nervous System
03B02	Tranexamic acid	C8H15NO2	157.21	Hematology
03B03	Etofylline	C9H12N4O3	224.22	Cardiovascular
03B04	Tranylcypromine hydrochloride	C9H12ClN	169.66	Central Nervous System
03B05	Alverine citrate salt	C26H35NO7	473.57	Neuromuscular
03B06	Aceclofenac	C16H13Cl2NO4	354.19	Central Nervous System
03B07	Iproniazide phosphate	C9H16N3O5P	277.22	Cardiovascular
03B08	Sulfamethoxazole	C10H11N3O3S	253.28	Infectiology
03B09	Mephenesin	C10H14O3	182.22	Central Nervous System
03B10	Phenformin hydrochloride	C10H16ClN5	241.73	Endocrinology
03B11	Flutamide	C11H11F3N2O3	276.22	Oncology
03C02	Ampyrone	C11H13N3O	203.25	Central Nervous System
03C03	Levamisole hydrochloride	C11H13ClN2S	240.76	Immunology
03C04	Pargyline hydrochloride	C11H14ClN	195.69	Cardiovascular
03C05	Methocarbamol	C11H15NO5	241.25	Central Nervous System
03C06	Aztreonam	C13H17N5O8S2	435.44	Infectiology
03C07	Cloxacillin sodium salt	C19H17ClN3NaO5S	457.87	Infectiology

03C08	Catharanthine	C21H24N2O2	336.44	Oncology
03C09	Pentolinium bitartrate	C23H42N2O12	538.60	Cardiovascular
03C10	Aminopurine, 6-benzyl	C12H11N5	225.25	Endocrinology
03C11	Tolbutamide	C12H18N2O3S	270.35	Endocrinology
03D02	Midodrine hydrochloride	C12H19ClN2O4	290.75	Cardiovascular
03D03	Thalidomide	C13H10N2O4	258.24	Central Nervous System
03D04	Oxolinic acid	C13H11NO5	261.24	Metabolism
03D05	Nimesulide	C13H12N2O5S	308.31	Metabolism
03D06	Asenapine maleate	C21H20ClNO5	401.85	Central Nervous System
03D07	Pentoxifylline	C13H18N4O3	278.31	Cardiovascular
03D08	Metaraminol bitartrate	C17H25NO14	467.39	Cardiovascular
03D09	Salbutamol	C13H21NO3	239.32	Neuromuscular
03D10	Prilocaine hydrochloride	C13H21ClN2O	256.78	Neuromuscular
03D11	Camptothecine (S,+)	C20H16N2O4	348.36	Oncology
03E02	Ranitidine hydrochloride	C13H23ClN4O3S	350.87	Gastroenterology
03E03	Tiratricol, 3,3',5-triiodothyroacetic acid	C14H9I3O4	621.94	Endocrinology
03E04	Flufenamic acid	C14H10F3NO2	281.24	Central Nervous System
03E05	Flumequine	C14H12FNO3	261.26	Infectiology
03E06	Tolfenamic acid	C14H12ClNO2	261.71	Central Nervous System
03E07	Meclofenamic acid sodium salt monohydrate	C14H12Cl2NNaO3	336.15	Central Nervous System
03E08	Tibolone	C21H28O2	312.46	Endocrinology
03E09	Trimethoprim	C14H18N4O3	290.32	Infectiology
03E10	Metoclopramide monohydrochloride	C14H23Cl2N3O2	336.26	Central Nervous System
03E11	Fenbendazole	C15H13N3O2S	299.35	Infectiology
03F02	Piroxicam	C15H13N3O4S	331.35	Central Nervous System
03F03	Pyrantel tartrate	C15H20N2O6S	356.40	Infectiology
03F04	Fenspiride hydrochloride	C15H21ClN2O2	296.80	Respiratory
03F05	Gemfibrozil	C15H22O3	250.34	Metabolism
03F06	Mefexamide hydrochloride	C15H25ClN2O3	316.83	Central Nervous System
03F07	Tiapride hydrochloride	C15H25ClN2O4S	364.89	Central Nervous System
03F08	Mebendazole	C16H13N3O3	295.30	Infectiology
03F09	Fenbufen	C16H14O3	254.29	Central Nervous System
03F10	Ketoprofen	C16H14O3	254.29	Central Nervous System
03F11	Indapamide	C16H16ClN3O3S	365.84	Metabolism
03G02	Norfloxacin	C16H18FN3O3	319.34	Infectiology
03G03	Antimycin A	C28H40N2O9	548.64	Infectiology
03G04	Xylometazoline hydrochloride	C16H25ClN2	280.84	Cardiovascular
03G05	Oxymetazoline hydrochloride	C16H25ClN2O	296.84	Respiratory
03G06	Nifenazone	C17H16N4O2	308.34	Central Nervous System
03G07	Griseofulvin	C17H17ClO6	352.77	Infectiology
03G08	Clemizole hydrochloride	C19H21Cl2N3	362.31	Allergology
03G09	Tropicamide	C17H20N2O2	284.36	Neuromuscular
03G10	Nefopam hydrochloride	C17H20ClNO	289.81	Central Nervous System
03G11	Phentolamine hydrochloride	C17H20ClN3O	317.82	Cardiovascular
03H02	Etodolac	C17H21NO3	287.36	Central Nervous System
03H03	Scopolamin-N-oxide hydrobromide	C17H22BrNO5	400.27	Neuromuscular
03H04	Hyoscyamine (L)	C17H23NO3	289.38	Central Nervous System

03H05	Chlorphensin carbamate	C10H12ClNO4	245.66	Central Nervous System
03H06	Fadrozole hydrochloride	C14H14ClN3	259.74	Oncology
03H07	Dilazep dihydrochloride	C31H46Cl2N2O10	677.63	Cardiovascular
03H08	Ofloxacin	C18H20FN3O4	361.38	Infectiology
03H09	Lomefloxacin hydrochloride	C17H20ClF2N3O3	387.82	Infectiology
03H10	Orphenadrine hydrochloride	C18H24ClNO	305.85	Allergology
03H11	Proglumide	C18H26N2O4	334.42	Gastroenterology
04A02	Mexiletine hydrochloride	C11H18ClNO	215.73	Cardiovascular
04A03	Flavoxate hydrochloride	C24H26ClNO4	427.93	Metabolism
04A04	Bufexamac	C12H17NO3	223.27	Central Nervous System
04A05	Glutethimide, para-amino	C13H16N2O2	232.28	Oncology
04A06	Dropropizine (R,S)	C13H20N2O2	236.32	Respiratory
04A07	Pinacidil	C13H19N5	245.33	Cardiovascular
04A08	Albendazole	C12H15N3O2S	265.34	Metabolism
04A09	Clonidine hydrochloride	C9H10Cl3N3	266.56	Cardiovascular
04A10	Bupropion hydrochloride	C13H19Cl2NO	276.21	Central Nervous System
04A11	Alprenolol hydrochloride	C15H24ClNO2	285.82	Cardiovascular
04B02	Chlorothiazide	C7H6ClN3O4S2	295.72	Metabolism
04B03	Diphenidol hydrochloride	C21H28ClNO	345.92	Central Nervous System
04B04	Norethindrone	C20H26O2	298.43	Endocrinology
04B05	Nortriptyline hydrochloride	C19H22ClN	299.85	Central Nervous System
04B06	Niflumic acid	C13H9F3N2O2	282.22	Central Nervous System
04B07	Isotretinoin	C20H28O2	300.44	Dermatology
04B08	Retinoic acid	C20H28O2	300.44	Dermatology
04B09	Antazoline hydrochloride	C17H20ClN3	301.82	Allergology
04B10	Ethacrynic acid	C13H12Cl2O4	303.14	Metabolism
04B11	Praziquantel	C19H24N2O2	312.42	Infectiology
04C02	Ethisterone	C21H28O2	312.46	Endocrinology
04C03	Triprolidine hydrochloride	C19H23ClN2	314.86	Allergology
04C04	Doxepin hydrochloride	C19H22ClNO	315.85	Allergology
04C05	Dyclonine hydrochloride	C18H28ClNO2	325.88	Neuromuscular
04C06	Dimenhydrinate	C24H28ClN5O3	469.98	Allergology
04C07	Disopyramide	C21H29N3O	339.48	Cardiovascular
04C08	Clotrimazole	C22H17ClN2	344.85	Infectiology
04C09	Vinpocetine	C22H26N2O2	350.46	Cardiovascular
04C10	Clomipramine hydrochloride	C19H24Cl2N2	351.32	Central Nervous System
04C11	Fendiline hydrochloride	C23H26ClN	351.92	Cardiovascular
04D02	Vincamine	C21H26N2O3	354.45	Central Nervous System
04D03	Indomethacin	C19H16ClNO4	357.80	Central Nervous System
04D04	Cortisone	C21H28O5	360.45	Endocrinology
04D05	Prednisolone	C21H28O5	360.45	Endocrinology
04D06	Fenofibrate	C20H21ClO4	360.84	Metabolism
04D07	Bumetanide	C17H20N2O5S	364.42	Metabolism
04D08	Labetalol hydrochloride	C19H25ClN2O3	364.88	Cardiovascular
04D09	Cinnarizine	C26H28N2	368.53	Allergology
04D10	Methylprednisolone, 6-alpha	C22H30O5	374.48	Endocrinology
04D11	Quinidine hydrochloride monohydrate	C20H27ClN2O3	378.90	Cardiovascular
04E02	Fludrocortisone acetate	C23H31FO6	422.50	Dermatology
04E03	Fenoterol hydrobromide	C17H22BrNO4	384.27	Neuromuscular

04E04	Homochlorcyclizine dihydrochloride	C19H25Cl3N2	387.78	Allergology
04E05	Diethylcarbamazine citrate	C16H29N3O8	391.42	Infectiology
04E06	Chenodiol	C24H40O4	392.58	Gastroenterology
04E07	Perhexiline maleate	C23H39NO4	393.57	Cardiovascular
04E08	Oxybutynin chloride	C22H32ClNO3	393.96	Neuromuscular
04E09	Spiperone	C23H26FN3O2	395.48	Central Nervous System
04E10	Pyrilamine maleate	C21H27N3O5	401.47	Allergology
04E11	Sulfinpyrazone	C23H20N2O3S	404.49	Hematology
04F02	Dantrolene sodium salt	C14H9N4NaO5	336.24	Neuromuscular
04F03	Trazodone hydrochloride	C19H23Cl2N5O	408.33	Central Nervous System
04F04	Glafenine hydrochloride	C19H18Cl2N2O4	409.27	Metabolism
04F05	Pimethixene maleate	C23H23NO4S	409.51	Allergology
04F06	Pergolide mesylate	C20H30N2O3S2	410.60	Central Nervous System
04F07	Acemetacin	C21H18ClNO6	415.83	Metabolism
04F08	Benzydamine hydrochloride	C19H24ClN3O	345.88	Central Nervous System
04F09	Fipexide hydrochloride	C20H22Cl2N2O4	425.32	Central Nervous System
04F10	Mifepristone	C29H35NO2	429.61	Endocrinology
04F11	Diperodon hydrochloride	C22H28ClN3O4	433.94	Neuromuscular
04G02	Lisinopril	C21H35N3O7	441.53	Cardiovascular
04G03	Lincomycin hydrochloride	C18H35ClN2O6S	443.01	Infectiology
04G04	Telenzepine dihydrochloride	C19H24Cl2N4O2S	443.40	Gastroenterology
04G05	Econazole nitrate	C18H16Cl3N3O4	444.70	Infectiology
04G06	Bupivacaine hydrochloride	C18H29ClN2O	324.90	Neuromuscular
04G07	Clemastine fumarate	C25H30ClNO5	459.97	Allergology
04G08	Oxytetracycline dihydrate	C22H28N2O11	496.48	Infectiology
04G09	Pimozide	C28H29F2N3O	461.56	Central Nervous System
04G10	Amodiaquin dihydrochloride dihydrate	C20H28Cl3N3O3	464.82	Infectiology
04G11	Mebeverine hydrochloride	C25H36ClNO5	466.02	Neuromuscular
04H02	Ifenprodil tartrate	C25H33NO8	475.54	Cardiovascular
04H03	Flunarizine dihydrochloride	C26H28Cl2F2N2	477.43	Central Nervous System
04H04	Trifluoperazine dihydrochloride	C21H26Cl2F3N3S	480.43	Central Nervous System
04H05	Enalapril maleate	C24H32N2O9	492.53	Cardiovascular
04H06	Minocycline hydrochloride	C23H28ClN3O7	493.95	Infectiology
04H07	Glibenclamide	C23H28ClN3O5S	494.01	Endocrinology
04H08	Guanethidine sulfate	C20H46N8O4S	494.70	Central Nervous System
04H09	Quinacrine dihydrochloride dihydrate	C23H36Cl3N3O3	508.92	Infectiology
04H10	Clofilium tosylate	C28H44ClNO3S	510.18	Cardiovascular
04H11	Fluphenazine dihydrochloride	C22H28Cl2F3N3OS	510.45	Central Nervous System
05A02	Streptomycin sulfate	C42H84N14O36S3	1457.40	Infectiology
05A03	Alfuzosin hydrochloride	C19H28ClN5O4	425.92	Cardiovascular
05A04	Chlorpropamide	C10H13ClN2O3S	276.74	Endocrinology
05A05	Phenylpropanolamine hydrochloride	C9H14ClNO	187.67	Respiratory
05A06	Ascorbic acid	C6H8O6	176.13	Metabolism
05A07	Methyldopa (L,-)	C10H13NO4	211.22	Cardiovascular
05A08	Cefoperazone dihydrate	C25H31N9O10S2	681.71	Infectiology
05A09	Zoxazolamine	C7H5ClN2O	168.58	Metabolism
05A10	Tacrine hydrochloride	C13H15ClN2	234.73	Central Nervous System
05A11	Bisoprolol fumarate	C40H66N2O12	766.98	Cardiovascular
05B02	Tremorine dihydrochloride	C12H22Cl2N2	265.23	Central Nervous System

05B03	Practolol	C14H22N2O3	266.34	Cardiovascular
05B04	Zidovudine, AZT	C10H13N5O4	267.25	Infectiology
05B05	Sulfisoxazole	C11H13N3O3S	267.31	Infectiology
05B06	Zaprinast	C13H13N5O2	271.28	Cardiovascular
05B07	Chlormezanone	C11H12ClNO3S	273.74	Central Nervous System
05B08	Procainamide hydrochloride	C13H22ClN3O	271.79	Cardiovascular
05B09	N6-methyladenosine	C11H15N5O4	281.27	Oncology
05B10	Guanfacine hydrochloride	C9H10Cl3N3O	282.56	Cardiovascular
05B11	Domperidone	C22H24ClN5O2	425.92	Central Nervous System
05C02	Furosemide	C12H11ClN2O5S	330.75	Metabolism
05C03	Methapyrilene hydrochloride	C14H20ClN3S	297.85	Allergology
05C04	Desipramine hydrochloride	C18H23ClN2	302.85	Central Nervous System
05C05	Clorgyline hydrochloride	C13H16Cl3NO	308.64	Central Nervous System
05C06	Clenbuterol hydrochloride	C12H19Cl3N2O	313.66	Neuromuscular
05C07	Maprotiline hydrochloride	C20H24ClN	313.87	Central Nervous System
05C08	Thioguanosine	C10H13N5O4S	299.31	Metabolism
05C09	Chlorprothixene hydrochloride	C18H19Cl2NS	352.33	Central Nervous System
05C10	Ritodrine hydrochloride	C17H22ClNO3	323.82	Neuromuscular
05C11	Clozapine	C18H19ClN4	326.83	Central Nervous System
05D02	Chlorthalidone	C14H11ClN2O4S	338.77	Metabolism
05D03	Dobutamine hydrochloride	C18H24ClNO3	337.85	Cardiovascular
05D04	Moclobemide	C13H17ClN2O2	268.75	Central Nervous System
05D05	Clopamide	C14H20ClN3O3S	345.85	Metabolism
05D06	Hycanthone	C20H24N2O2S	356.49	Infectiology
05D07	Adenosine 5'-monophosphate monohydrate	C10H16N5O8P	365.24	Cardiovascular
05D08	Amoxicillin	C16H19N3O5S	365.41	Metabolism
05D09	Pemirolast potassium	C10H7KN6O	266.31	Ophthalmology
05D10	Dextromethorphan hydrobromide monohydrate	C18H28BrNO2	370.33	Central Nervous System
05D11	Droperidol	C22H22FN3O2	379.44	Central Nervous System
05E02	Bambuterol hydrochloride	C18H30ClN3O5	403.91	Neuromuscular
05E03	Betamethasone	C22H29FO5	392.47	Endocrinology
05E04	Colchicine	C22H25NO6	399.45	Metabolism
05E05	Metergoline	C25H29N3O2	403.53	Central Nervous System
05E06	Brinzolamide	C12H21N3O5S3	383.51	Metabolism
05E07	Ambroxol hydrochloride	C13H19Br2ClN2O	414.57	Respiratory
05E08	Benfluorex hydrochloride	C19H21ClF3NO2	387.83	Central Nervous System
05E09	Bepridil hydrochloride	C24H35ClN2O	403.01	Cardiovascular
05E10	Meloxicam	C14H13N3O4S2	351.41	Metabolism
05E11	Benzbromarone	C17H12Br2O3	424.09	Cardiovascular
05F02	Ketotifen fumarate	C23H23NO5S	425.51	Allergology
05F03	Debrisoquin sulfate	C20H28N6O4S	448.55	Cardiovascular
05F04	Amethopterin (R,S)	C20H22N8O5	454.45	Immunology
05F05	Methylergometrine maleate	C24H29N3O6	455.52	Neuromuscular
05F06	Methiothepin maleate	C24H28N2O4S2	472.63	Central Nervous System
05F07	Clofazimine	C27H22Cl2N4	473.41	Infectiology
05F08	Nafronyl oxalate	C26H35NO7	473.57	Cardiovascular
05F09	Bezafibrate	C19H20ClNO4	361.83	Metabolism

05F10	Nefazodone HCl	C25H33Cl2N5O2	506.48	Central Nervous System
05F11	Clebopride maleate	C24H28ClN3O6	489.96	Central Nervous System
05G02	Lidoflazine	C30H35F2N3O	491.63	Cardiovascular
05G03	Betaxolol hydrochloride	C18H30ClNO3	343.90	Cardiovascular
05G04	Nicardipine hydrochloride	C26H30ClN3O6	516.00	Cardiovascular
05G05	Probucol	C31H48O2S2	516.86	Metabolism
05G06	Mitoxantrone dihydrochloride	C22H30Cl2N4O6	517.41	Oncology
05G07	GBR 12909 dihydrochloride	C28H34Cl2F2N2O	523.50	Central Nervous System
05G08	Carbetapentane citrate	C26H39NO10	525.60	Central Nervous System
05G09	Dequalinium dichloride	C30H40Cl2N4	527.59	Infectiology
05G10	Ketoconazole	C26H28Cl2N4O4	531.44	Infectiology
05G11	Fusidic acid sodium salt	C31H47NaO6	538.71	Infectiology
05H02	Terbutaline hemisulfate	C24H40N2O10S	548.66	Respiratory
05H03	Ketanserin tartrate hydrate	C26H30FN3O10	563.54	Cardiovascular
05H04	Hemicholinium bromide	C24H34Br2N2O4	574.36	Neuromuscular
05H05	Kanamycin A sulfate	C18H38N4O15S	582.59	Infectiology
05H06	Amikacin hydrate	C22H47N5O15	621.64	Infectiology
05H07	Etoposide	C29H32O13	588.57	Oncology
05H08	Clomiphene citrate (Z,E)	C32H36ClNO8	598.10	Endocrinology
05H09	Oxantel pamoate	C49H48N4O8	820.95	Infectiology
05H10	Prochlorperazine dimaleate	C28H32ClN3O8S	606.10	Central Nervous System
05H11	Hesperidin	C28H34O15	610.57	Oncology
06A02	Testosterone propionate	C22H32O3	344.50	Endocrinology
06A03	Haloprogin	C9H4Cl3IO	361.40	Central Nervous System
06A04	Thyroxine (L)	C15H11I4NO4	776.88	Endocrinology
06A05	Idebenone	C19H30O5	338.45	Oncology
06A06	Pepstatin A	C34H63N5O9	685.91	Infectiology
06A07	Morpholinoethylamino-3-benzocyclohepta-(5,6-c)-pyridazine dihydrochloride	C19H26Cl2N4O	397.35	
06A08	Adamantamine fumarate	C24H38N2O4	418.58	Infectiology
06A09	Butoconazole nitrate	C19H18Cl3N3O3S	474.80	Infectiology
06A10	Amiodarone hydrochloride	C25H30ClI2NO3	681.78	Cardiovascular
06A11	Amphotericin B	C47H73NO17	924.10	Infectiology
06B02	Androsterone	C19H30O2	290.45	Endocrinology
06B03	Amifostine	C5H15N2O3PS	214.22	Diagnostic
06B04	Carbarsone	C7H9AsN2O4	260.08	Infectiology
06B05	Amlodipine	C20H25ClN2O5	408.89	Cardiovascular
06B06	Modafinil	C15H15NO2S	273.36	Central Nervous System
06B07	Bacampicillin hydrochloride	C21H28ClN3O7S	501.99	Infectiology
06B08	Lamivudine	C8H11N3O3S	229.26	Infectiology
06B09	Biotin	C10H16N2O3S	244.31	Metabolism
06B10	Bisacodyl	C22H19NO4	361.40	Gastroenterology
06B11	Erlotinib	C22H23N3O4	393.45	Oncology
06C02	Suloctidil	C20H35NOS	337.57	Neuromuscular
06C03	Zotepine	C18H18ClNOS	331.87	Central Nervous System
06C04	Carisoprodol	C12H24N2O4	260.34	Central Nervous System
06C05	Cephalosporanic acid, 7-amino	C10H12N2O5S	272.28	Infectiology
06C06	Chicago sky blue 6B	C34H24N6Na4O16S4	992.82	Central Nervous System

06C07	Buflomedil hydrochloride	C17H26ClNO4	343.85	Cardiovascular
06C08	Dibenzepine hydrochloride	C18H22ClN3O	331.85	Central Nervous System
06C09	Roxatidine Acetate HCl	C19H29ClN2O4	384.91	Gastroenterology
06C10	Valacyclovir hydrochloride	C13H21ClN6O4	360.80	Infectiology
06C11	Cisapride	C23H29ClFN3O4	465.96	Gastroenterology
06D02	Pefloxacine	C17H20FN3O3	333.37	Infectiology
06D03	Corticosterone	C21H30O4	346.47	Endocrinology
06D04	Cyanocobalamin	C63H88CoN14O14P	1355.40	Metabolism
06D05	Cefadroxil	C16H17N3O5S	363.39	Infectiology
06D06	Cyclosporin A	C62H111N11O12	1202.64	Immunology
06D07	Digitoxigenin	C23H34O4	374.53	Cardiovascular
06D08	Digoxin	C41H64O14	780.96	Cardiovascular
06D09	Doxorubicin hydrochloride	C27H30ClNO11	579.99	Infectiology
06D10	Carbimazole	C7H10N2O2S	186.23	Metabolism
06D11	Epiandrosterone	C19H30O2	290.45	Endocrinology
06E02	Estradiol-17 beta	C18H24O2	272.39	Endocrinology
06E03	Clobutinol hydrochloride	C14H23Cl2NO	292.25	Central Nervous System
06E04	Gabazine bromide	C15H18BrN3O3	368.23	Central Nervous System
06E05	Oxcarbazepine	C15H12N2O2	252.28	Central Nervous System
06E06	Cyclobenzaprine hydrochloride	C20H22ClN	311.86	Neuromuscular
06E07	Carteolol hydrochloride	C16H25ClN2O3	328.84	Cardiovascular
06E08	Hydrocortisone base	C21H30O5	362.47	Endocrinology
06E09	Hydroxytacrine maleate (R,S)	C17H18N2O5	330.34	Central Nervous System
06E10	Pilocarpine nitrate	C11H17N3O5	271.28	Ophthalmology
06E11	Dicloxacillin sodium salt hydrate	C19H18Cl2N3NaO6S	510.33	Infectiology
06F02	Alizapride HCl	C16H22ClN5O2	351.84	Central Nervous System
06F03	Stanozolol	C21H32N2O	328.50	Endocrinology
06F04	Calcipotriene	C27H40O3	412.62	Dermatology
06F05	Linezolid	C16H20FN3O4	337.35	Infectiology
06F06	Mebhydroline 1,5-naphtalenedisulfonate	C48H48N4O6S2	841.07	Allergology
06F07	Meclocycline sulfosalicylate	C29H27ClN2O14S	695.06	Infectiology
06F08	Meclozine dihydrochloride	C25H29Cl3N2	463.88	Allergology
06F09	Melatonin	C13H16N2O2	232.28	Central Nervous System
06F10	Butalbital	C11H16N2O3	224.26	Central Nervous System
06F11	Dinoprost trometamol	C24H45NO8	475.63	Endocrinology
06G02	Tropisetron HCl	C17H21ClN2O2	320.82	Central Nervous System
06G03	Cefixime	C16H15N5O7S2	453.46	Infectiology
06G04	Metrizamide	C18H22I3N3O8	789.10	Diagnostic
06G05	Quetiapine hemifumarate	C46H54N6O8S2	883.11	Central Nervous System
06G06	Tosufloxacin hydrochloride	C19H16ClF3N4O3	440.81	Infectiology
06G07	Efavirenz	C14H9ClF3NO2	315.68	Infectiology
06G08	Rifapentine	C47H64N4O12	877.05	Infectiology
06G09	Neostigmine bromide	C12H19BrN2O2	303.20	Diagnostic
06G10	Niridazole	C6H6N4O3S	214.20	Infectiology
06G11	Ceforanide	C20H21N7O6S2	519.56	Infectiology
06H02	Vatalanib	C20H15ClN4	346.82	Oncology
06H03	Itopride	C20H26N2O4	358.44	Metabolism
06H04	Cefotetan	C17H17N7O8S4	575.62	Infectiology

06H05	Fentiazac	C17H12ClNO2S	329.81	Metabolism
06H06	Brompheniramine maleate	C20H23BrN2O4	435.32	Allergology
06H07	Primaquine diphosphate	C15H27N3O9P2	455.34	Infectiology
06H08	Progesterone	C21H30O2	314.47	Endocrinology
06H09	Felodipine	C18H19Cl2NO4	384.26	Neuromuscular
06H10	Raclopride	C15H20Cl2N2O3	347.24	Central Nervous System
06H11	Closantel	C22H14Cl2I2N2O2	663.08	Infectiology
07A02	Serotonin hydrochloride	C10H13ClN2O	212.68	Central Nervous System
07A03	Cefotiam hydrochloride	C18H24ClN9O4S3	562.09	Infectiology
07A04	Rofecoxib	C17H14O4S	314.36	Metabolism
07A05	Benperidol	C22H24FN3O2	381.45	Central Nervous System
07A06	Cefaclor hydrate	C15H16ClN3O5S	385.83	Infectiology
07A07	Colistin sulfate	C52H100N16O17S	1253.54	Infectiology
07A08	Daunorubicin hydrochloride	C27H30ClNO10	563.99	Infectiology
07A09	Dosulepin hydrochloride	C19H22ClNS	331.91	Central Nervous System
07A10	Ceftazidime pentahydrate	C22H32N6O12S2	636.66	Infectiology
07A11	Iobenguane sulfate	C8H12IN3O4S	373.17	Oncology
07B02	Metixene hydrochloride	C20H24ClNS	345.94	Central Nervous System
07B03	Nitrofural	C6H6N4O4	198.14	Infectiology
07B04	Omeprazole	C17H19N3O3S	345.42	Gastroenterology
07B05	Propylthiouracil	C7H10N2OS	170.23	Metabolism
07B06	Terconazole	C26H31Cl2N5O3	532.47	Infectiology
07B07	Tiaprofenic acid	C14H12O3S	260.31	Central Nervous System
07B08	Vancomycin hydrochloride	C66H76Cl3N9O24	1485.75	Infectiology
07B09	Artemisinin	C15H22O5	282.34	Infectiology
07B10	Propafenone hydrochloride	C21H28ClNO3	377.92	Cardiovascular
07B11	Ethamivan	C12H17NO3	223.27	Central Nervous System
07C02	Vigabatrin	C6H11NO2	129.16	Central Nervous System
07C03	Biperiden hydrochloride	C21H30ClNO	347.93	Central Nervous System
07C04	Cetirizine dihydrochloride	C21H27Cl3N2O3	461.82	Allergology
07C05	Etifenin	C16H22N2O5	322.36	Diagnostic
07C06	Metaproterenol sulfate, orciprenaline sulfate	C22H36N2O10S	520.60	Respiratory
07C07	Sisomicin sulfate	C19H39N5O11S	545.61	Infectiology
07C08	Sibutramine HCl	C17H27Cl2N	316.32	Central Nervous System
07C09	Acenocoumarol	C19H15NO6	353.33	Hematology
07C10	Bromperidol	C21H23BrFNO2	420.33	Central Nervous System
07C11	Cyclizine hydrochloride	C18H23ClN2	302.85	Allergology
07D02	Fluoxetine hydrochloride	C17H19ClF3NO	345.80	Central Nervous System
07D03	Iohexol	C19H26I3N3O9	821.15	Diagnostic
07D04	Norcyclobenzaprine	C19H19N	261.37	Gastroenterology
07D05	Pyrazinamide	C5H5N3O	123.12	Infectiology
07D06	Trimethadione	C6H9NO3	143.14	Central Nervous System
07D07	Lovastatin	C24H36O5	404.55	Metabolism
07D08	Nystatine	C47H75NO17	926.12	Infectiology
07D09	Budesonide	C25H34O6	430.55	Endocrinology
07D10	Imipenem	C12H17N3O4S	299.35	Infectiology
07D11	Sulfasalazine	C18H14N4O5S	398.40	Infectiology
07E02	Lofexidine	C11H12Cl2N2O	259.14	Cardiovascular

07E03	Thiostrepton	C72H85N19O18S5	1664.92	Infectiology
07E04	Miglitol	C8H17NO5	207.23	Endocrinology
07E05	Tiabendazole	C10H7N3S	201.25	Infectiology
07E06	Rifampicin	C43H58N4O12	822.96	Infectiology
07E07	Ethionamide	C8H10N2S	166.25	Infectiology
07E08	Tenoxicam	C13H11N3O4S2	337.38	Central Nervous System
07E09	Triflusal	C10H7F3O4	248.16	Hematology
07E10	Mesoridazine besylate	C27H32N2O4S3	544.76	Central Nervous System
07E11	Trolox	C14H18O4	250.30	Metabolism
07F02	Pirenperone	C23H24FN3O2	393.47	Central Nervous System
07F03	Isoquinoline, 6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydro, hydrochloride	C12H18ClNO2	243.74	
07F04	Phenacetin	C10H13NO2	179.22	Central Nervous System
07F05	Atovaquone	C22H19ClO3	366.85	Infectiology
07F06	Methoxamine hydrochloride	C11H18ClNO3	247.72	Cardiovascular
07F07	(S)-(-)-Atenolol	C14H22N2O3	266.34	Cardiovascular
07F08	Piracetam	C6H10N2O2	142.16	Central Nervous System
07F09	Phenindione	C15H10O2	222.25	Hematology
07F10	Thiocolchicoside	C27H33NO10S	563.63	Central Nervous System
07F11	Clorsulon	C8H8Cl3N3O4S2	380.66	Infectiology
07G02	Ciclopirox ethanolamine	C14H24N2O3	268.36	Infectiology
07G03	Probenecid	C13H19NO4S	285.36	Metabolism
07G04	Betahistine mesylate	C10H20N2O6S2	328.41	Allergology
07G05	Tobramycin	C18H37N5O9	467.52	Infectiology
07G06	Tetramisole hydrochloride	C11H13ClN2S	240.76	Immunology
07G07	Pregnenolone	C21H32O2	316.49	Endocrinology
07G08	Molsidomine	C9H14N4O4	242.24	Cardiovascular
07G09	Chloroquine diphosphate	C18H32ClN3O8P2	515.87	Metabolism
07G10	Trimetazidine dihydrochloride	C14H24Cl2N2O3	339.26	Cardiovascular
07G11	Parthenolide	C15H20O3	248.32	Metabolism
07H02	Hexetidine	C21H45N3	339.61	Infectiology
07H03	Selegiline hydrochloride	C13H18ClN	223.75	Central Nervous System
07H04	Pentamidine isethionate	C23H36N4O10S2	592.69	Infectiology
07H05	Tolazamide	C14H21N3O3S	311.41	Metabolism
07H06	Nifuroxazide	C12H9N3O5	275.22	Infectiology
07H07	Mirtazapine	C17H19N3	265.36	Central Nervous System
07H08	Dirithromycin	C42H78N2O14	835.09	Infectiology
07H09	Gliclazide	C15H21N3O3S	323.42	Metabolism
07H10	DO 897/99	C26H33Cl2N3O2	490.48	Central Nervous System
07H11	Prenylamine lactate	C27H33NO3	419.57	Cardiovascular
08A02	Ziprasidone Hydrochloride	C21H22Cl2N4OS	449.41	Central Nervous System
08A03	Mevastatin	C23H34O5	390.52	Cardiovascular
08A04	Pyridostigmine iodide	C9H13IN2O2	308.12	Central Nervous System
08A05	Pentobarbital	C11H18N2O3	226.28	Central Nervous System
08A06	Atropine sulfate monohydrate	C34H50N2O11S	694.85	Ophthalmology
08A07	Eserine hemisulfate salt	C30H44N6O8S	648.78	Central Nervous System
08A08	Itraconazole	C35H38Cl2N8O4	705.65	Infectiology
08A09	Acarbose	C25H43NO18	645.62	Endocrinology
08A10	Entacapone	C14H15N3O5	305.29	Central Nervous System

08A11	Nicotinamide	C6H6N2O	122.13	Dermatology
08B02	Tetracaïne hydrochloride	C15H25ClN2O2	300.83	Neuromuscular
08B03	Mometasone furoate	C27H30Cl2O6	521.44	Endocrinology
08B04	Troglitazone	C24H27NO5S	441.55	Metabolism
08B05	Dacarbazine	C6H10N6O	182.19	Oncology
08B06	Tenatoprazole	C16H18N4O3S	346.41	Metabolism
08B07	Acetopromazine maleate salt	C23H26N2O5S	442.54	Central Nervous System
08B08	Escitalopram	C22H23FN2O5	414.44	Central Nervous System
08B09	Ropinirole HCl	C16H25ClN2O	296.84	Central Nervous System
08B10	Lacidipine	C26H33NO6	455.56	Cardiovascular
08B11	Argatroban	C23H36N6O5S	508.64	Hematology
08C02	Reboxetine mesylate	C20H27NO6S	409.51	Central Nervous System
08C03	Camylofine chlorhydrate	C19H34Cl2N2O2	393.40	
08C04	Papaverine hydrochloride	C20H22ClNO4	375.86	Cardiovascular
08C05	Yohimbine hydrochloride	C21H27ClN2O3	390.91	Cardiovascular
08C06	Voriconazole	C16H14F3N5O	349.32	Infectiology
08C07	Alfacalcidol	C27H44O2	400.65	Metabolism
08C08	Cilostazol	C20H27N5O2	369.47	Hematology
08C09	Galanthamine hydrobromide	C17H22BrNO3	368.27	Central Nervous System
08C10	Azelastine HCl	C22H25Cl2N3O	418.37	Immunology
08C11	Etretinate	C23H30O3	354.49	Dermatology
08D02	Emedastine	C25H34N4O9	534.57	Allergology
08D03	Etofenamate	C18H18F3NO4	369.34	Metabolism
08D04	Zaleplon	C17H15N5O	305.34	Central Nervous System
08D05	Diclofenac sodium	C14H10Cl2NNaO2	318.14	Central Nervous System
08D06	Exemestane	C20H24O2	296.41	Endocrinology
08D07	Fomepizole	C4H6N2	82.11	Metabolism
08D08	Temozolomide	C6H6N6O2	194.15	Oncology
08D09	Xylazine	C12H16N2S	220.34	Central Nervous System
08D10	Celiprolol HCl	C20H34ClN3O4	415.96	Cardiovascular
08D11	Zopiclone	C17H17ClN6O3	388.82	Central Nervous System
08E02	Tranilast	C18H17NO5	327.34	Allergology
08E03	Tizanidine HCl	C9H9Cl2N5S	290.18	Metabolism
08E04	Zafirlukast	C31H33N3O6S	575.69	Respiratory
08E05	Butenafine Hydrochloride	C23H28ClN	353.94	Infectiology
08E06	Carbadox	C11H10N4O4	262.23	Infectiology
08E07	Rimantadine Hydrochloride	C12H22ClN	215.77	Infectiology
08E08	Eburnamonine (-)	C19H22N2O	294.40	Central Nervous System
08E09	Oxibendazol	C12H15N3O3	249.27	Metabolism
08E10	Ipsapirone	C19H23N5O3S	401.49	Central Nervous System
08E11	Hydroxychloroquine sulfate	C18H28ClN3O5S	433.96	Metabolism
08F02	Loracarbef	C16H16ClN3O4	349.78	Infectiology
08F03	Fenipentol	C11H16O	164.25	Metabolism
08F04	Diosmin	C28H32O15	608.56	Cardiovascular
08F05	Carbidopa	C10H14N2O4	226.23	Central Nervous System
08F06	(-)-Emtricitabine	C8H10FN3O3S	247.25	Infectiology
08F07	Demecarium bromide	C32H52Br2N4O4	716.60	Ophthalmology
08F08	Quipazine dimaleate salt	C21H23N3O8	445.43	Central Nervous System
08F09	Acipimox	C6H6N2O3	154.13	Metabolism

08F10	Diflorasone Diacetate	C26H32F2O7	494.54	Endocrinology
08F11	Acamprosate calcium	C10H20CaN2O8S2	400.49	Central Nervous System
08G02	Mizolastine	C24H25FN6O	432.50	Allergology
08G03	Amisulpride	C17H27N3O4S	369.49	Central Nervous System
08G04	Pyridoxine hydrochloride	C8H12ClNO3	205.64	Metabolism
08G05	Mercaptopurine	C5H4N4S	152.18	Immunology
08G06	Cytarabine	C9H13N3O5	243.22	Oncology
08G07	Racecadotril	C21H23NO4S	385.49	Gastroenterology
08G08	Folic acid	C19H19N7O6	441.41	Metabolism
08G09	Benazepril HCl	C24H29ClN2O5	460.96	Cardiovascular
08G10	Aniracetam	C12H13NO3	219.24	Central Nervous System
08G11	Dimethisoquin hydrochloride	C17H25ClN2O	308.85	Neuromuscular
08H02	Alendronate sodium	C4H12NNaO7P2	271.08	Metabolism
08H03	Dipivefrin hydrochloride	C19H30ClNO5	387.91	Ophthalmology
08H04	Thiorphan	C12H15NO3S	253.32	Gastroenterology
08H05	Tomoxetine hydrochloride	C17H22ClNO	291.82	Central Nervous System
08H06	Aceclidine Hydrochloride	C9H16ClNO2	205.69	Ophthalmology
08H07	Penciclovir	C10H15N5O3	253.26	Infectiology
08H08	Levetiracetam	C8H14N2O2	170.21	Central Nervous System
08H09	Dexfenfluramine hydrochloride	C12H17ClF3N	267.72	Central Nervous System
08H10	Etoricoxib	C18H15ClN2O2S	358.85	Central Nervous System
08H11	Sertindole	C24H26ClFN4O	440.95	Central Nervous System
09A02	Sulmazole	C14H13N3O2S	287.34	Cardiovascular
09A03	Gefitinib	C22H24ClFN4O3	446.91	Oncology
09A04	Flunisolide	C24H31FO6	434.51	Endocrinology
09A05	N-Acetyl-DL-homocysteine Thiolactone	C6H9NO2S	159.21	Respiratory
09A06	Flurandrenolide	C24H33FO6	436.53	Dermatology
09A07	Oxiconazole Nitrate	C18H14Cl4N4O4	492.15	Infectiology
09A08	Rebamipide	C19H15ClN2O4	370.80	Metabolism
09A09	Nilvadipine	C19H19N3O6	385.38	Cardiovascular
09A10	Etanidazole	C7H10N4O4	214.18	Oncology
09A11	Pinaverium bromide	C26H41Br2NO4	591.43	Neuromuscular
09B02	Glimepiride	C24H34N4O5S	490.63	Endocrinology
09B03	Picrotoxinin	C15H16O6	292.29	Central Nervous System
09B04	Mepenzolate bromide	C21H26BrNO3	420.35	Neuromuscular
09B05	Benfotiamine	C19H23N4O6PS	466.46	Metabolism
09B06	Halcinonide	C24H32ClFO5	454.97	Dermatology
09B07	Lanatoside C	C49H76O20	985.14	Cardiovascular
09B08	Benzamil hydrochloride	C13H15Cl2N7O	356.22	Metabolism
09B09	Suxibuzone	C24H26N2O6	438.48	Central Nervous System
09B10	6-Furfurylaminopurine	C10H9N5O	215.22	Dermatology
09B11	Avermectin B1a	C48H72O14	873.10	Infectiology
09C02	Pranlukast	C27H23N5O4	481.52	Respiratory
09C03	Penicillamine	C5H11NO2S	149.21	Central Nervous System
09C04	Zileuton	C11H12N2O2S	236.29	Respiratory
09C05	Loratadine	C22H23ClN2O2	382.89	Allergology
09C06	Tetraethylenepentamine pentahydrochloride	C8H28Cl5N5	371.61	Metabolism

09C07	Nisoldipine	C20H24N2O6	388.42	Cardiovascular
09C08	Acefylline	C9H10N4O4	238.20	Central Nervous System
09C09	Acitretin	C21H26O3	326.44	Dermatology
09C10	Zonisamide	C8H8N2O3S	212.23	Central Nervous System
09C11	Irsogladine maleate	C13H11Cl2N5O4	372.17	Metabolism
09D02	Dydrogesterone	C21H28O2	312.46	Endocrinology
09D03	Sumatriptan succinate	C18H27N3O6S	413.50	Central Nervous System
09D04	Opipramol dihydrochloride	C23H31Cl2N3O	436.43	Central Nervous System
09D05	Nalidixic acid sodium salt	C12H11N2NaO3	254.22	Infectiology
09D06	Oxacillin sodium	C19H18N3NaO5S	423.43	Infectiology
09D07	Beta-Escin	C55H86O24	1131.28	Metabolism
09D08	Thiamine hydrochloride	C12H18Cl2N4OS	337.27	Immunology
09D09	Tazobactam	C10H12N4O5S	300.29	Infectiology
09D10	Ibandronate sodium	C9H22NNaO7P2	341.22	Metabolism
09D11	Warfarin	C19H16O4	308.34	Hematology
09E02	Pranoprofen	C15H13NO3	255.28	Metabolism
09E03	Secnidazole	C7H11N3O3	185.18	Infectiology
09E04	Pempidine tartrate	C14H27NO6	305.37	Cardiovascular
09E05	Clodronate	CH2Cl2Na2O6P2	288.86	Metabolism
09E06	Ibutilide fumarate	C44H76N4O10S2	885.25	Cardiovascular
09E07	Thimerosal	C9H9HgNaO2S	404.81	Infectiology
09E08	Tramadol hydrochloride	C16H26ClNO2	299.84	Central Nervous System
09E09	Estropipate	C22H32N2O5S	436.57	Endocrinology
09E10	Butylscopolammonium (n-) bromide	C21H30BrNO4	440.38	Central Nervous System
09E11	Irinotecan hydrochloride trihydrate	C33H45ClN4O9	677.20	Oncology
09F02	Tylosin	C46H77NO17	916.12	Infectiology
09F03	Citalopram Hydrobromide	C20H22BrFN2O	405.31	Central Nervous System
09F04	Promazine hydrochloride	C17H21ClN2S	320.89	Central Nervous System
09F05	Sulfamerazine	C11H12N4O2S	264.31	Infectiology
09F06	Venlafaxine	C17H27NO2	277.41	Central Nervous System
09F07	Ethotoin	C11H12N2O2	204.23	Central Nervous System
09F08	3-alpha-Hydroxy-5-beta-androstan-17-one	C19H30O2	290.45	Endocrinology
09F09	Tetrahydrozoline hydrochloride	C13H17ClN2	236.75	Cardiovascular
09F10	Hexestrol	C18H22O2	270.37	Endocrinology
09F11	Cefmetazole sodium salt	C15H16N7NaO5S3	493.52	Infectiology
09G02	Trihexyphenidyl-D,L Hydrochloride	C20H32ClNO	337.94	Central Nervous System
09G03	Succinylsulfathiazole	C13H13N3O5S2	355.39	Infectiology
09G04	Famprofazone	C24H31N3O	377.53	Central Nervous System
09G05	Bromopride	C14H22BrN3O2	344.25	Central Nervous System
09G06	Methyl benzethonium chloride	C28H44ClNO2	462.12	Infectiology
09G07	Chlorcyclizine hydrochloride	C18H22Cl2N2	337.30	Allergology
09G08	Diphenylpyraline hydrochloride	C19H24ClNO	317.86	Allergology
09G09	Benzethonium chloride	C27H42ClNO2	448.09	Infectiology
09G10	Trioxsalen	C14H12O3	228.25	Dermatology
09G11	Doxofylline	C11H14N4O4	266.26	Respiratory
09H02	Sulfabenzamide	C13H12N2O3S	276.32	Infectiology
09H03	Benzocaine	C9H11NO2	165.19	Neuromuscular
09H04	Dipyrone	C13H16N3NaO4S	333.34	Central Nervous System

09H05	Isosorbide dinitrate	C6H8N2O8	236.14	Cardiovascular
09H06	Sulfachloropyridazine	C10H9ClN4O2S	284.73	Infectiology
09H07	Pramoxine hydrochloride	C17H28ClNO3	329.87	Neuromuscular
09H08	Finasteride	C23H36N2O2	372.56	Endocrinology
09H09	Fluorometholone	C22H29FO4	376.47	Endocrinology
09H10	Cephalothin sodium salt	C16H15N2NaO6S2	418.43	Infectiology
09H11	Cefuroxime sodium salt	C16H15N4NaO8S	446.37	Infectiology
10A02	Althiazide	C11H14ClN3O4S3	383.90	Metabolism
10A03	Isopyrin hydrochloride	C14H20ClN3O	281.79	Central Nervous System
10A04	Phenethicillin potassium salt	C17H19KN2O5S	402.52	Infectiology
10A05	Sulfamethoxypyridazine	C11H12N4O3S	280.31	Infectiology
10A06	Deferoxamine mesylate	C26H52N6O11S	656.80	Diagnostic
10A07	Mephentermine hemisulfate	C22H36N2O4S	424.61	Cardiovascular
10A08	Liranaftate	C18H20N2O2S	328.44	Infectiology
10A09	Sulfadimethoxine	C12H14N4O4S	310.33	Infectiology
10A10	Sulfanilamide	C6H8N2O2S	172.21	Infectiology
10A11	Balsalazide Sodium	C17H13N3Na2O6	401.29	Gastroenterology
10B02	Sulfaquinoxaline sodium salt	C14H11N4NaO2S	322.32	Infectiology
10B03	Streptozotocin	C8H15N3O7	265.22	Oncology
10B04	Metoprolol-(+,-) (+)-tartrate salt	C34H56N2O12	684.83	Cardiovascular
10B05	Flumethasone	C22H28F2O5	410.46	Metabolism
10B06	Flecainide acetate	C19H24F6N2O5	474.40	Cardiovascular
10B07	Cefazolin sodium salt	C14H13N8NaO4S3	476.49	Metabolism
10B08	Atractyloside potassium salt	C30H44K2O16S2	803.01	Oncology
10B09	Folinic acid calcium salt	C20H21CaN7O7	511.51	Hematology
10B10	Levonordefrin	C9H13NO3	183.21	Cardiovascular
10B11	Ebselen	C13H9NOSe	274.18	Metabolism
10C02	Nadide	C21H27N7O14P2	663.44	Metabolism
10C03	Sulfamethizole	C9H10N4O2S2	270.33	Metabolism
10C04	Medrysone	C22H32O3	344.50	Metabolism
10C05	Flunixin meglumine	C21H28F3N3O7	491.47	Central Nervous System
10C06	Spiramycin	C43H73N2O14R	842.07	Metabolism
10C07	Glycopyrrolate	C19H28BrNO3	398.34	Gastroenterology
10C08	Aprepitant	C23H21F7N4O3	534.44	Metabolism
10C09	Monensin sodium salt	C36H61NaO11	692.87	Infectiology
10C10	Isoetharine mesylate salt	C14H25NO6S	335.42	Respiratory
10C11	Mevalonic-D, L acid lactone	C6H10O3	130.14	Cardiovascular
10D02	Terazosin hydrochloride	C19H26ClN5O4	423.90	Cardiovascular
10D03	Phenazopyridine hydrochloride	C11H12ClN5	249.70	Central Nervous System
10D04	Demeclocycline hydrochloride	C21H22Cl2N2O8	501.32	Metabolism
10D05	Fenoprofen calcium salt dihydrate	C30H30CaO8	558.65	Metabolism
10D06	Piperacillin sodium salt	C23H26N5NaO7S	539.55	Metabolism
10D07	Diethylstilbestrol	C18H20O2	268.36	Endocrinology
10D08	Chlorotrianisene	C23H21ClO3	380.88	Endocrinology
10D09	Ribostamycin sulfate salt	C17H36N4O14S	552.56	Metabolism
10D10	Methacholine chloride	C8H18ClNO2	195.69	Diagnostic
10D11	Pipenzolate bromide	C22H28BrNO3	434.38	Gastroenterology
10E02	Butamben	C11H15NO2	193.25	Central Nervous System
10E03	Sulfapyridine	C11H11N3O2S	249.29	Metabolism

10E04	Meclofenoxate hydrochloride	C12H17Cl2NO3	294.18	Central Nervous System
10E05	Furaltadone hydrochloride	C13H17ClN4O6	360.76	Infectiology
10E06	Ethoxyquin	C14H19NO	217.31	Metabolism
10E07	Tinidazole	C8H13N3O4S	247.27	Infectiology
10E08	Guanadrel sulfate	C20H40N6O8S	524.64	Cardiovascular
10E09	Vidarabine	C10H13N5O4	267.25	Metabolism
10E10	Sulfameter	C11H12N4O3S	280.31	Metabolism
10E11	Isopropamide iodide	C23H33IN2O	480.44	Metabolism
10F02	Alclometasone dipropionate	C28H37ClO7	521.06	Metabolism
10F03	Leflunomide	C12H9F3N2O2	270.21	Immunology
10F04	Norgestrel-(-)-D	C21H28O2	312.46	Endocrinology
10F05	Fluocinonide	C26H32F2O7	494.54	Metabolism
10F06	Sulfamethazine sodium salt	C12H13N4NaO2S	300.32	Metabolism
10F07	Guaifenesin	C10H14O4	198.22	Respiratory
10F08	Alexidine dihydrochloride	C26H58Cl2N10	581.73	Infectiology
10F09	Proadifen hydrochloride	C23H32ClNO2	389.97	Neuromuscular
10F10	Zomepirac sodium salt	C15H13ClNNaO3	313.72	Metabolism
10F11	Cinoxacin	C12H10N2O5	262.22	Metabolism
10G02	Clobetasol propionate	C25H32ClFO5	466.98	Metabolism
10G03	Podophyllotoxin	C22H22O8	414.42	Metabolism
10G04	Clofibric acid	C10H11ClO3	214.65	Metabolism
10G05	Bendroflumethiazide	C15H14F3N3O4S2	421.42	Cardiovascular
10G06	Dicumarol	C19H12O6	336.30	Hematology
10G07	Methimazole	C4H6N2S	114.17	Endocrinology
10G08	Merbromin	C20H8Br2HgNa2O6	750.66	Infectiology
10G09	Hexylcaine hydrochloride	C16H24ClNO2	297.83	Dermatology
10G10	Drofenine hydrochloride	C20H32ClNO2	353.94	Neuromuscular
10G11	Cycloheximide	C15H23NO4	281.35	Infectiology
10H02	(R) -Naproxen sodium salt	C14H13NaO3	252.25	Metabolism
10H03	Propidium iodide	C27H34I2N4	668.41	Infectiology
10H04	Cloperastine hydrochloride	C20H25Cl2NO	366.33	Respiratory
10H05	Eucatropine hydrochloride	C17H26ClNO3	327.85	Neuromuscular
10H06	Isocarboxazid	C12H13N3O2	231.26	Central Nervous System
10H07	Lithocholic acid	C24H40O3	376.58	Metabolism
10H08	Methotrimeprazine maleat salt	C23H28N2O5S	444.55	Central Nervous System
10H09	Dienestrol	C18H18O2	266.34	Endocrinology
10H10	Pridinol methanesulfonate salt	C21H29NO4S	391.53	Central Nervous System
10H11	Amrinone	C10H9N3O	187.20	Cardiovascular
11A02	Carbinoxamine maleate salt	C20H23ClN2O5	406.87	Allergology
11A03	Methazolamide	C5H8N4O3S2	236.27	Metabolism
11A04	Pyrithyldione	C9H13NO2	167.21	Central Nervous System
11A05	Spectinomycin dihydrochloride	C14H26Cl2N2O7	405.28	Metabolism
11A06	Piromidic acid	C14H16N4O3	288.31	Metabolism
11A07	Trimipramine maleate salt	C24H30N2O4	410.52	Central Nervous System
11A08	Chloropyramine hydrochloride	C16H21Cl2N3	326.27	Allergology
11A09	Furazolidone	C8H7N3O5	225.16	Metabolism
11A10	Dichlorphenamide	C6H6Cl2N2O4S2	305.16	Ophthalmology
11A11	Sulconazole nitrate	C18H16Cl3N3O3S	460.77	Metabolism
11B02	Auranofin	C20H34AuO9PS	678.49	Metabolism

11B03	Cromolyn disodium salt	C23H14Na2O11	512.34	Allergology
11B04	Bucladesine sodium salt	C18H23N5NaO8P	491.38	Cardiovascular
11B05	Cefsulodin sodium salt	C22H19N4NaO8S2	554.54	Metabolism
11B06	Fosfosal	C7H7O6P	218.10	Central Nervous System
11B07	Suprofen	C14H12O3S	260.31	Central Nervous System
11B08	Deflazacort	C25H31NO6	441.53	Immunology
11B09	Nadolol	C17H27NO4	309.41	Cardiovascular
11B10	Moxalactam disodium salt	C20H18N6Na2O9S	564.44	Metabolism
11B11	Aminophylline	C16H24N10O4	420.43	Cardiovascular
11C02	Azlocillin sodium salt	C20H22N5NaO6S	483.48	Metabolism
11C03	Clidinium bromide	C22H26BrNO3	432.36	Neuromuscular
11C04	Sulfamonomethoxine	C11H12N4O3S	280.31	Metabolism
11C05	Benzthiazide	C15H14ClN3O4S3	431.94	Cardiovascular
11C06	Trichlormethiazide	C8H8Cl3N3O4S2	380.66	Cardiovascular
11C07	Oxalamine citrate salt	C20H27N3O8	437.45	Central Nervous System
11C08	Propantheline bromide	C23H30BrNO3	448.40	Neuromuscular
11C09	Viloxazine hydrochloride	C13H20ClNO3	273.76	Central Nervous System
11C10	Dimethadione	C5H7NO3	129.12	Central Nervous System
11C11	Ethaverine hydrochloride	C24H30ClNO4	431.96	Central Nervous System
11D02	Butacaine	C18H30N2O2	306.45	Dermatology
11D03	Cefoxitin sodium salt	C16H16N3NaO7S2	449.44	Metabolism
11D04	Ifosfamide	C7H15Cl2N2O2P	261.09	Oncology
11D05	Novobiocin sodium salt	C31H35N2NaO11	634.62	Metabolism
11D06	Tetrahydroxy-1,4-quinone monohydrate	C6H6O7	190.11	Dermatology
11D07	Indoprofen	C17H15NO3	281.31	Central Nervous System
11D08	Carbenoxolone disodium salt	C34H48Na2O7	614.74	Metabolism
11D09	Iocetamic acid	C12H13I3N2O3	613.96	Diagnostic
11D10	Ganciclovir	C9H13N5O4	255.24	Metabolism
11D11	Ethopropazine hydrochloride	C19H25ClN2S	348.94	Central Nervous System
11E02	Olanzapine	C17H20N4S	312.44	Central Nervous System
11E03	Trimeprazine tartrate	C40H50N4O6S2	747.00	Allergology
11E04	Nafcillin sodium salt monohydrate	C21H23N2NaO6S	454.48	Metabolism
11E05	Procyclidine hydrochloride	C19H30ClNO	323.91	Central Nervous System
11E06	Amiprilose hydrochloride	C14H28ClNO6	341.84	Immunology
11E07	Ethynylestradiol 3-methyl ether	C21H26O2	310.44	Endocrinology
11E08	(-) -Levobunolol hydrochloride	C17H26ClNO3	327.85	Ophthalmology
11E09	Iodixanol	C35H44I6N6O15	1550.20	Diagnostic
11E10	Clinafloxacin	C17H17ClFN3O3	365.79	Infectiology
11E11	Equilin	C18H20O2	268.36	Endocrinology
11F02	Paroxetine Hydrochloride	C19H21ClFNO3	365.84	Central Nervous System
11F03	Nylidrin	C19H25NO2	299.42	Cardiovascular
11F04	Liothyronine	C15H12I3NO4	650.98	Endocrinology
11F05	Roxithromycin	C41H76N2O15	837.07	Metabolism
11F06	Beclomethasone dipropionate	C28H37ClO7	521.06	Metabolism
11F07	Tolmetin sodium salt dihydrate	C15H18NNaO5	315.30	Metabolism
11F08	(+) -Levobunolol hydrochloride	C17H26ClNO3	327.85	Ophthalmology
11F09	Doxazosin mesylate	C24H29N5O8S	547.59	Cardiovascular
11F10	Fluvastatin sodium salt	C24H25FNNaO4	433.46	Cardiovascular

11F11	Methylhydantoin-5-(L)	C4H6N2O2	114.10	Central Nervous System
11G02	Gabapentin	C9H17NO2	171.24	Central Nervous System
11G03	Raloxifene hydrochloride	C28H28ClNO4S	510.06	Endocrinology
11G04	Etidronic acid, disodium salt	C2H6Na2O7P2	249.99	Metabolism
11G05	Methylhydantoin-5-(D)	C4H6N2O2	114.10	
11G06	Simvastatin	C25H38O5	418.58	Cardiovascular
11G07	Azacytidine-5	C8H12N4O5	244.21	Oncology
11G08	Paromomycin sulfate	C23H47N5O18S	713.72	Metabolism
11G09	Acetaminophen	C8H9NO2	151.17	Central Nervous System
11G10	Phthalylsulfathiazole	C17H13N3O5S2	403.44	Metabolism
11G11	Luteolin	C15H10O6	286.24	Respiratory
11H02	Iopamidol	C17H22I3N3O8	777.09	Diagnostic
11H03	Iopromide	C18H24I3N3O8	791.12	Diagnostic
11H04	Theophylline monohydrate	C7H10N4O3	198.18	Cardiovascular
11H05	Theobromine	C7H8N4O2	180.17	Cardiovascular
11H06	Reserpine	C33H40N2O9	608.69	Central Nervous System
11H07	Bicalutamide	C18H14F4N2O4S	430.38	Endocrinology
11H08	Scopolamine hydrochloride	C17H22ClNO4	339.82	Central Nervous System
11H09	Ioversol	C18H24I3N3O9	807.12	Diagnostic
11H10	Rabeprazole Sodium salt	C18H21N3NaO3S	382.44	Metabolism
11H11	Carbachol	C6H15ClN2O2	182.65	Cardiovascular
12A02	Niacin	C6H5NO2	123.11	Cardiovascular
12A03	Bemegride	C8H13NO2	155.20	Central Nervous System
12A04	Digoxigenin	C23H34O5	390.52	Diagnostic
12A05	Meglumine	C7H17NO5	195.22	Metabolism
12A06	Dolasetron mesilate	C20H26N2O7S	438.50	Central Nervous System
12A07	Clioquinol	C9H5ClINO	305.50	Metabolism
12A08	Oxybenzone	C14H12O3	228.25	Dermatology
12A09	Promethazine hydrochloride	C17H21ClN2S	320.89	Allergology
12A10	Diacerein	C19H12O8	368.30	Immunology
12A11	Esmolol hydrochloride	C16H26ClNO4	331.84	Cardiovascular
12B02	Cortisol acetate	C23H32O6	404.51	Metabolism
12B03	Flubendazol	C16H12FN3O3	313.29	Metabolism
12B04	Felbinac	C14H12O2	212.25	Central Nervous System
12B05	Butylparaben	C11H14O3	194.23	Metabolism
12B06	Aminohippuric acid	C9H10N2O3	194.19	Diagnostic
12B07	N-Acetyl-L-leucine	C8H15NO3	173.21	Central Nervous System
12B08	Pipemidic acid	C14H17N5O3	303.32	Metabolism
12B09	Dioxybenzone	C14H12O4	244.25	Dermatology
12B10	Adrenosterone	C19H24O3	300.40	Endocrinology
12B11	Methylatropine nitrate	C18H26N2O6	366.42	Neuromuscular
12C02	Hymecromone	C10H8O3	176.17	Metabolism
12C03	Abacavir Sulfate	C28H38N12O6S	670.76	Infectiology
12C04	Diloxanide furoate	C14H11Cl2NO4	328.15	Metabolism
12C05	Metyrapone	C14H14N2O	226.28	Endocrinology
12C06	Urapidil hydrochloride	C20H30ClN5O3	423.95	Cardiovascular
12C07	Fluspirilen	C29H31F2N3O	475.59	Central Nervous System
12C08	S-(+)-ibuprofen	C13H18O2	206.29	Central Nervous System
12C09	Ethynodiol diacetate	C24H32O4	384.52	Endocrinology

12C10	Nabumetone	C15H16O2	228.29	Central Nervous System
12C11	Nisoxetine hydrochloride	C17H22ClNO2	307.82	Central Nervous System
12D02	(+)-Isoproterenol (+)-bitartrate salt	C15H23NO9	361.35	Respiratory
12D03	Monobenzone	C13H12O2	200.24	Dermatology
12D04	2-Aminobenzenesulfonamide	C6H8N2O2S	172.21	Metabolism
12D05	Estrone	C18H22O2	270.37	Endocrinology
12D06	Lorglumide sodium salt	C22H31Cl2N2NaO4	481.40	Metabolism
12D07	Nitrendipine	C18H20N2O6	360.37	Cardiovascular
12D08	Flurbiprofen	C15H13FO2	244.27	Central Nervous System
12D09	Nimodipine	C21H26N2O7	418.45	Cardiovascular
12D10	Bacitracin	C66H103N17O16S	1422.73	Metabolism
12D11	L(-)-vesamicol hydrochloride	C17H26ClNO	295.86	Neuromuscular
12E02	Nizatidine	C12H21N5O2S2	331.46	Metabolism
12E03	Thioperamide maleate	C19H28N4O4S	408.52	Central Nervous System
12E04	Xamoterol hemifumarate	C36H54N6O14	794.86	Cardiovascular
12E05	Rolipram	C16H21NO3	275.35	Central Nervous System
12E06	Thonzonium bromide	C32H55BrN4O	591.73	Dermatology
12E07	Idazoxan hydrochloride	C11H13ClN2O2	240.69	Central Nervous System
12E08	Quinapril HCl	C25H31ClN2O5	474.99	Cardiovascular
12E09	Nilutamide	C12H10F3N3O4	317.23	Oncology
12E10	Ketorolac tromethamine	C19H24N2O6	376.41	Central Nervous System
12E11	Protriptyline hydrochloride	C19H22ClN	299.85	Central Nervous System
12F02	Propofol	C12H18O	178.28	Central Nervous System
12F03	S(-)Eticlopride hydrochloride	C17H26Cl2N2O3	377.31	Central Nervous System
12F04	Primidone	C12H14N2O2	218.26	Central Nervous System
12F05	Flucytosine	C4H4FN3O	129.09	Metabolism
12F06	(-)-MK 801 hydrogen maleate	C20H19NO4	337.38	Central Nervous System
12F07	Bephenium hydroxynaphthoate	C28H29NO4	443.55	Metabolism
12F08	Dehydroisoandosterone 3-acetate	C21H30O3	330.47	Endocrinology
12F09	Benserazide hydrochloride	C10H16ClN3O5	293.71	Central Nervous System
12F10	Iodipamide	C20H14I6N2O6	1139.77	Diagnostic
12F11	Allopurinol	C5H4N4O	136.11	Metabolism
12G02	Pentetic acid	C14H23N3O10	393.35	Oncology
12G03	Bretylium tosylate	C18H24BrNO3S	414.36	Cardiovascular
12G04	Pralidoxime chloride	C7H9ClN2O	172.62	Neuromuscular
12G05	Phenoxybenzamine hydrochloride	C18H23Cl2NO	340.30	Cardiovascular
12G06	Salmeterol	C25H37NO4	415.58	Respiratory
12G07	Altretamine	C9H18N6	210.28	Oncology
12G08	Prazosin hydrochloride	C19H22ClN5O4	419.87	Cardiovascular
12G09	Timolol maleate salt	C17H28N4O7S	432.50	Cardiovascular
12G10	(+,-)-Octopamine hydrochloride	C8H12ClNO2	189.64	Cardiovascular
12G11	Stavudine	C10H12N2O4	224.22	Infectiology
12H02	Crotamiton	C13H17NO	203.29	Dermatology
12H03	Toremifene	C26H28ClNO	405.97	Endocrinology
12H04	(R)-(+)-Atenolol	C14H22N2O3	266.34	Cardiovascular
12H05	Tyloxapol	C59H96O12	997.42	Respiratory
12H06	Florfenicol	C12H14Cl2FNO4S	358.22	Metabolism
12H07	Megestrol acetate	C24H32O4	384.52	Endocrinology
12H08	Deoxycorticosterone	C21H30O3	330.47	Endocrinology

12H09	Urosiol	C24H40O4	392.58	Metabolism
12H10	Proparacaine hydrochloride	C16H27ClN2O3	330.86	Central Nervous System
12H11	Aminocaproic acid	C6H13NO2	131.18	Allergology
13A02	Denatonium benzoate	C28H34N2O3	446.59	Neuromuscular
13A03	Canrenone	C22H28O3	340.47	Endocrinology
13A04	Enilconazole	C14H14Cl2N2O	297.19	Metabolism
13A05	Methacycline hydrochloride	C22H23ClN2O8	478.89	Metabolism
13A06	Floxuridine	C9H11FN2O5	246.20	Oncology
13A07	Sotalol hydrochloride	C12H21ClN2O3S	308.83	Cardiovascular
13A08	Gestrinone	C21H24O2	308.42	Endocrinology
13A09	Decamethonium bromide	C16H38Br2N2	418.30	Neuromuscular
13A10	Darifenacin hydrobromide	C28H31BrN2O2	507.48	Neuromuscular
13A11	Indatraline hydrochloride	C16H16Cl3N	328.67	Central Nervous System
13B02	Remoxipride Hydrochloride	C16H24BrClN2O3	407.74	Central Nervous System
13B03	THIP Hydrochloride	C6H9ClN2O2	176.60	Central Nervous System
13B04	Pirlindole mesylate	C16H22N2O3S	322.43	Central Nervous System
13B05	Pronethalol hydrochloride	C15H20ClNO	265.79	Cardiovascular
13B06	Naftopidil dihydrochloride	C24H30Cl2N2O3	465.42	Cardiovascular
13B07	Tracazolate hydrochloride	C16H25ClN4O2	340.86	Central Nervous System
13B08	Zardaverine	C12H10F2N2O3	268.22	Respiratory
13B09	Memantine Hydrochloride	C12H22ClN	215.77	Central Nervous System
13B10	Ozagrel hydrochloride	C13H13ClN2O2	264.71	Cardiovascular
13B11	Piribedil hydrochloride	C16H19ClN4O2	334.81	Cardiovascular
13C02	Nitrocaramiphen hydrochloride	C18H27ClN2O4	370.88	Central Nervous System
13C03	Nandrolone	C18H26O2	274.41	Endocrinology
13C04	Dimaprit dihydrochloride	C6H17Cl2N3S	234.19	Metabolism
13C05	Oxfendazol	C15H13N3O3S	315.35	Metabolism
13C06	Guaiacol	C7H8O2	124.14	Respiratory
13C07	Proscillaridin A	C30H42O8	530.66	Cardiovascular
13C08	Pramipexole	C10H17N3S	211.33	Central Nervous System
13C09	Norgestimate	C23H31NO3	369.51	Endocrinology
13C10	Chlormadinone acetate	C23H29ClO4	404.94	Endocrinology
13C11	Phenylbutazone	C19H20N2O2	308.38	Metabolism
13D02	Gliquidone	C27H33N3O6S	527.64	Endocrinology
13D03	Pizotifen malate	C23H27NO5S	429.54	Allergology
13D04	Ribavirin	C8H12N4O5	244.21	Metabolism
13D05	Cyclopenthiazide	C13H18ClN3O4S2	379.89	Cardiovascular
13D06	Fluvoxamine maleate	C19H25F3N2O6	434.42	Central Nervous System
13D07	Prothionamide	C9H12N2S	180.27	Infectiology
13D08	Fluticasone propionate	C25H31F3O5S	500.58	Cardiovascular
13D09	Zuclopenthixol dihydrochloride	C22H27Cl3N2OS	473.90	Central Nervous System
13D10	Proguanil hydrochloride	C11H17Cl2N5	290.20	Metabolism
13D11	Lymecycline	C29H38N4O10	602.65	Metabolism
13E02	Alfadolone acetate	C23H34O5	390.52	Central Nervous System
13E03	Alfaxalone	C21H32O3	332.49	Central Nervous System
13E04	Azapropazone	C16H20N4O2	300.36	Central Nervous System
13E05	Meptazinol hydrochloride	C15H24ClNO	269.82	Central Nervous System
13E06	Apramycin	C21H41N5O11	539.59	Metabolism
13E07	Epitiostanol	C19H30OS	306.51	Oncology

13E08	Fursultiamine Hydrochloride	C17H27ClN4O3S2	435.01	Metabolism
13E09	Gabexate mesilate	C17H27N3O7S	417.48	Hematology
13E10	Pivampicillin	C22H29N3O6S	463.56	Metabolism
13E11	Talampicillin hydrochloride	C24H24ClN3O6S	517.99	Metabolism
13F02	Flucloxacillin sodium	C19H16ClFN3NaO5S	475.86	Metabolism
13F03	Trapidil	C10H15N5	205.26	Cardiovascular
13F04	Deptropine citrate	C29H35NO8	525.60	Allergology
13F05	Sertraline	C17H17Cl2N	306.24	Central Nervous System
13F06	Ethamsylate	C10H17NO5S	263.31	Cardiovascular
13F07	Moxonidine	C9H12ClN5O	241.68	Cardiovascular
13F08	Etilefrine hydrochloride	C10H16ClNO2	217.70	Cardiovascular
13F09	Alprostadil	C20H34O5	354.49	Cardiovascular
13F10	Tribenoside	C29H34O6	478.59	Cardiovascular
13F11	Rimexolone	C24H34O3	370.54	Metabolism
13G02	Isradipine	C19H21N3O5	371.40	Cardiovascular
13G03	Tiletamine hydrochloride	C12H18ClNOS	259.80	Central Nervous System
13G04	Isometheptene mucate	C24H48N2O8	492.66	Cardiovascular
13G05	Nifurtimox	C10H13N3O5S	287.30	Metabolism
13G06	Letrozole	C17H11N5	285.31	Oncology
13G07	Arbutin	C12H16O7	272.26	Metabolism
13G08	Tocainide hydrochloride	C11H17ClN2O	228.72	Cardiovascular
13G09	Benzathine benzylpenicillin	C48H56N6O10S2	941.14	Metabolism
13G10	Risperidone	C23H27FN4O2	410.50	Central Nervous System
13G11	Torsemide	C16H20N4O3S	348.43	Cardiovascular
13H02	Halofantrine hydrochloride	C26H31Cl3F3NO	536.90	Metabolism
13H03	Articaine hydrochloride	C13H21ClN2O3S	320.84	Central Nervous System
13H04	Nomegestrol acetate	C23H30O4	370.49	Endocrinology
13H05	Pancuronium bromide	C35H60Br2N2O4	732.69	Neuromuscular
13H06	Molindone hydrochloride	C16H25ClN2O2	312.84	Central Nervous System
13H07	Alcuronium chloride	C44H50Cl2N4O2	737.82	Neuromuscular
13H08	Zalcitabine	C9H13N3O3	211.22	Metabolism
13H09	Methyldopate hydrochloride	C12H18ClNO4	275.73	Cardiovascular
13H10	Levocabastine hydrochloride	C26H30ClFN2O2	456.99	Allergology
13H11	Pyrvinium pamoate	C75H70N6O6	1151.43	Metabolism
14A02	Etomidate	C14H16N2O2	244.30	Central Nervous System
14A03	Tridihexethyl chloride	C21H36ClNO	353.98	Neuromuscular
14A04	Penbutolol sulfate	C36H60N2O8S	680.95	Cardiovascular
14A05	Prednicarbate	C27H36O8	488.58	Metabolism
14A06	Sertaconazole nitrate	C20H16Cl3N3O4S	500.79	Metabolism
14A07	Repaglinide	C27H36N2O4	452.60	Endocrinology
14A08	Piretanide	C17H18N2O5S	362.41	Cardiovascular
14A09	Piperacetazine	C24H30N2O2S	410.58	Central Nervous System
14A10	Oxyphenbutazone	C19H20N2O3	324.38	Metabolism
14A11	Quinethazone	C10H12ClN3O3S	289.74	Cardiovascular
14B02	Moricizine hydrochloride	C22H26ClN3O4S	463.99	Cardiovascular
14B03	Iopanoic acid	C11H12I3NO2	570.94	Diagnostic
14B04	Pivmecillinam hydrochloride	C21H34ClN3O5S	476.04	Metabolism
14B05	Levopropoxyphene napsylate	C32H37NO5S	547.72	Central Nervous System
14B06	Piperidolate hydrochloride	C21H26ClNO2	359.90	Neuromuscular

14B07	Trifluridine	C10H11F3N2O5	296.20	Metabolism
14B08	Oxprenolol hydrochloride	C15H24ClNO3	301.82	Cardiovascular
14B09	Ondansetron Hydrochloride	C18H20ClN3O	329.83	Central Nervous System
14B10	Propoxycaine hydrochloride	C16H27ClN2O3	330.86	Central Nervous System
14B11	Oxaprozin	C18H15NO3	293.33	Central Nervous System
14C02	Phensuximide	C11H11NO2	189.22	Central Nervous System
14C03	Ioxaglic acid	C24H21I6N5O8	1268.89	Diagnostic
14C04	Naftifine hydrochloride	C21H22ClN	323.87	Infectiology
14C05	Meprylcaine hydrochloride	C14H22ClNO2	271.79	Neuromuscular
14C06	Milrinone	C12H9N3O	211.23	Cardiovascular
14C07	Methantheline bromide	C21H26BrNO3	420.35	Neuromuscular
14C08	Ticarcillin sodium	C15H15N2NaO6S2	406.41	Infectiology
14C09	Thiethylperazine dimalate	C30H41N3O10S2	667.80	Central Nervous System
14C10	Mesalamine	C7H7NO3	153.14	Metabolism
14C11	Vorinostat	C14H20N2O3	264.33	Oncology
14D02	Imidurea	C11H16N8O8	388.30	Infectiology
14D03	Lansoprazole	C16H14F3N3O2S	369.37	Metabolism
14D04	Bethanechol chloride	C7H17ClN2O2	196.68	Metabolism
14D05	Cyproterone acetate	C24H29ClO4	416.95	Endocrinology
14D06	(R)-Propranolol hydrochloride	C16H22ClNO2	295.81	Cardiovascular
14D07	Ciprofibrate	C13H14Cl2O3	289.16	Metabolism
14D08	Formestane	C19H26O3	302.42	Endocrinology
14D09	Benzylpenicillin sodium	C16H17N2NaO4S	356.38	Infectiology
14D10	Chlorambucil	C14H19Cl2NO2	304.22	Oncology
14D11	Methiazole	C12H15N3O2S	265.34	Infectiology
14E02	(S)-propranolol hydrochloride	C16H22ClNO2	295.81	Cardiovascular
14E03	(-)-Eseroline fumarate salt	C17H22N2O5	334.38	Central Nervous System
14E04	Isosorbide mononitrate	C6H9NO6	191.14	Cardiovascular
14E05	Levalbuterol hydrochloride	C13H22ClNO3	275.78	Respiratory
14E06	Topiramate	C12H21NO8S	339.37	Central Nervous System
14E07	D-cycloserine	C3H6N2O2	102.09	Infectiology
14E08	2-Chloropyrazine	C4H3ClN2	114.53	Cardiovascular
14E09	(+,-)-Synephrine	C9H13NO2	167.21	Cardiovascular
14E10	(S)-(-)-Cycloserine	C3H6N2O2	102.09	Infectiology
14E11	Homosalate	C16H22O3	262.35	Dermatology
14F02	Spaglumic acid	C11H16N2O8	304.26	Allergology
14F03	Ranolazine	C24H33N3O4	427.55	Cardiovascular
14F04	Misoprostol	C22H38O5	382.55	Metabolism
14F05	Sulfadoxine	C12H14N4O4S	310.33	Infectiology
14F06	Cyclopentolate hydrochloride	C17H26ClNO3	327.85	Metabolism
14F07	Estriol	C18H24O3	288.39	Endocrinology
14F08	(-)-Isoproterenol hydrochloride	C11H18ClNO3	247.72	Cardiovascular
14F09	Sarafloxacin	C20H17F2N3O3	385.37	Infectiology
14F10	Nialamide	C16H18N4O2	298.35	Central Nervous System
14F11	Toltrazuril	C18H14F3N3O4S	425.39	Infectiology
14G02	Perindopril	C19H32N2O5	368.48	Cardiovascular
14G03	Fexofenadine HCl	C32H40ClNO4	538.13	Allergology
14G04	4-aminosalicylic acid	C7H7NO3	153.14	Infectiology
14G05	Clonixin Lysinate	C19H25ClN4O4	408.89	Central Nervous System

14G06	Verteporfin	C41H42N4O8	718.81	Ophthalmology
14G07	Meropenem	C17H25N3O5S	383.47	Infectiology
14G08	Ramipril	C23H32N2O5	416.52	Cardiovascular
14G09	Mephenytoin	C12H14N2O2	218.26	Central Nervous System
14G10	Rifabutin	C46H62N4O11	847.03	Infectiology
14G11	Parbendazole	C13H17N3O2	247.30	Infectiology
14H02	Mecamylamine hydrochloride	C11H22ClN	203.76	Cardiovascular
14H03	Procarbazine hydrochloride	C12H20ClN3O	257.77	Oncology
14H04	Viomycin sulfate	C25H45N13O14S	783.78	Infectiology
14H05	Saquinavir mesylate	C39H54N6O8S	766.96	Immunology
14H06	Ronidazole	C6H8N4O4	200.16	Infectiology
14H07	Dorzolamide hydrochloride	C10H17ClN2O4S3	360.90	Cardiovascular
14H08	Azaperone	C19H22FN3O	327.41	Central Nervous System
14H09	Cefepime hydrochloride	C19H25ClN6O5S2	517.03	Infectiology
14H10	Clocortolone pivalate	C27H36ClFO5	495.04	Endocrinology
14H11	Nadifloxacin	C19H21FN2O4	360.39	Infectiology
15A02	Buspirone hydrochloride	C21H32ClN5O2	421.97	Central Nervous System
15A03	Anastrozole	C17H19N5	293.37	Oncology
15A04	Doxycycline hydrochloride	C22H25ClN2O8	480.91	Metabolism
15A05	Sulbactam	C8H11NO5S	233.24	Infectiology
15A06	Fleroxacin	C17H18F3N3O3	369.35	Infectiology
15A07	Clavulanate potassium salt	C8H8KNO5	237.26	Infectiology
15A08	Valproic acid	C8H16O2	144.22	Central Nervous System
15A09	Mepivacaine hydrochloride	C15H23ClN2O	282.82	Neuromuscular
15A10	Rifaximin	C43H51N3O11	785.90	Infectiology
15A11	Estradiol Valerate	C23H32O3	356.51	Endocrinology
15B02	Acetylcysteine	C5H9NO3S	163.20	Metabolism
15B03	Melengestrol acetate	C25H32O4	396.53	Endocrinology
15B04	Bromhexine hydrochloride	C14H21Br2ClN2	412.60	Respiratory
15B05	Anethole-trithione	C10H8OS3	240.37	Metabolism
15B06	Amcinonide	C28H35FO7	502.59	Metabolism
15B07	Caffeine	C8H10N4O2	194.19	Central Nervous System
15B08	Carvedilol	C24H26N2O4	406.49	Cardiovascular
15B09	Methenamine	C6H12N4	140.19	Infectiology
15B10	Phentermine hydrochloride	C10H16ClN	185.70	Central Nervous System
15B11	Diclazuril	C17H9Cl3N4O2	407.65	Metabolism
15C02	Famciclovir	C14H19N5O4	321.34	Infectiology
15C03	Dopamine hydrochloride	C8H12ClNO2	189.64	Cardiovascular
15C04	Cefdinir	C14H13N5O5S2	395.42	Infectiology
15C05	Carprofen	C15H12ClNO2	273.72	Metabolism
15C06	Celecoxib	C17H14F3N3O2S	381.38	Metabolism
15C07	Candesartan	C24H20N6O3	440.47	Cardiovascular
15C08	Fludarabine	C10H12FN5O4	285.24	Oncology
15C09	Cladribine	C10H12ClN5O3	285.69	Oncology
15C10	Vardenafil	C23H32N6O4S	488.61	Cardiovascular
15C11	Fluconazole	C13H12F2N6O	306.28	Metabolism
15D02	5-fluorouracil	C4H3FN2O2	130.08	Oncology
15D03	Mesna	C2H5NaO3S2	164.18	Oncology
15D04	Mitotane	C14H10Cl4	320.05	Endocrinology

15D05	Ambrisentan	C22H22N2O4	378.43	Cardiovascular
15D06	Triclosan	C12H7Cl3O2	289.55	Infectiology
15D07	Enoxacin	C15H17FN4O3	320.33	Infectiology
15D08	Olopatadine hydrochloride	C21H24ClNO3	373.88	Allergology
15D09	Granisetron	C18H24N4O	312.42	Endocrinology
15D10	Anthralin	C14H10O3	226.23	Dermatology
15D11	Lamotrigine	C9H7Cl2N5	256.10	Central Nervous System
15E02	Clofibrate	C12H15ClO3	242.70	Metabolism
15E03	Cyclophosphamide	C7H15Cl2N2O2P	261.09	Immunology
15E04	Aripiprazole	C23H27Cl2N3O2	448.40	Central Nervous System
15E05	Ethinylestradiol	C20H24O2	296.41	Endocrinology
15E06	Fluocinolone acetonide	C24H30F2O6	452.50	Metabolism
15E07	Sparfloxacin	C19H22F2N4O3	392.41	Infectiology
15E08	Desloratadine	C19H19ClN2	310.83	Allergology
15E09	Clarithromycin	C38H69NO13	747.97	Infectiology
15E10	Tripelennamine hydrochloride	C16H22ClN3	291.83	Allergology
15E11	Tulobuterol	C12H18ClNO	227.74	Respiratory
15F02	Topotecan	C23H23N3O5	421.46	Oncology
15F03	Atorvastatin	C33H35FN2O5	558.66	Metabolism
15F04	Azithromycin	C38H72N2O12	749.00	Infectiology
15F05	Ibudilast	C14H18N2O	230.31	Metabolism
15F06	Losartan	C22H23ClN6O	422.92	Cardiovascular
15F07	Benztropine mesylate	C22H29NO4S	403.54	Central Nervous System
15F08	Vecuronium bromide	C34H57BrN2O4	637.75	Metabolism
15F09	Telmisartan	C33H30N4O2	514.63	Cardiovascular
15F10	Nalmefene hydrochloride	C21H26ClNO3	375.90	Central Nervous System
15F11	Bifonazole	C22H18N2	310.40	Infectiology
15G02	Gatifloxacin	C19H22FN3O4	375.40	Infectiology
15G03	Bosentan	C27H29N5O6S	551.63	Cardiovascular
15G04	Gemcitabine	C9H11F2N3O4	263.20	Oncology
15G05	Olmesartan	C29H30N6O6	558.60	Cardiovascular
15G06	Racepinephrine HCl	C9H14ClNO3	219.67	Cardiovascular
15G07	Montelukast	C35H36ClNO3S	586.20	Respiratory
15G08	Docetaxel	C43H53NO14	807.90	Oncology
15G09	Cilnidipine	C27H28N2O7	492.53	Cardiovascular
15G10	Imiquimod	C14H16N4	240.31	Dermatology
15G11	Fosinopril	C30H46NO7P	563.68	Cardiovascular
15H02	Imatinib	C29H31N7O	493.62	Oncology
15H03	Moxifloxacin	C21H24FN3O4	401.44	Infectiology
15H04	Formoterol fumarate	C42H52N4O12	804.90	Respiratory
15H05	Rufloxacin	C17H18FN3O3S	363.41	Infectiology
15H06	Pravastatin	C23H36O7	424.54	Metabolism
15H07	Rosiglitazone Hydrochloride	C18H20ClN3O3S	393.90	Metabolism
15H08	Rivastigmine	C14H22N2O2	250.34	Central Nervous System
15H09	Sildenafil	C22H30N6O4S	474.59	Cardiovascular
15H10	Acetylsalicylic acid	C9H8O4	180.16	Central Nervous System
15H11	Hexachlorophene	C13H6Cl6O2	406.91	Infectiology

Bibliography

1. Louis, D.N., et al., *The 2007 WHO classification of tumours of the central nervous system.* Acta Neuropathol, 2007. **114**(2): p. 97-109.
2. Wen, P.Y. and S. Kesari, *Malignant gliomas in adults.* N Engl J Med, 2008. **359**(5): p. 492-507.
3. Jovcevska, I., N. Kocevar, and R. Komel, *Glioma and glioblastoma - how much do we (not) know?* Mol Clin Oncol, 2013. **1**(6): p. 935-941.
4. Davis, F.G., et al., *Survival rates in patients with primary malignant brain tumors stratified by patient age and tumor histological type: an analysis based on Surveillance, Epidemiology, and End Results (SEER) data, 1973-1991.* J Neurosurg, 1998. **88**(1): p. 1-10.
5. Parney, I.F.a.P.M.D., *Glioblastoma Multiforme*. Textbook of Neuro-Oncology, ed. M.S. Berger. 2009: Elsevier Saunders.
6. Fisher, J.L., et al., *Epidemiology of brain tumors.* Neurol Clin, 2007. **25**(4): p. 867-90, vii.
7. Furnari, F.B., et al., *Malignant astrocytic glioma: genetics, biology, and paths to treatment.* Genes Dev, 2007. **21**(21): p. 2683-710.
8. Hou, L.C., et al., *Recurrent glioblastoma multiforme: a review of natural history and management options.* Neurosurg Focus, 2006. **20**(4): p. E5.
9. Iacob, G. and E.B. Dinca, *Current data and strategy in glioblastoma multiforme.* J Med Life, 2009. **2**(4): p. 386-93.
10. Weingart, J.D., *High-Grade Astrocytoma/Glioblastoma*, in *Neuro-Oncology of CNS Tumors*. 2006, Springer. p. 127-138.
11. Sathornsumetee, S., et al., *Molecularly targeted therapy for malignant glioma.* Cancer, 2007. **110**(1): p. 13-24.
12. Stupp, R., et al., *Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma.* N Engl J Med, 2005. **352**(10): p. 987-96.
13. Cohen, M.H., et al., *FDA drug approval summary: bevacizumab (Avastin) as treatment of recurrent glioblastoma multiforme.* Oncologist, 2009. **14**(11): p. 1131-8.
14. Reardon, D.A., et al., *Phase I pharmacokinetic study of the vascular endothelial growth factor receptor tyrosine kinase inhibitor vatalanib (PTK787) plus imatinib and hydroxyurea for malignant glioma.* Cancer, 2009. **115**(10): p. 2188-98.
15. Goldbrunner, R.H., et al., *PTK787/ZK222584, an inhibitor of vascular endothelial growth factor receptor tyrosine kinases, decreases glioma growth and vascularization.* Neurosurgery, 2004. **55**(2): p. 426-32; discussion 432.
16. Jost, L.M., et al., *Metabolism and disposition of vatalanib (PTK787/ZK-222584) in cancer patients.* Drug Metab Dispos, 2006. **34**(11): p. 1817-28.
17. Pelloski, C.E., et al., *Epidermal growth factor receptor variant III status defines clinically distinct subtypes of glioblastoma.* J Clin Oncol, 2007. **25**(16): p. 2288-94.
18. Rich, J.N., et al., *Phase II trial of gefitinib in recurrent glioblastoma.* J Clin Oncol, 2004. **22**(1): p. 133-42.
19. Bai, R.Y., V. Staedtke, and G.J. Riggins, *Molecular targeting of glioblastoma: Drug discovery and therapies.* Trends Mol Med, 2011. **17**(6): p. 301-12.

20. Bangert, A., et al., *Histone deacetylase inhibitors sensitize glioblastoma cells to TRAIL-induced apoptosis by c-myc-mediated downregulation of cFLIP.* Oncogene, 2012. **31**(44): p. 4677-88.
21. Chinnaiyan, P., et al., *Modulation of radiation response by histone deacetylase inhibition.* Int J Radiat Oncol Biol Phys, 2005. **62**(1): p. 223-9.
22. Yin, D., et al., *Proteasome inhibitor PS-341 causes cell growth arrest and apoptosis in human glioblastoma multiforme (GBM).* Oncogene, 2005. **24**(3): p. 344-54.
23. Cloughesy, T.F., et al., *Antitumor Activity of Rapamycin in a Phase I Trial for Patients with Recurrent PTEN-Deficient Glioblastoma.* PLoS Med, 2008. **5**(1): p. e8.
24. Falschlehner, C., et al., *TRAIL signalling: decisions between life and death.* Int J Biochem Cell Biol, 2007. **39**(7-8): p. 1462-75.
25. Wang, S. and W.S. El-Deiry, *TRAIL and apoptosis induction by TNF-family death receptors.* Oncogene, 2003. **22**(53): p. 8628-33.
26. Johnstone, R.W., A.A. Ruefli, and S.W. Lowe, *Apoptosis: A link between cancer genetics and chemotherapy.* Cell, 2002. **108**(2): p. 153-164.
27. Stuckey, D.W. and K. Shah, *TRAIL on trial: preclinical advances in cancer therapy.* Trends Mol Med, 2013. **19**(11): p. 685-94.
28. Pitti, R.M., et al., *Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family.* J Biol Chem, 1996. **271**(22): p. 12687-90.
29. Janssen, E.M., et al., *CD4+ T-cell help controls CD8+ T-cell memory via TRAIL-mediated activation-induced cell death.* Nature, 2005. **434**(7029): p. 88-93.
30. Smyth, M.J., et al., *Nature's TRAIL--on a path to cancer immunotherapy.* Immunity, 2003. **18**(1): p. 1-6.
31. Hayakawa, Y., et al., *NK cell TRAIL eliminates immature dendritic cells in vivo and limits dendritic cell vaccination efficacy.* J Immunol, 2004. **172**(1): p. 123-9.
32. Kuijlen, J.M., et al., *Review: on TRAIL for malignant glioma therapy?* Neuropathol Appl Neurobiol, 2010. **36**(3): p. 168-82.
33. Degli-Esposti, M.A., et al., *The novel receptor TRAIL-R4 induces NF-kappaB and protects against TRAIL-mediated apoptosis, yet retains an incomplete death domain.* Immunity, 1997. **7**(6): p. 813-20.
34. Pan, G., et al., *An Antagonist Decoy Receptor and a Death Domain-Containing Receptor for TRAIL.* Science, 1997. **277**(5327): p. 815-818.
35. Pan, G., et al., *The receptor for the cytotoxic ligand TRAIL.* Science, 1997. **276**(5309): p. 111-3.
36. Sheridan, J.P., et al., *Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors.* Science, 1997. **277**(5327): p. 818-21.
37. Wiley, S.R., et al., *Identification and characterization of a new member of the TNF family that induces apoptosis.* Immunity, 1995. **3**(6): p. 673-82.
38. Ashkenazi, A., et al., *Safety and antitumor activity of recombinant soluble Apo2 ligand.* J Clin Invest, 1999. **104**(2): p. 155-62.
39. Walczak, H., et al., *Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo.* Nat Med, 1999. **5**(2): p. 157-63.
40. Ashkenazi, A. and V.M. Dixit, *Apoptosis control by death and decoy receptors.* Curr Opin Cell Biol, 1999. **11**(2): p. 255-60.
41. Lawrence, D., et al., *Differential hepatocyte toxicity of recombinant Apo2L/TRAIL versions.* Nat Med, 2001. **7**(4): p. 383-5.

42. Yang, J.K., *FLIP as an anti-cancer therapeutic target.* Yonsei Med J, 2008. **49**(1): p. 19-27.
43. Deveraux, Q.L. and J.C. Reed, *IAP family proteins--suppressors of apoptosis.* Genes Dev, 1999. **13**(3): p. 239-52.
44. Dimberg, L.Y., et al., *On the TRAIL to successful cancer therapy? Predicting and counteracting resistance against TRAIL-based therapeutics.* Oncogene, 2013. **32**(11): p. 1341-50.
45. MacFarlane, M., et al., *TRAIL receptor-selective mutants signal to apoptosis via TRAIL-R1 in primary lymphoid malignancies.* Cancer Res, 2005. **65**(24): p. 11265-70.
46. Wagner, K.W., et al., *Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL.* Nat Med, 2007. **13**(9): p. 1070-7.
47. Kuijlen, J.M., et al., *TRAIL-receptor expression is an independent prognostic factor for survival in patients with a primary glioblastoma multiforme.* J Neurooncol, 2006. **78**(2): p. 161-71.
48. Nagane, M., et al., *Increased death receptor 5 expression by chemotherapeutic agents in human gliomas causes synergistic cytotoxicity with tumor necrosis factor-related apoptosis-inducing ligand in vitro and in vivo.* Cancer Res, 2000. **60**(4): p. 847-53.
49. Bagci-Onder, T., et al., *Real-time imaging of the dynamics of death receptors and therapeutics that overcome TRAIL resistance in tumors.* Oncogene, 2013. **32**(23): p. 2818-2827.
50. Koschny, R., et al., *Bortezomib sensitizes primary human astrocytoma cells of WHO grades I to IV for tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis.* Clin Cancer Res, 2007. **13**(11): p. 3403-12.
51. Brooks, A.D., et al., *The proteasome inhibitor bortezomib (Velcade) sensitizes some human tumor cells to Apo2L/TRAIL-mediated apoptosis.* Ann N Y Acad Sci, 2005. **1059**: p. 160-7.
52. Unterkircher, T., et al., *Bortezomib primes glioblastoma, including glioblastoma stem cells, for TRAIL by increasing tBid stability and mitochondrial apoptosis.* Clin Cancer Res, 2011. **17**(12): p. 4019-30.
53. Jane, E.P., D.R. Premkumar, and I.F. Pollack, *Bortezomib sensitizes malignant human glioma cells to TRAIL, mediated by inhibition of the NF-{kappa}B signaling pathway.* Mol Cancer Ther, 2011. **10**(1): p. 198-208.
54. Vucic, D., V.M. Dixit, and I.E. Wertz, *Ubiquitylation in apoptosis: a post-translational modification at the edge of life and death.* Nat Rev Mol Cell Biol, 2011. **12**(7): p. 439-52.
55. Styczynski, J., et al., *Activity of bortezomib in glioblastoma.* Anticancer Res, 2006. **26**(6b): p. 4499-503.
56. Koehn, F.E. and G.T. Carter, *The evolving role of natural products in drug discovery.* Nat Rev Drug Discov, 2005. **4**(3): p. 206-20.
57. Brekke, O.H. and I. Sandlie, *Therapeutic antibodies for human diseases at the dawn of the twenty-first century.* Nat Rev Drug Discov, 2003. **2**(1): p. 52-62.
58. Waterhouse, P.M., M.B. Wang, and T. Lough, *Gene silencing as an adaptive defence against viruses.* Nature, 2001. **411**(6839): p. 834-42.
59. Lombardino, J.G. and J.A. Lowe, 3rd, *The role of the medicinal chemist in drug discovery--then and now.* Nat Rev Drug Discov, 2004. **3**(10): p. 853-62.
60. Chong, C.R. and D.J. Sullivan, Jr., *New uses for old drugs.* Nature, 2007. **448**(7154): p. 645-6.

61. Jin, G. and S.T. Wong, *Toward better drug repositioning: prioritizing and integrating existing methods into efficient pipelines.* Drug Discov Today, 2014. **19**(5): p. 637-644.
62. Fry, D.W., et al., *A specific inhibitor of the epidermal growth factor receptor tyrosine kinase.* Science, 1994. **265**(5175): p. 1093-5.
63. Wang, L., *Identification of novel small-molecule inhibitors of glioblastoma cell growth and invasion by high-throughput screening.* BioScience Trends, 2012. **6**(4): p. 192-200.
64. Hothi, P., et al., *High-throughput chemical screens identify disulfiram as an inhibitor of human glioblastoma stem cells.* Oncotarget, 2012. **3**(10): p. 1124-36.
65. Karamanakos, P. *Disulfiram/Copper Combination In The Treatment of Newly Diagnosed Glioblastoma Multiform (GLIODIS)*. 2013.
66. Badr, C.E., T. Wurdinger, and B.A. Tannous, *Functional drug screening assay reveals potential glioma therapeutics.* Assay Drug Dev Technol, 2011. **9**(3): p. 281-9.
67. Bagci-Onder, T., et al., *A Dual PI3K/mTOR Inhibitor, PI-103, Cooperates with Stem Cell-Delivered TRAIL in Experimental Glioma Models.* Cancer Research, 2011. **71**(1): p. 154-163.
68. Oliver, F.J., et al., *Importance of poly(ADP-ribose) polymerase and its cleavage in apoptosis. Lesson from an uncleavable mutant.* J Biol Chem, 1998. **273**(50): p. 33533-9.
69. Baginski, M. and J. Czub, *Amphotericin B and Its New Derivatives - Mode of Action.* Current Drug Metabolism, 2009. **10**(5): p. 459-469.
70. Ennis, H.L. and M. Lubin, *CYCLOHEXIMIDE: ASPECTS OF INHIBITION OF PROTEIN SYNTHESIS IN MAMMALIAN CELLS.* Science, 1964. **146**(3650): p. 1474-6.
71. van Dijk, M., et al., *Resistance to TRAIL in non-transformed cells is due to multiple redundant pathways.* Cell Death Dis, 2013. **4**: p. e702.
72. Krishnamoorthy, B., et al., *Glioma cells deficient in urokinase plaminogen activator receptor expression are susceptible to tumor necrosis factor-alpha-related apoptosis-inducing ligand-induced apoptosis.* Clin Cancer Res, 2001. **7**(12): p. 4195-201.
73. Dimarco, A., et al., *DAUNOMYCIN: A NEW ANTIBIOTIC WITH ANTITUMOR ACTIVITY.* Cancer Chemother Rep, 1964. **38**: p. 31-8.
74. Fornari, F.A., et al., *Interference by doxorubicin with DNA unwinding in MCF-7 breast tumor cells.* Mol Pharmacol, 1994. **45**(4): p. 649-56.
75. Oh, Y., et al., *Regulation in the targeting of TRAIL receptor 1 to cell surface via GODZ for TRAIL sensitivity in tumor cells.* Cell Death Differ, 2012. **19**(7): p. 1196-207.
76. Yip, K.W., et al., *Benzethonium chloride: a novel anticancer agent identified by using a cell-based small-molecule screen.* Clin Cancer Res, 2006. **12**(18): p. 5557-69.
77. Huczynski, A., *Polyether ionophores-promising bioactive molecules for cancer therapy.* Bioorganic & Medicinal Chemistry Letters, 2012. **22**(23): p. 7002-7010.
78. Yoon, M.J., et al., *Monensin, a polyether ionophore antibiotic, overcomes TRAIL resistance in glioma cells via endoplasmic reticulum stress, DR5 upregulation and c-FLIP downregulation.* Carcinogenesis, 2013. **34**(8): p. 1918-28.
79. Pommier, Y., et al., *DNA topoisomerases and their poisoning by anticancer and antibacterial drugs.* Chem Biol, 2010. **17**(5): p. 421-33.
80. Guo, L., et al., *TRAIL and doxorubicin combination enhances anti-glioblastoma effect based on passive tumor targeting of liposomes.* J Control Release, 2011. **154**(1): p. 93-102.
81. La Ferla-Bruhl, K., et al., *NF-kappaB-independent sensitization of glioblastoma cells for TRAIL-induced apoptosis by proteasome inhibition.* Oncogene, 2007. **26**(4): p. 571-82.

82. Doughty-Shenton, D., et al., *Pharmacological targeting of the mitochondrial phosphatase PTPMT1.* J Pharmacol Exp Ther, 2010. **333**(2): p. 584-92.
83. Jin, Y., et al., *Antineoplastic mechanisms of niclosamide in acute myelogenous leukemia stem cells: inactivation of the NF-kappaB pathway and generation of reactive oxygen species.* Cancer Res, 2010. **70**(6): p. 2516-27.
84. Chen, M., et al., *The anti-helminthic niclosamide inhibits Wnt/Frizzled1 signaling.* Biochemistry, 2009. **48**(43): p. 10267-74.
85. Ehsanian, R., C. Van Waes, and S.M. Feller, *Beyond DNA binding - a review of the potential mechanisms mediating quinacrine's therapeutic activities in parasitic infections, inflammation, and cancers.* Cell Communication and Signaling, 2011. **9**: p. 18.
86. Wang, W., et al., *Quinacrine sensitizes hepatocellular carcinoma cells to TRAIL and chemotherapeutic agents.* Cancer Biol Ther, 2011. **12**(3): p. 229-38.
87. Yu, Z., et al., *Allosteric modulators of the hERG K(+) channel: radioligand binding assays reveal allosteric characteristics of dofetilide analogs.* Toxicol Appl Pharmacol, 2014. **274**(1): p. 78-86.
88. Hamed, H.A., et al., *Sorafenib and HDAC inhibitors synergize with TRAIL to kill tumor cells.* J Cell Physiol, 2013. **228**(10): p. 1996-2005.
89. Yaseen, A., et al., *Resveratrol sensitizes acute myelogenous leukemia cells to histone deacetylase inhibitors through reactive oxygen species-mediated activation of the extrinsic apoptotic pathway.* Mol Pharmacol, 2012. **82**(6): p. 1030-41.
90. Kim, D.R., et al., *Combination of vorinostat and adenovirus-TRAIL exhibits a synergistic antitumor effect by increasing transduction and transcription of TRAIL in lung cancer cells.* Cancer Gene Ther, 2011. **18**(7): p. 467-77.
91. Morales, J.C., et al., *HDAC inhibitors with different gene regulation activities depend on the mitochondrial pathway for the sensitization of leukemic T cells to TRAIL-induced apoptosis.* Cancer Lett, 2010. **297**(1): p. 91-100.
92. Kollmannsberger, C., et al., *Topotecan - A novel topoisomerase I inhibitor: pharmacology and clinical experience.* Oncology, 1999. **56**(1): p. 1-12.
93. Petrucci, E., et al., *A small molecule SMAC mimic LBW242 potentiates TRAIL- and anticancer drug-mediated cell death of ovarian cancer cells.* PLoS One, 2012. **7**(4): p. e35073.
94. Griffith, T.S. and T.J. Kemp, *The topoisomerase I inhibitor topotecan increases the sensitivity of prostate tumor cells to TRAIL/Apo-2L-induced apoptosis.* Cancer Chemother Pharmacol, 2003. **52**(3): p. 175-84.
95. Ciusani, E., et al., *In vitro effects of topotecan and ionizing radiation on TRAIL/Apo2L-mediated apoptosis in malignant glioma.* J Neurooncol, 2005. **71**(1): p. 19-25.
96. Borodovsky, A., et al., *5-azacytidine reduces methylation, promotes differentiation and induces tumor regression in a patient-derived IDH1 mutant glioma xenograft.* Oncotarget, 2013. **4**(10): p. 1737-1747.
97. Venturelli, S., et al., *Dual antitumour effect of 5-azacytidine by inducing a breakdown of resistance-mediating factors and epigenetic modulation.* Gut, 2011. **60**(2): p. 156-65.
98. Fox, E.J., *Mechanism of action of mitoxantrone.* Neurology, 2004. **63**(12 Suppl 6): p. S15-8.
99. Taylor, D.J., et al., *Parallel screening of FDA-approved antineoplastic drugs for identifying sensitizers of TRAIL-induced apoptosis in cancer cells.* BMC Cancer, 2011. **11**: p. 470.

100. Loebinger, M.R., et al., *TRAIL-expressing mesenchymal stem cells kill the putative cancer stem cell population.* Br J Cancer, 2010. **103**(11): p. 1692-7.
101. Luzzio, M.J., et al., *Synthesis and antitumor activity of novel water soluble derivatives of camptothecin as specific inhibitors of topoisomerase I.* J Med Chem, 1995. **38**(3): p. 395-401.
102. Christen, M.O., *Action of pinaverium bromide, a calcium-antagonist, on gastrointestinal motility disorders.* Gen Pharmacol, 1990. **21**(6): p. 821-5.
103. Ravi Kumar, A. and P.A. Kurup, *Digoxin and membrane sodium potassium ATPase inhibition in cardiovascular disease.* Indian Heart J, 2000. **52**(3): p. 315-8.
104. Bielawski, K., K. Winnicka, and A. Bielawska, *Inhibition of DNA topoisomerases I and II, and growth inhibition of breast cancer MCF-7 cells by ouabain, digoxin and proscillaridin A.* Biol Pharm Bull, 2006. **29**(7): p. 1493-7.
105. Badr, C.E., et al., *Lanatoside C sensitizes glioblastoma cells to tumor necrosis factor-related apoptosis-inducing ligand and induces an alternative cell death pathway.* Neuro Oncol, 2011. **13**(11): p. 1213-24.
106. Contreras, R.G., et al., *Ouabain binding to Na+,K+-ATPase relaxes cell attachment and sends a specific signal (NACos) to the nucleus.* J Membr Biol, 2004. **198**(3): p. 147-58.
107. Perotti, V., et al., *NFATc2 is a potential therapeutic target in human melanoma.* J Invest Dermatol, 2012. **132**(11): p. 2652-60.
108. Xu, W., et al., *The Antihelmintic Drug Pyrvinium Pamoate Targets Aggressive Breast Cancer.* PLoS ONE, 2013. **8**(8): p. e71508.
109. Ishii, I., Y. Harada, and T. Kasahara, *Reprofiling a classical anthelmintic, pyrvinium pamoate, as an anti-cancer drug targeting mitochondrial respiration.* Front Oncol, 2012. **2**: p. 137.
110. Chan, C.Y., et al., *Inhibitors of V-ATPase proton transport reveal uncoupling functions of tether linking cytosolic and membrane domains of V0 subunit a (Vph1p).* J Biol Chem, 2012. **287**(13): p. 10236-50.
111. Cory, S. and J.M. Adams, *The Bcl2 family: regulators of the cellular life-or-death switch.* Nat Rev Cancer, 2002. **2**(9): p. 647-56.
112. Prassas, I. and E.P. Diamandis, *Novel therapeutic applications of cardiac glycosides.* Nat Rev Drug Discov, 2008. **7**(11): p. 926-35.
113. Hyman, B.T. and J. Yuan, *Apoptotic and non-apoptotic roles of caspases in neuronal physiology and pathophysiology.* Nat Rev Neurosci, 2012. **13**(6): p. 395-406.
114. Pennarun, B., et al., *Targeting FLIP and Mcl-1 using a combination of aspirin and sorafenib sensitizes colon cancer cells to TRAIL.* J Pathol, 2013. **229**(3): p. 410-21.
115. Newman, R.A., et al., *Cardiac glycosides as novel cancer therapeutic agents.* Mol Interv, 2008. **8**(1): p. 36-49.
116. Bezecny, P., *Histone deacetylase inhibitors in glioblastoma: pre-clinical and clinical experience.* Med Oncol, 2014. **31**(6): p. 985.
117. Chinnaiyan, P., et al., *Phase I trial of vorinostat combined with bevacizumab and CPT-11 in recurrent glioblastoma.* Neuro Oncol, 2012. **14**(1): p. 93-100.
118. Asklund, T., et al., *Synergistic killing of glioblastoma stem-like cells by bortezomib and HDAC inhibitors.* Anticancer Res, 2012. **32**(7): p. 2407-13.
119. Huxtable, R.J., *The erroneous pharmacology of a cat.* Mol Interv, 2001. **1**(2): p. 75-7.
120. Langenhan, J.M., et al., *Enhancing the anticancer properties of cardiac glycosides by neoglycorandomization.* Proc Natl Acad Sci U S A, 2005. **102**(35): p. 12305-10.

121. Haux, J., *Digitoxin is a potential anticancer agent for several types of cancer.* Med Hypotheses, 1999. **53**(6): p. 543-8.
122. Mijatovic, T., F. Dufrasne, and R. Kiss, *Cardiotonic steroids-mediated targeting of the Na(+)/K(+)-ATPase to combat chemoresistant cancers.* Curr Med Chem, 2012. **19**(5): p. 627-46.
123. Xie, Z. and T. Cai, *Na+-K+--ATPase-mediated signal transduction: from protein interaction to cellular function.* Mol Interv, 2003. **3**(3): p. 157-68.
124. Sreenivasan, Y., A. Sarkar, and S.K. Manna, *Oleandrin suppresses activation of nuclear transcription factor-kappa B and activator protein-1 and potentiates apoptosis induced by ceramide.* Biochem Pharmacol, 2003. **66**(11): p. 2223-39.
125. Manna, S.K., et al., *Oleandrin suppresses activation of nuclear transcription factor-kappaB, activator protein-1, and c-Jun NH2-terminal kinase.* Cancer Res, 2000. **60**(14): p. 3838-47.
126. Sreenivasan, Y., P.B. Raghavendra, and S.K. Manna, *Oleandrin-mediated expression of Fas potentiates apoptosis in tumor cells.* J Clin Immunol, 2006. **26**(4): p. 308-22.
127. Frese, S., et al., *Cardiac glycosides initiate Apo2L/TRAIL-induced apoptosis in non-small cell lung cancer cells by up-regulation of death receptors 4 and 5.* Cancer Res, 2006. **66**(11): p. 5867-74.
128. Newman, R.A., et al., *Oleandrin-mediated oxidative stress in human melanoma cells.* J Exp Ther Oncol, 2006. **5**(3): p. 167-81.
129. Hollman, A., *Digoxin comes from Digitalis lanata*. Vol. 312. 1996. 912.

Vita

Filiz Şenbabaoğlu was born in Istanbul, Turkey in 1989. After graduating from Darüşşafaka High School, she received her Bachelor of Science Degree in Biology from Middle East Technical University. From 2012 to 2014, she attended Bio-medical Sciences and Engineering M.Sc. program at Koç University where she has worked as a research and teaching assistant.