Molecular Docking Based Screening of BRAF for Improved Inhibitors

[a]Rekha Kandula, [b]B.V.Sandeep
[a]Research Scholar, Department of Biotechnology, Andhra University, India
[b]Department of Biotechnology, Andhra University, Visakhapatnam, India

Abstract

B-Raf is a 766 Amino acid protein, regulated signal transduction serine/threonine protein kinase. The structure of B-Raf (PDB ID: 4MNF) with domains CR1 to CR3 is known. Therefore, it is of interest to identify binding sites of B-Raf for inhibiting cell proliferation of late stage melanoma. Therefore, suitable inhibitors for inhibiting B-Raf are identified. Previous reports suggest the use of vemurafenib like compounds for inhibiting B-Raf protein. Our interest is to identify improved compounds to destabilize B-Raf. We used a 14 compounds against B-Raf using molecular docking based screening. This resulted in designing 5 new compounds (MDD,MD1 to MD5) that were further analyzed for pharmacological properties. The hit MD-4 shows fitting binding properties with B-Raf with very less undesirable pharmacological properties such as toxicity but shows immunotoxicity.

KEYWORDS: B-Raf, Melanoma, Molecular docking, Drug discovery

B-Raf is a protein encoded by a proto oncogene B-raf located on human chromosome 7p11-7qt.[1,2] B-raf which is also known as serine/threonine-protein kinase regulates the cytoplasmic MAP kinase/ERK signaling pathway, which affects cell division, differentiation, and secretion.[3] It has three conserved domains conserved region 1 (CR1), a Ras-GTP –binding self-regulatory domain, conserved region 2 (CR2), a serine -rich hinge region, and conserved region 3 (CR3), a catalytic protein kinase domain that phosphorylates concenses sequence on protein substrates.

B-raf gene when mutated leads to the change in the structure of its B-raf protein resulting in the proliferation of cells causing melanoma (eg, cutaneous, acral).

Vemurafenib (Zelboraf), dabrafenib (Tafinlar), and encorafenib (Braftovi) are the inhibitors of B-raf protein(BRAF). These drugs are shrink or slow the growth of metastatic melanoma that has a BRAF gene mutation. Vemurafenib,dabrafenib,encorafenib are synthetic compounds and has side effects like skin reactions, photosensitivity, headache, arthralgia, hyperkeratosis, pyrexia. In this context this paper attempts to vitually screen few Withanolides which are natural compounds isolated from three members of the *Solanaceae*: *Physalis longifolia*, *Vassobia breviflora*, and *Withania somnifera* to be used as inhibitors. The “withanolides” are family of C28 ergostane-type steroidal δ-lactones (derived from a parent 22-hydroxy-26-oic acid). The withanolides studied for docking in this paper is the work of Huaping Zhang et al.[10]

Methodology

Protein and ligand structures

The X-ray crystal structure of 658 amino acids polymer of B-raf is obtained from protein data bank (www.rcsb.org/pdb/home/home.do) with pdb access code of 4MNF .[11]The provided structure is a dimer in complex with inhibitor2-{4-[(1E)-1-(hydroxyimino)-2,3-dihydro-1H-inden-5-yl]-3-(pyridin-4-yl)-1H-pyrazol-1-yl}ethanol . The Withanolide ligands for virtual screening were obtained from the work of Huaping Zhang et al.[10] . Virtual screening was performed on14 ligands and top successive hits were selected for rational drug design purpose.

Virtual screening

PyRX software [12]was used as the virtual screening software. PyRX includes Autodock vina [13]with a Lamarckian genetic algorithm as scoring algorithm.

Pharmacokinetic analysis and rational drug design

SWISSADME server [1] was used to analyze the absorption, distribution, and metabolism properties of all the 14 virtual screening hits. The toxicity properties were analyzed using the PROTOX web server[1] . New ligands were designed based on the structure of top hits retrieved from virtual screening process. New rationally designed ligands were then analyzed regarding ADME and toxicity to reach optimal scores.

Results and Discussion

Among 14 drug-like ligands, the 5 highest binding affinity hits were selected for the further evaluation. These top 5 poses, which indicated more negative binding affinity, were examined for pharmacokinetic analysis and rational drug design purpose. The selected hits reached the binding affinity equal to -8.7 for hit 9, -8.6 for hit 5, 8.2 for hit 3 and 10, -8.1 for hit 2 and 6 respectively. Based on Lipinski rule of 5 the the hits were analysed for their pharmacological properties. In Table 1 the pharmacological properties of these top hits are presented and smiles given in Table 2.

To reach the best inhibitor, active site obtained from Ligsite csc[1] was used as shown in Fig2. The H-bonds formed with different ligands are shown in Table3.

For acquiring the specific binding affinity we tried to design specific ligands, which could tightly bind to active site. To do this, the hit 9 was chosen because of high binding affinity and appropriate molecular weight. Thus, hit 9 initially was modified to reach its optimal pharmacological properties.

Based on hits 17,9,10,5 and 2 various ligands(MDD,MD1,MD2,MD3,MD4 and MD5) were rationally designed. The structurally modifications and substitutions changed the pharmacophore model increasing the binding to -10.5. The final structure of MD-4 in contact with BRAF following its binding pockets is depicted in Figure 1.

In order to calculate the LD50 and probable nonspecific targets, PROTOX webserver was used. PROTOX prediction indicated LD50 of MD4 is 5105 mg/kg with toxicity class 6.

This performance operated in average similarity 51.74%and prediction accuracy of 7.38%. Interestingly Immunotoxic was found by PROTOX. As PROTOX indicated, the MD-4 is a lead compound and can serve as a new drug to inhibit B-Raf.

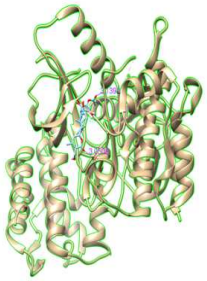

Fig1: 4MNF with MD4

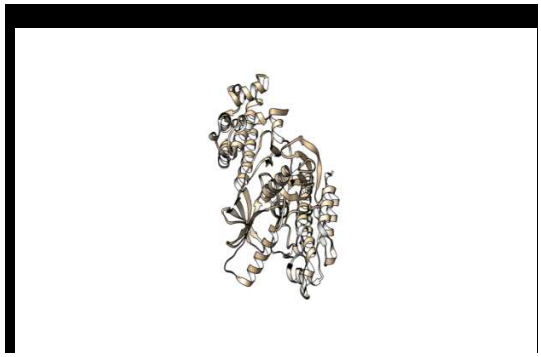

Fig1: 4MNF

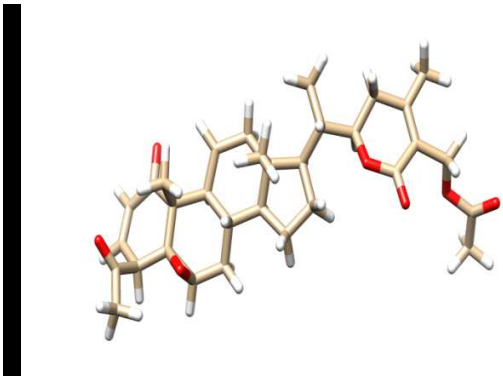

Fig1:Compound 2

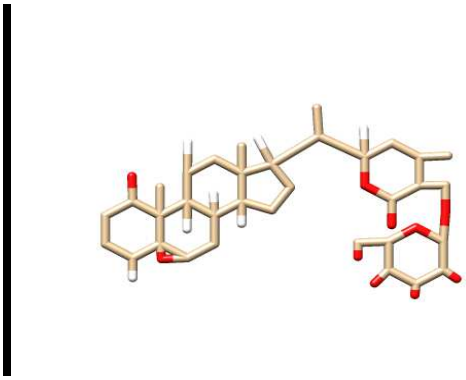

Fig1:Compound 3

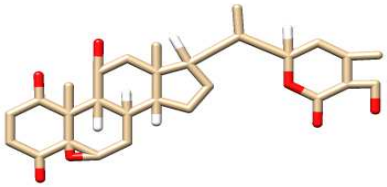

Fig1:Compound 5

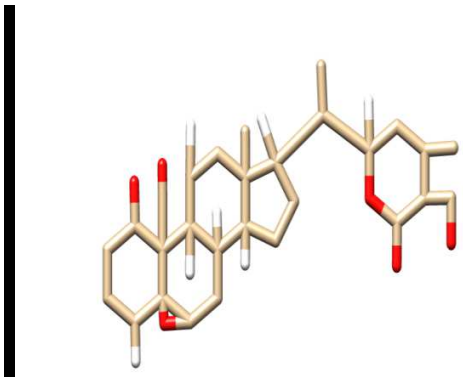

Fig1 :Compound 6

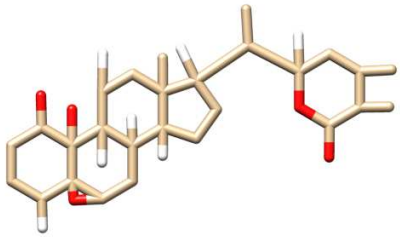

Fig1 :Compound 9

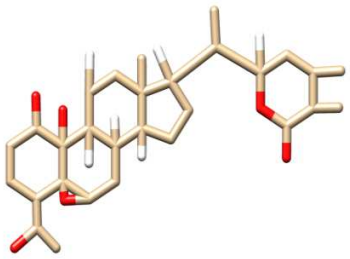

Fig1: Compound 10

Fig 1: MD4

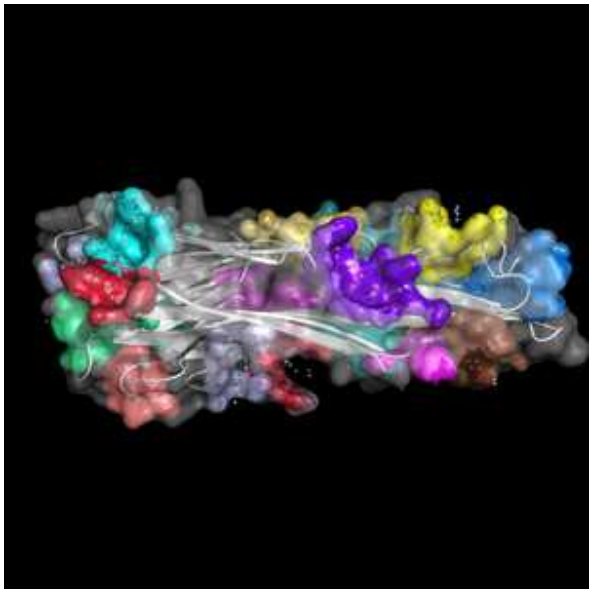

Fig 2: Pockets of 4MNF

S.No	Formula	Molecular weight (g/mol)	Num. heavy atoms	Num. arom. heavy atoms	Num. rotatable bonds	Num. H-bond acceptors	Num. H-bond donors	Total charge	Binding Affinity
1	C28H38O6	470.60	34	0	3	6	2	0	-7.9
2	C32H42O7	538.67	39	0	6	7	0	0	-8.1
3	C34H48O10	616.74	44	0	6	10	4	0	-8.2
5	C28H38O7	486.60	35	0	3	7	3	0	-8.6
6	C28H38O6	470.60	34	0	4	6	2	0	-8.1

7	C32H42O8	554.67	40	0	7	8	1	0	-7.6
8	C33H42O9	582.68	42	0	8	9	0	0	-8.0
9	C27H36O5	440.57	32	0	2	5	1	0	-8.7
10	C29H38O6	482.61	35	0	3	6	1	0	-8.2
11	C31H40O7	524.65	38	0	5	7	0	0	-7.7
16	C29H40O8	516.62	37	0	5	8	3	0	-7.5
17	C29H40O7	500.62	36	0	4	7	2	0	-8.0
21	C38H38O10S	566.66	39	0	5	10	4	0	-7.9
22	C28H38O9S	550.66	38	0	4	9	3	0	-7.8
MDD	C32H38O9	566.64	41	0	6	9	3	0	-8.5
MD1	C29H34O7	494.58	36	0	4	7	3	0	-8.3
MD2	C24H22O9	454.43	33	0	1	9	6	0	-8.8
MD3	C28H32O9	512.55	37	0	5	9	5	0	-7.6
MD4	C29H32O9	524.56	38	0	5	9	4	0	-10.2
MD5	C28H27O8	481.51	35	6	2	8	5	0	-9.4

Table 1: The pharmacological properties of the Withanolide ligands and designed ligands

Table 2: Smiles of the ligands

S.No	smiles
1	[C@]12([C@@]3([C@H](C=CC1=O)O)[C@@H](C[C@@H]1[C@@H]2CC[C@]2([C@H]1CC[C@@H]2[C@@H]([C@H]1CC(=C(C(=O)O1)CO)C)C)C)O3)C
2	[C@]12([C@@]3([C@H](C=CC1=O)C(=O)C)[C@@H](C[C@@H]1[C@@H]2CC[C@]2([C@H]1CC[C@@H]2[C@@H]([C@H]1CC(=C(C(=O)O1)COC(=O)C)C)C)C)O3)C
3	[C@]12([C@@]3(CC=CC1=O)[C@@H](C[C@@H]1[C@@H]2CC[C@]2([C@H]1CC[C@@H]2[C@@H]([C@H]1CC(=C(C(=O)O1)CO[C@H]1[C@@H]([C@H]([C@H]([C@@H](O1)CO)O)O)O)C)C)C)O3)C
5	[C@]12([C@@]3([C@H](C=CC1=O)O)[C@@H](C[C@@H]1[C@@H]2[C@H](C[C@]2([C@H]1CC[C@@H]2[C@@H]([C@H]1CC(=C(C(=O)O1)CO)C)C)C)O)O3)C
6	[C@]12([C@@]3(CC=CC1=O)[C@@H](C[C@@H]1[C@@H]2CC[C@]2([C@H]1CC[C@@H]2[C@@H]([C@H]1CC(=C(C(=O)O1)CO)C)C)C)O3)CO

7	[C@]12([C@@]3([C@H](C=CC1=O)C(=O)C)[C@@H](C[C@@H]1[C@@H]2CC[C@]2([C@H]1CC[C@@H]2[C@@H]([C@H]1CC(=C(C(=O)O1)COC(=O)C)C)C)C)O3)CO
8	[C@]12([C@@]3([C@H](C=CC1=O)C(=O)C)[C@@H](C[C@@H]1[C@@H]2CC[C@]2([C@H]1CC[C@@H]2[C@@H]([C@H]1CC(=C(C(=O)O1)COC(=O)C)C)C)C)O3)OC(=O)C
9	[C@]12([C@@]3(CC=CC1=O)[C@@H](C[C@@H]1[C@@H]2CC[C@]2([C@H]1CC[C@@H]2[C@@H]([C@H]1CC(=C(C(=O)O1)C)C)C)C)O3)O
10	[C@]12([C@@]3([C@H](C=CC1=O)C(=O)C)[C@@H](C[C@@H]1[C@@H]2CC[C@]2([C@H]1CC[C@@H]2[C@@H]([C@H]1CC(=C(C(=O)O1)C)C)C)C)O3)O
11	[C@]12([C@@]3([C@H](C=CC1=O)C(=O)C)[C@@H](C[C@@H]1[C@@H]2CC[C@]2([C@H]1CC[C@@H]2[C@@H]([C@H]1CC(=C(C(=O)O1)C)C)C)C)O3)OC(=O)C
16	[C@]12([C@@]3([C@H](C(=CC1=O)OC)O)[C@@H](C[C@@H]1[C@@H]2CC[C@]2([C@H]1CC[C@@H]2[C@@H]([C@H]1CC(=C(C(=O)O1)CO)C)C)C)O3)CO
17	[C@]12([C@@]3([C@H](C(=CC1=O)OC)O)[C@@H](C[C@@H]1[C@@H]2CC[C@]2([C@H]1CC[C@@H]2[C@@H]([C@H]1CC(=C(C(=O)O1)CO)C)C)C)O3)C
21	[C@]12([C@@]3([C@H](C(=CC1=O)S(=O)(=O)O)O)[C@@H](C[C@@H]1[C@@H]2CC[C@]2([C@H]1CC[C@@H]2[C@@H]([C@H]1CC(=C(C(=O)O1)CO)C)C)C)O3)CO
22	[C@]12([C@@]3([C@H](C(=CC1=O)S(=O)(=O)O)O)[C@@H](C[C@@H]1[C@@H]2CC[C@]2([C@H]1CC[C@@H]2[C@@H]([C@H]1CC(=C(C(=O)O1)CO)C)C)C)O3)C
MDD	C1(=C[C@@H](/C(=C(/C)\O)/[C@]23[C@]1([C@H]1[C@H](C=C2O3)[C@@H]2[C@@](C=C1)([C@@H](C=C2)[C@H]([C@H]1CC(=C(C(=O)O1)COC(=O)C)C)C)C)O)OC)O
MD1	C1(=CC(=C([C@@]23[C@@]1([C@H]1[C@@H](C=C2O3)[C@H]2[C@@](C=C1)([C@H](C=C2)[C@@H]([C@@H]1CC(=C(C(=O)O1)CO)C)C)C)C)O)OC)O
MD2	C1(=CC(=C([C@@]23[C@]1([C@H]1[C@@H](C=C2O3)[C@@H]2[C@@H](C=C1)[C@@H](C=C2)/C(=C\1/CC(=C(C(=O)O1)O)O)/C)O)O)O)O
MD3	C1(=CC(=C([C@]23[C@@]1([C@@H]1[C@H](C=C2O3)[C@H]2[C@](C=C1)([C@H](C=C2)[C@@H]([C@@H]1CC(=C(C(=O)O1)CO)CO)C)C)O)O)OC)O

MD4	C1(=CC(=C([C@@]23[C@]1([C@H]1[C@@H](C=C2O3)[C@H]2[C@](C=C1)([C@H](C=C2)[C@@H]([C@H]1CC(=C(C(=O)O1)CO)C)C)C)O)O)OC(=O)C)O
MD5	C1(=C[C@@H](/C(=C(/C)\O)/[C@@]23[C@H]1[C@H]1C(=C[C@@H]2O3)[C@@H]2[C@@](C=C1)([C@@H](C=C2)[C@H](c1cc(c(c(=O)o1)O)O)C)C)O)O

Table3: H-bonds formed with different ligands in the binding sites of 4MNF.For other ligands there were no H-bonds

S.No of the ligand	Aminoacid of 4MNF to which it forms H-Bonds	H-Bond Length (A˚)
3	Asp-479	2.963
11	Asp-479	2.962
21	Asp-479	2.632
22	His-477	2.235
MD1	Asp-479, Trp- 531	2.217, 3.177
MD2	Cys-532	2.409
MD3	Trp- 531	3.381
MD4	Phe-595 Ser-536	3.139 3.050
MD5	His-477	2.755

Conclusion:

Identification of an B-Raf inhibitor to inhibit the proliferation of meyloma cells is of interest. B-Raf inhibitor named MD-4 was described after identification, modification and design and propose to further consider the fitting properties of the same. It should be noted that further in vitro studies are needed to confirm binding and inhibition of B-Raf.

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