BIOINFORMATION
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Volume 6(3)

In silico studies on marine actinomycetes as potential inhibitors for Glioblastoma multiforme

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Received December 28, 2010; Accepted February 21, 2011; Published April 22, 2011

Abstract:
Glioblastoma multiforme (GBM) is considered to be the most common and often deadly disorder which affects the brain. It is caused by the over expression of proteins such as ephrin type-A receptor 2 (EphA2), epidermal growth factor receptor (EGFR) and EGFRvIII. These 3 proteins are considered to be the potential therapeutic targets for GBM. Among these, EphA2 is reported to be over-expressed in ~90% of GBM. Herein we selected 35 compounds from marine actinomycetes, 5 *in vitro* and *in vivo* studied drug candidates and 4 commercially available drugs for GBM which were identified from literature and analysed by using comparative docking studies. Based on the glide scores and other *in silico* parameters available in Schrödinger, two selected marine actinomycetes compounds which include *Tetracenomycin D* and *Chartreusin* exhibited better binding energy among all the compounds studied in comparative docking. In this study we have demonstrated the inhibition of the 3 selected targets by the two bioactive compounds from marine actinomycetes through *in-silico* docking studies. Furthermore molecular dynamics simulation were also been performed to check the stability and the amino acids interacted with the 3 molecular targets (EphA2 receptor, EGFR, EGFRvIII) for GBM. Our results suggest that *Tetracinomycin D* and *Chartreusin* are the novel and potential inhibitor for the treatment of GBM.

Keywords: Glioblastoma multiforme, EphA2, EGFR, EGFRvIII, docking and molecular dynamics.

Background:
Glioblastoma multiforme (GBM) is the most common and aggressive type of primary brain tumor in humans **[1]**. GBM is a fast-growing type of central nervous system (CNS) tumor arises mainly from glial tissue of the brain and spinal cord. GBM usually occurs in adults and affects the brain more often than the spinal cord **[2]**. It has been proved that 17,000 primary brain tumors diagnosed in the United States each year, out of which approximately 60% are gliomas **[3]**. Gliomas are a group of central nervous system (CNS) neoplasms with various histological characteristics. They are classified into two major groups as astrocytomas and oligodendrogliomas based on their morphological and histological resemblances between malignant and normal cells **[4]**. The most common form of gliomas in human is the astrocytoma, and the most aggressive type is GBM **[5]**.

Molecular markers that are found on tumour cells and are also over-expressed on malignant cells were nearly absent on normal cells which facilitates as attractive drug targets. Along these lines we found ephrin-A2 (EphA2), epidermal growth factor receptor (EGFR) and EGFRvIII are considered to be novel molecular targets for this pathological condition, because it is expressed in high quantities in GBM **[6]**. EphA2 is a type of tyrosine kinase family **[3]** and this receptor is over expressed in various cancers of brain, breast, cervix, colon, oesophagus, head, neck, liver, lungs, ovary and skin **[7-16]**. The EphA2 is believed to be an ultimate target in many cancers **[17]**. It plays a critical role in embryonic patterning, neuronal targeting, and vascular development during normal embryogenesis, cell proliferation and migration **[18]**. Eph receptor tyrosine kinases (Eph RTKs) and their ligands, ephrins are frequently over expressed in a various types of cancer and tumor **[19]**. About fourteen Eph receptor and eight ephrin ligands are involved in the various type of cancer **[20-21]**. *In vitro* study has shown that modifications or a mutation in the EphA2 is responsible for GBM **[22]**.

EGFR is also a member of tyrosine kinase family containing proteins and it plays a major role in signal transduction pathway responsible for cell differentiation and proliferation **[23]**. Over expression of EGFR in GBM has been proven in many *in vitro* and *in vivo* studies **[24]**. It is also been over expressed in 50-60% of gliomas **[25]**. EGFRvIII is over expressed in most 24-67% of glioblastoma patients **[25]**. This is a mutated type of the tyrosine kinase receptor. This is caused by genetic loss of 270 amino acids from the EGFR. This mutated type protein is responsible for GBM disease in humans **[24]**. Multimodality therapy for this disease still remains unsatisfied **[26]**. In this study we adopted cheminformatics-based drug design approach to identify potential inhibitors against GBM. We conducted comparative docking studies using molecular modelling approach against a total of 44 drug-like molecules which includes (i) 35 drug-like molecules from the marine actinomycetes were selected through the available literature **[27]**, (ii) 5 drug-like molecules which are currently under *in vitro* and *in vivo* investigations **[28-31]** and (iii) 4 known commercially available inhibitors **[32-35]** were docked against the 3 molecular targets including EphA2, EGFR and EGFRvIII. From the docking experiments

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data obtained, we identified two potential bioactive drug-like molecules out of 35 ligands based on their better binding energies and pharmacokinetic properties than the other compounds utilized in this study.

Methodology:

Protein preparation of the 3 molecular targets of GBM:

Docking studies were conducted on the three dimensional (3D) structures of the 3 molecular targets including EphA2, EGFR and EGFRvIII (PDB ID: 1MQB, 1M17 and 1I8I) which were obtained from protein data bank **[36]**. Before performing docking, hydrogen atoms and charges were added to these crystal structures of 1MQB, 1M17 and 1I8I and then the complex was submitted to a series of restrained, partial minimizations using the optimized potential for liquid simulations-all atom (OPLS-2005) force field **[37]**. The 3D structures were then processed by use of the 'Protein Preparation module' with the 'preparation and refinement' option before docking. The missing loops in the structures were then filled in the respective protein molecules with the help of Prime, version 2.1 (Schrödinger, LLC, New York, 2009) **[38]**. Hydrogen atoms were added and all unwanted water molecules were removed from the structure. Partial charges were assigned according to OPLS-2005 force field. Charges and atom types were assigned.

Binding site prediction for EGFRvIII:

The binding sites for 1MQB and 1M17 were determined using PDBsum (http://www.ebi.ac.uk/pdbsum/). Since the active for EGFRvIII was not well defined; its binding site was predicted using SiteMap, version 2.3 (Schrödinger, LLC, NewYork, 2009) **[38]**. The SiteMap predicts the binding site in three stages, (i) a grid was assigned, and the points were grouped into sets according to various criteria to define the sites, (ii) the sites were mapped on another grid to produce files for visualization of the maps and (iii) finally, the properties were evaluated and sites has been written in a maestro-readable form. Each stage is accomplished by running an impact job and finally the best site was considered for the further docking study.

Ligand structure Preparation:

The 35 marine actinomycetes compounds which were retrieved from the literature **[27]**, the chemical structures of these molecules were downloaded from PubChem (http://pubchem.ncbi.nlm.nih.gov/), few of these structures are not available in PubChem, hence we used ChemSketch version 11.01 (http://www.acdlabs.com) to draw those structures. The chemical structures of the 5 compounds which are under *in vitro* and *in vivo* investigation along with the 4 commercially available compounds were also downloaded from PubChem and all these ligands were prepared for docking by using LigPrep, version 2.3 **[38]**. The tautomers for each of these ligands were generated and optimized. Partial atomic charges were computed using the OPLS-2005 force field.

Docking using Glide extra precision:

All the ligands which were prepared using LigPrep were then subjected for docking against the 3 molecular targets including EphA2, EGFR and EGFRvIII (PDB ID: 1MQB, 1M17 and 1I8I) using Glide extra-precision (XP), version 5.5 **[38]** mode. The grid-enclosing box was centered to the active sites of the corresponding 3D-structures of these 3 molecular targets to GBM; so as to enclose them within 3 Å from the centroid of these residues. A scaling factor of 1.0 was set to van der Waals (VDW) radii for these residue atoms, with the partial atomic charge less than 0.25. Glide XP mode determines all reasonable conformations for each low-energy conformer in the designated binding site. In the process, torsional degrees of each ligand are relaxed, though the protein conformation is fixed. During the docking process, the glide scoring function (G-score) was used to select the best conformation for each ligand. Final G-scores were analysed based on the conformation at which the ligands formed hydrogen bonds to at least one of the active site amino acid residues of the corresponding 3D-structures of these 3 molecular targets with optimal binding affinity. Herein, the data obtained from these dockings were used to analyse the molecular interactions and also to identify the residues involved in hydrogen bond formation with 1MQB, 1M17, and 1I8I. The glide scores and energies including van der Waals (VDW) and electrostatic were calculated for all the ligands against EphA2, EGFR and EGFRvIII. Finally the molecular interactions and functional role of the two selected marine actinomycetes compounds named and the commercially available drugs were proposed in detail. All these docking procedures were performed on a Dell RHEL 5.0 workstation.

Molecular Dynamics Simulation:

Molecular Dynamics simulations were done with all the 3 molecular targets of GBM against the two selected bioactive compounds. The MD simulation was performed by using Gromacs 3.3.2 **[39, 40]**. It works based on the leap-frog algorithm to integrate Newton equations. The NPT ensemble and Gromos96 force field were applied to the system. Each docking complex was placed in the center of a 72 Å × 72 Å × 72 Å cubic box and solvated by simple point charge water molecules (SPC/E). Na^+ counter ions were added to keep the system electrically neutral and the periodic boundary condition was also applied to the system. Energy minimization was carried out by using steepest-descent method **[41]**. Berendsen temperature and pressure coupling methods were applied to keep the system in stable environment (300 K, 1 Bar), and the coupling constants were set at 0.1 and 1.0 for temperature and pressure respectively. Cut-off method was employed for electrostatic and van der walls interactions; cut-off distance for the short-range neighbor list (rlist) and was set at 0.8, whereas coulomb cut-off (rcoulomb) and VDW cut-off (rvdw) was fixed at 1.4. The LINCS algorithm was used to constraint the bonds **[42]**. The simulation was performed with a time step of 2fs and the coordinates were saved every 1000 steps. 20 ps position restraining dynamics simulation was carried to relieve close contacts and to equilibrate the protein in the medium; finally 1ns molecular dynamics simulation and further analysis were performed. The dynamics results were visualized using VMD **[43]**.

Assessment of drug-like properties of selected optimized ligands:

The selected optimized molecules were studied for their drug-like properties based on Lipinski parameters using QikProp version 3.2 **[38]**, and also the percentage of their human oral absorption was also predicted to determine the toxicity levels, by use of QikProp **[37]**.

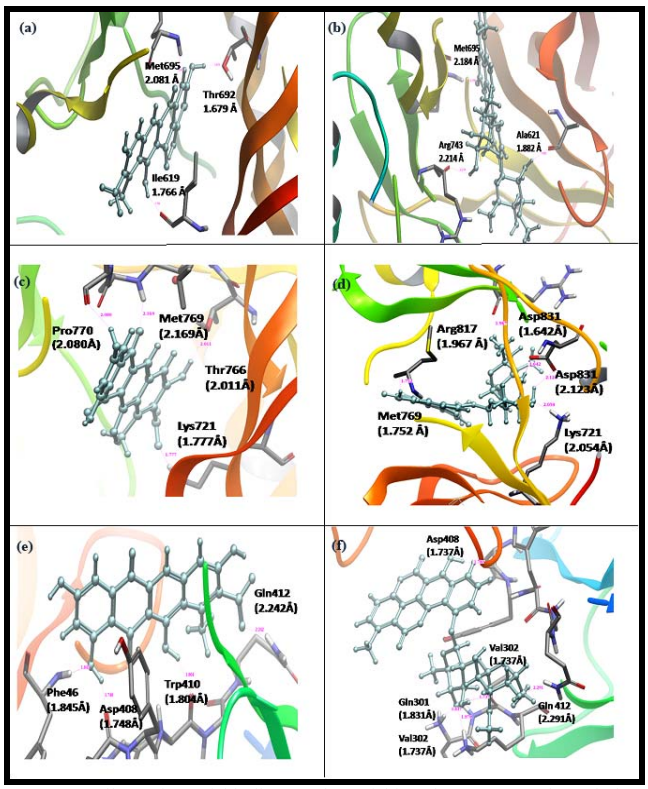

Figure 1: Illustration of binding pocket of bioactive compounds and drug targets a) EphA2 –*Tetracenomycin D*, b) EphA2- *Chartreusin*, c) EGFR-*Tetracenomycin D*, d) EGFR- *Chartreusin*, e) EGFRvIII –*Tetracenomycin D* and f) EGFRvIII- *Chartreusin.* Binding poses of the six lead molecules. The proposed binding modes of the two bioactive compounds with 3 molecular targets of the GBM are shown. The two bioactive compounds are shown in ball and stick display. Critical residues for binding are shown as sticks colored by atom types. Hydrogen bonds are shown as dotted pink lines with the distance between donor and acceptor atoms indicated. Atom type colour code: red for oxygen, blue for nitrogen, grey for carbon and yellow for sulphur atoms respectively. (a) The EphA2 docked with the *Tetracenomycin D.* (b) The EphA2 docked with the *Chartreusin*. (c) The EGFR docked with the *Tetracenomycin D.* (d) The EGFR docked with the *Chartreusin*. (e) The EGFRvIII docked with the *Tetracenomycin D.* (f) The EGFRvIII docked with the *Chartreusin*.

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Results and Discussion:

In this study, we conducted a comparative docking and molecular dynamics simulation between the two selected bioactive molecules that include *Tetracenomycin D* and *Chartreusin* which was obtained from 35 marine actinomycetes compounds along with the 5 compounds derived from the experimental studies and 4 commercially available drugs against GBM. The *in silico* results revealed that the two bioactive molecules exhibited better binding affinity than the commercially available drugs against the 3 molecular targets of GBM including EphA2, EGFR and EGFRvIII.

Binding site analysis for the molecular targets against GBM:

The binding site for the two molecular targets including EphA2 and EGFR (PDB ID: 1MQB, 1M17) are known and were determined using PDBsum. But EGFRvIII (PDB ID: 1I8I) does not have any defined active site and hence it was predicted using SiteMap program in Schrödinger. The predicted amino acids were identified to be Asp408, Gln412, Trp410, Phe46, Gln301, Val302, and Gln412. Docking studies were performed with the two bioactive molecules against the 3 molecular targets based on their corresponding co-crystallized ligands available in their 3D-structures. All binding pockets of protein- ligand complexes were shown in the **Figure 1**.

Analysis of Glide XP and Molecular Dynamics simulation results:

The comparative docking analysis on the 35 marine actinomycetes **[27]**, 5 *in vitro* and *in vivo* **[36-39]** compounds including i) Nimodipine- 3-(2-methoxyethyl) 5-propan-2-yl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, (ii) Gallic acid - 3,4,5-trihydroxybenzoic acid, (iii) Verapamil- (*RS*)-2-(3,4-dimethoxyphenyl)-5-{2-(3,4-dimethoxyphenyl)ethyl-(methyl)amino}-2-prop-2ylpentanenitrile, (iv) Perrilyl alcohol-((4-prop-1-en-2-yl-1-cyclohexenyl) methanol), (v) Gambogic acid and 4 known commercial inhibitors **[40-43]** including Temazolomide, Sunitinib, Carmustine and Thalidomide **(Supplementary Figure 1)** against the 3 molecular target proteins of GBM was performed using Glide XP application. The glide scores for i) 35 selected marine actinomycetes **[27]** drug-like molecules possessed ranged between ~ -8.5 kcal/mol to ~ -2.8 kcal/mol, ii) 5 *in vitro* and *in vivo* compounds **[36-39]** ranged from ~ -7.5 kcal/mol to ~ -3.7 kcal/mol and iii) 4 commercial compounds **[40-43]** ranged from ~ -7.1 kcal/mol to ~ -5.3 kcal/mol. By comparing their respective glide scores and hydrogen bond interactions, it was found that only two compounds including *Tetracenomycin D* and *Chartreusin* **(Figure 2)** out of 35 marine actinomycetes compounds exhibited better binding energies than the other drug-like molecules **(Table 1 see Supplementary material)**. The docking scores of the two bioactive molecules including *Tetracenomycin D* and *Chartreusin* against the EphA2, EGFR and EGFRvIII are shown in **Table 2 (see Supplementary material)**. All hydrogen bond interactions formed in the exterior/hydrophilic portion of the protein, since both the bioactive molecules are polar in nature. This may be due to the electric charge of the compound leading to the electric dipole.

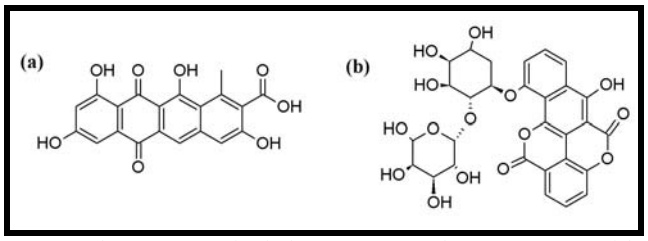

Figure 2: The 2D structure for the best two compounds. **(a)** 2D structure of the Tetracenomycin D **(b)** Chartreusin respectively.

The amino acids Met695 and Thr692 of PDB ID: 1MQB were identified to form hydrogen bonds with its co-crystallized ligand Phosphoaminophosphonic acid-adenylate ester present in the 3D-structure of this protein. Whereas the docking data after simulation with *Tetracenomycin D* and *Chartreusin* revealed that the amino acids Met695, Asn744 and Arg743 formed hydrogen bonds with EphA2. The amino acids Asn744 and Arg743 are the active site residues of 1MQB. Hence it has been observed that the binding of the two active bio molecules against EphA2 can block the active site region of this protein. Whereas for EGFR (PDB ID: 1M17) the amino acid Met769 is involved in hydrogen bonding with the co-crystallized ligand 6, 7-bis(2-methoxy-ethoxy)quinazoline-4-yl]-(3- ethynylphenyl)amine [Erlotinib]. The residues interacted with EGFR with the two bioactive molecules were found to be Met769 which is found to interact with the co-crystallized ligand of 1M17. On the other hand for EGFRvIII (PDB ID: 1I8I) the amino acids interacted and formed hydrogen bonds after simulation are Asp408, Phe46, Val302, Gln301, Lys303, Gln412 and Trp410 out of which none of them are found to interact with the co-crystallized peptide of 1I8I. However these amino acids are found to be in close contact with EGFRvIII. Finally the simulation results revealed that the two bioactive molecules can efficiently block EphA2 than when compared to EGFR and EGFRvIII without much conformational change in the active site after 1ns simulation **(Table 3 see Supplementary material)**. The temperature and pressure does not imply any changes in the conformation of the structure. The hydrogen bonds that have been formed between the protein and ligand after simulation are mostly present in the β-sheets and loop regions of the protein which contains the active site region for the catalysis of the substrate binding. Since they form the hydrogen bonds with by blocking the active site region of the protein, the docking results suggests that the two bioactive molecules could efficiently inhibit the functional activity of the target proteins of GBM.

ADME or pharmacokinetics prediction of the ligands:

Predict pharmacokinetic properties using the QikProp module of the Schrödinger 2009 software. QikProp settings determine which molecules are flagged as being dissimilar to other 95% of the known drugs. Predicted significant ADMET properties such as permeability through MDCK cells (QPlogMDCK), QikProp predicted log IC50 value for blockage of K^+ channels (QPlogHERG), QikProp predicted gut-blood barrier (QPPCaco) and violations of the Lipinski's rule of five (LROF) were reported in **Table 4 (see Supplementary material)**. The number of stars indicates the deviations from the 95% of the known drugs. Percent of Human Oral absorption is based on number of metabolites, number of rotatable bonds, logP, solubility and cell permeability.

In accordance with Lipinski's rule of five, QikProp was used to evaluate the drug-likeness of the lead molecules by assessing their physicochemical properties. Their molecular weights were < 500 Daltons with < 5 hydrogen bond donors, < 10 hydrogen bond acceptors and a log p of < 5 **(Table 4 see Supplementary material)**; these properties are well within the acceptable range of the Lipinski rule for drug-like molecules. These compounds were further evaluated for their drug-like behavior through analysis of pharmacokinetic parameters required for absorption, distribution, metabolism, excretion and toxicity (ADMET) by use of QikProp. For the two bioactive compounds, the partition coefficient (QPlogPo/w) and water solubility (QPlogS), critical for estimation of absorption and distribution of drugs within the body, ranged between ~ 0.7 and ~ 2043, cell permeability (QPP_{Caco}), a key factor governing drug metabolism and its access to biological membranes, ranged from 0.004 to 2050, while the bioavailability and toxicity were from ~ 3.4 to ~ 0.4. Overall, the percentage human oral absorption for the compounds ranged from ~ 36 to ~ 79%. All these pharmacokinetic parameters are within the acceptable range defined for human use **(Table 4 see Supplementary material)**, thereby indicating the selected two drug-like compounds their potential as drug- like molecules could be a potential inhibitor of therapeutic targets of GBM disease and further analysis can be performed through various experimental studies. Among various commercially available drugs against GBM, the best two bio active compounds from marine actinomycetes have good interactions with the GBM targets. *Tetracenomycin D* and *Chartreusin* have also been confirmed in both *in vitro* and *in vivo* studies for the different cancer treatments **[27]**. ADMET properties of these two compounds are under acceptable range. So, these drugs can be a potential inhibitor of therapeutic targets of GBM disease and further analysis can be performed through various experimental studies.

Conclusion:

In the present study we have performed comparative docking analysis of various compounds using Glide and the results are interpreted. EphA2 was identified as good target for GBM. For the first time we proposed *in silico* study to identify the potential small molecule inhibitor for EphA2, EGFR, EGFRvIII proteins against GBM.

Acknowledgment:

The authors are grateful to the Department of Bioinformatics, Alagappa University and Department of Obstetrics and Gynecology, Yong Loo Lin School of Medicine, National University of Singapore for providing laboratory assistance.

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Edited by A Cherkosav
Citation: Kirubakaran *et al.* Bioinformation 6(3): 100-106 (2011)
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Supplementary material:

Table 1: Glide scores of the 35 marine actinomycetes, 5 *in vitro* and *in vivo* studied compounds and 4 commercially available compounds docked against EphA2 receptor by using Schrödinger 9.0.

Compound ID[a]	Glide Scores[b]	Amino acids interacted[c]
196730	-8.49826	**Met695 (2.081),** Thr692 (1.679), Ile619 (1.766)
5281394	-8.42768	**Met695 (2.184), Arg743 (2.214),** Ala621 (1.882)
8378	-8.40021	Asn744 (2.035), Glu623 (2.098 and 1.688), Lys778 (1.675), Ala621 (1.801), Gly759 (1.863), Glu663 (1.836)
159572	-8.28561	Met695 (1.714), Glu696 (2.002), Ala699 (2.470), Lys646 (1.720), Asp757 (2.194)
198084	-7.26372	Met695 (1.741), Ile619 (2.107), Lys646 (2.178)
6436271	-7.20094	Ala621 (2.041), Arg743 (2.296), Ser756 (2.138), Lys778 (2.155), Lys646 (2.185 and 2.054)
5282060	-6.8135	Met695 (2.496), Ala621 (1.892), Lys646 (1.727),
656677	-6.75792	Arg743 (1.944 and 2.234), Asn744 (1.772), Asp754 (1.962), Glu663 (3.170)
Aureoverticlluatum*	-6.55117	Lys646 (2.258), Arg743 (2.016), Asn744 (1.989), Lys778 (2.462), Asp757 (1.987)
6438330	-6.42631	Arg743 (1.877 and 2.252), Lys646 (1.929), Asp757 (1.903)
44418843	-6.36831	Lys778 (1.881), Asp757 (1.919), Asp739 (2.148), Arg743 (1.969), Ala621 (1.840 and 1.878)
485475	-6.24722	Ile619 (2.134), Ala699 (2.410), Arg743 (2.090), Asn744 (1.591)
11695330	-6.17333	Met695 (2.168), Ile619 (2.161)
Arenicolide_A*	-6.03126	Ala621 (1.956), Arg743 (2.186, 2.160)
Arenamide_A*	-6.02925	Lys646 (2.358), Asn744 (2.090), Asp757 (2.499)
65556	-5.7631	Met695 (2.056), Glu693 (1.876)
Streptopyrrolidine*	-5.69317	Met695 (2.085)
72725	-5.63672	Met695 (1.925), Glu693 (2.336) Thr692 (2.336)
44259	-5.56443	Lys646 (1.751), Ala699 (2.018)
5458191	-5.53807	Arg743 (2.080)
6437838	-5.4544	Met695 (1.902), Glu623 (1.709)
6780	-5.288	Met695 (2.074)
5896	-5.15812	Ala699 (2.139), Ile619 (1.859)
15939615	-5.08984	Met695 (2.066 and 2.065)
4650	-4.48456	Met695 (1.801)
3815	-4.21491	Met695 (2.405)
10341	-3.941	Met695 (2.036)
72519	-2.83629	Ala699 (1.899)
72542	-5.25208	Met695 (2.075), Ala699 (2.182), Ile619 (1.798)
In vivo and in vitro compounds		
370	-7.520973	Met695 (2.172 and 1.921), Lys646 (1.879), Ile619 (2.328)
4497	-6.133939	Met695 (2.499), Lys646 (2.208, 1.708 and 1.824)
2520	-4.633991	Glu623 (2.011), Lys646 (2.317)
5353639	-4.290444	Lys778 (1.776)
369312	-3.731156	Lys646 (1.986)
Commercially available compounds		
5329012	-7.133880	Ile619 (1.972), Lys646 (1.947), Met695 (1.840)
5426	-6.824796	Met695 (2.093 and 1.747)
5394	-6.060448	Met695 (1.95), Glu693 (2.163)
2578	-5.271020	Met695 (1.962 and 1.990), Lys646 (2.415)

* Chemical structures drawn in ChemSketch (PubChem ID's are not available), [a] Ligand IDs from pubchem database, [b] binding energies are calculated using glide scores, [c] residues involved in the Docking against EphA2 receptor {the distance between the amino acid and ligand are calculated in Angstrom (Å)}.

Table 2: Docking results of bioactive compounds with GBM molecular target before simulation.

PDB Id[a]	Compound (Pubchem ID)[b]	Docking Score[c]	Docking Energy[d] (kcal/mol)	Binding residues[e]	Hydrophillic/ Hydrophobic[f]
1MQB (EphA2)	196730	-8.498263	-43.758053	Met695 (2.085), Thr692(1.679) and Ile619(1.766)	Hydrophilic
	5281394	-8.427678	-56.356547	Met695 (2.184), Ala621 (1.882) and Arg743 (2.214).	Hydrophilic
1M17 (EGFR)	196730	-9.383792	-44.072346	Pro770 (2.080), Met769 (2.169), Thr766 (2.011) and Lys721 (1.777).	Hydrophilic
	5281394	-9.532162	-61.889792	Arg817 (1.967), Met769 (1.752), Asp831 (1.692), Asp831 (2.123) and Lys721 (2.054).	Hydrophilic
1I8I (EGFRvIII)	196730	-6.177133	-41.088783	Asp408 (1.748), Phe46 (1.845), Trp410 (1.804) and Gln412 (2.242)	Hydrophilic
	5281394	-8.865244	-55.201336	Asp408 (1.780), Phe46 (2.245), Val302 (1.737), Gln301 (1.837), Lys303 (1.979) and Gln412 (2.291).	Hydrophilic

[a] PDB id's 1MQB- EphA2; 1M17- EGFR; 1I8I- EGFRvIII, [b] Pubchem Compound id's 196730- Tetracenomycin D, 5281394- Chartreusin, [c] The score calculated for the docking, [d] The docking energy calculated in the Kcal/mol, [e] The residues involved in the Docking (The distance between the amino acid and ligands are calculated in Angstrom (Å)), [f] Polarity of the compound.

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Table 3: Docking results of bioactive compounds with GBM molecular targets after simulation.

Pdb Id[a]	Compound (Pubchem Id)[b]	Docking Score[c]	Docking Energy[d] (kcal/mol)	Binding residues[e]	Hydrophillic/ Hydrophobic[f]
1MQB	196730	-5.194349	-40.005452	Met695 (2.053) and Asn744 (2.118)	Hydrophillic
	5281394	-7.849478	-54.326594	Met695 (1.921) and Arg743 (1.764)	Hydrophillic
1M17	196730	-9.582641	-42.135803	Pro770 (1.992), Met769 (2.076), Thr766 (2.091) and Lys721 (1.882)	Hydrophilic
	5281394	-9.22831	-55.401185	Glu780 (2.489), Phe771 (2.268), Asp776 (1.847), Asp776 (1.847), Met769 (1.917) and Met769 (1.894)	Hydrophilic
1I8I	196730	-6.44557	-38.182559	Phe46 (1.960) and Pro44 (2.022)	Hydrophilic
	5281394	-5.152034	-50.618807	Arg55 (2.071), Tyr409 (1.898), Arg398 (2.328), Arg398 (1.682) and Gly326 (2.061)	Hydrophilic

[a] PDB id's 1MQB- EphA2;1M17- EGFR; 1I8I- EGFRvIII, [b] Pubchem Compound id's 196730- *Tetracenomycin D*, 5281394- *Chartreusin*, [c] The score calculated for the docking, [d] The docking energy calculated in the Kcal/mol, [e] The residues involved in the Docking {The distance between the amino acid and ligands are calculated in Angstrom (Å)}, [f] Polarity of the compound.

Table 4: QikProp results of best two marine actinomycetes compounds, commercially available compounds, *in vitro* and *in vivo* studied compounds.

Best two marine actinomycetes compounds					
Compound ID[a]	**QPPMDCK[b]**	**QPlogHERG[c]**	**QPPCaco[d]**	**Stars[e]**	**Rule of Five[f]**
5281394	39.209	-5.807	95.813	2	2
196730	0.748	-2.899	1.968	0	0
Commercially available compounds					
5329012	723.491	-5.595	1421.445	0	0
5426	73.075	-3.897	170.440	0	0
5394	23.845	-3.313	60.479	0	0
2578	2680.532	-2.220	608.912	2	0
***In vitro* and *in vivo* studied compounds**					
370	4.348	-1.396	10.027	0	1
4497	182.626	-4.463	397.739	0	1
2520	499.498	-5.453	918.914	0	0
5353639	25.237	-3.362	51.023	4	2
369312	1787.647	-3.339	3282.329	3	0

[a] Ligand IDs of pubchem database 5281394- Chartreusin, 196730- Tetracenomycin D, 5329012- Sunitinib, 5426- Thalidomide, 5394- Temozolamide, 2578- Carmustine, 370- Gallic acid, 4497- Nimodipine, 2520- Verapamil, 5353639- Gambogic acid, 369312- Perrilyl alcohol. [b] Predicted apparent MDCK cell permeability in nm/sec (acceptable range: < 25 is poor, >500 is high). [c] Predicted IC50 value for blockage of HERG K+ channels.(Concern below -5). [d] Predicted Caco-2 cell permeability in nm/s (acceptable range: < 25 is poor and > 500 is high). [e] Number of property or descriptor values that fall outside the 95% range of similar values for known drugs). [f] Number of violations of Lipinski's rule of five (maximum is 4).

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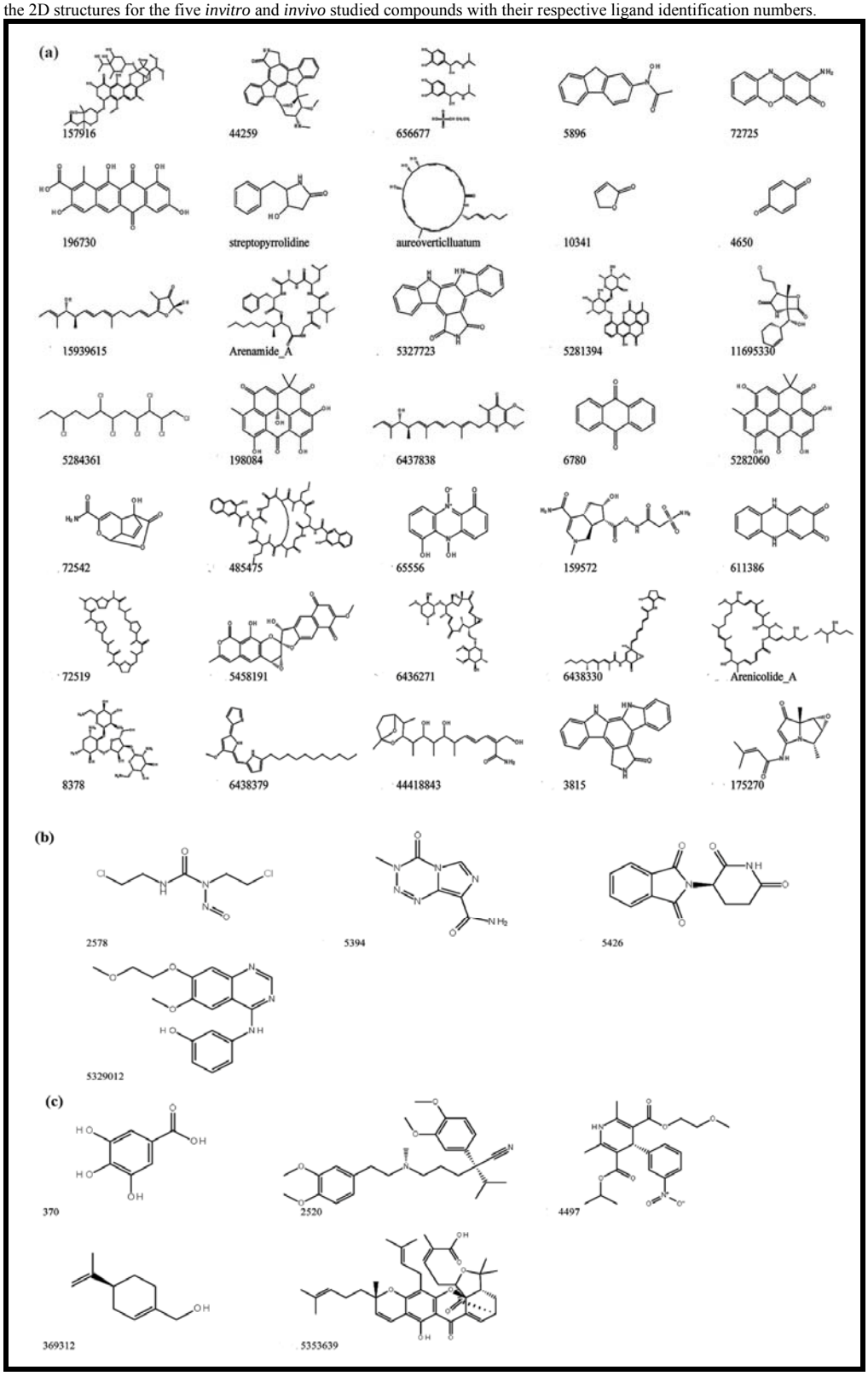
Supplementary Figure 1: The 2D structures for the 35 marine actinomycetes compounds, commercially available compounds, i*n vitro* and *in vivo* studied compounds. **(a)** The 2D structures for the best 35 marine actinomycetes compounds, **(b)** the 2D structures for the four commercially available compounds and **(c)** the 2D structures for the five *invitro* and *invivo* studied compounds with their respective ligand identification numbers.



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