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In-Silico Design Screening, Molecular Docking and Pharmacokinetics Studies of Some Indazole Derivatives as Anti-Cancer Agents

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ABSTRACT:

Cancer is the second leading cause of death worldwide according to the latest Cancer Report by WHO. Multiple routes involving different proteins that control the development of cancer. Tyrosine receptor kinases play an important role in a variety of cellular processes including growth, motility, differentiation, and metabolism. Tyrosine Kinase Inhibitors prevent and hamper the pathways by targeting signaling molecules that are necessary for cell survival. Docking studies are proved to be an essential tool that facilitates the structural diversity of natural products to be harnessed in an organized manner. In this study, we docked ten Tyrosine Kinase inhibitors with PDB ID: 5JFX to identify the potent inhibitor against the enzyme. The predictive binding of several forms of indazole compounds to Tyrosine Receptor Kinase inhibitor was analysed using docking analysis in an in silico model. Among the compounds, TRK-3 and TRK- 9 with significant docking scores may produce significant anti-breast cancer activity and further in-vitro and in-vivo investigations may prove their therapeutic potential.
The study was validated by docking of standard drug Entrectinib. The goal of this work is to use docking and molecular dynamic analysis to investigate the activity of Indazole derivate compounds in inhibiting TRK expression, which plays a key role in cancer cell progression.

Keywords: Cancer, Tyrosine Receptor Kinase, Docking, Indazole, Inhibitors**.**

1. INTRODUCTION

Cancer can be driven by a wide variety of clinically actionable alterations. The late 1990s and 2000s were marked by landmark regulatory approvals of targeted therapies that exploit these dependencies. These included the US Food and Drug Administration (FDA) approval of trastuzumab for HER-2 (human epidermal growth factor receptor) positive breast cancers in 1998, imatinib for Philadelphia chromosome-positive chronic myelogenous leukaemias in 2001, and gefitinib for EGFR- (estimated glomerular filtration rate) mutant lung cancers in 2009.[1]

Tropomyosin receptor kinase A (TrkA) is a type of receptor tyrosine kinase that consists of an extracellular ligand-binding domain, a transmembrane domain and an intracellular domain. The intracellular domain further consists of a juxtamembrane (JM) region, a kinase domain responsible for phosphorylation, and a C-terminal domain [2]. The tropomyosin receptor kinase (TrK) family of receptor tyrosine kinases are of interest as the NTRK genes that encode them are involved in gene fusions identified in a wide range of adult and pediatric tumors. [3]

TRKA, TRKB and TRKC are transmembrane proteins that comprise the TRK receptor family. TRKA is encoded by the NTRK1 gene located on chromosome 1q21-q22 [4]. TRKB is encoded by the NTRK2 gene located on chromosome 9q22.1 [5]. TRKC is encoded by the NTRK3 gene located on chromosome 15q25. [6]

Tropomyosin receptor kinase A (TrkA) is a type of receptor tyrosine kinase that consists of an extracellular ligand-binding domain, a transmembrane domain, and an intra cellular domain. The intracellular domain further consists of a juxta membrane (JM) region, a kinase domain responsible for phosphorylation, and a C-terminal domain. TrkA is activated by binding of the ligand nerve growth factor (NGF) to its extracellular domain, which ultimately causes neurone outgrowth and differentiation.[7]

Tropomyosin receptor kinase B (TrkB) as the highest affinity to the binding of brain-derived neurotrophic factor (BDNF) and NT-4. BDNF is a growth factor that has important roles in the survival and function of neurons in the central nervous system. The binding of BDNF to TrkB receptor causes many intracellular cascades to be activated, which regulate neuronal development and plasticity, long-term potentiation, and apoptosis.[3]

Tropomyosin receptor kinase C (TrkC) is ordinarily activated by binding with NT-3 and has little activation by other ligands. TrkC is mostly expressed by proprioceptive sensory neurons.[8]

Mechanism of action[9]

The tropomyosin receptor kinase (TRK) family of receptor tyrosine kinases are of interest as the NTRK genes that encode them are involved in gene fusions identified in a wide range of adult and paediatric tumours. The normal function and physiology of TRK receptors, the biology behind NTRK gene fusions, the mechanisms by which NTRK gene fusions become oncogenic drivers in cancer, and the incidence and prevalence of NTRK gene fusions in a variety of cancers. When a neurotrophic binds to a TRK receptor, the kinase domain is activated resulting in auto phosphorylation. Auto phosphorylation results in further activation of the kinase domain, leading to activation of three potential signalling cascades. It leads to MAPK (Mitogen Activated Protein Kinase), PI3K (Phosphoinositide 3- Kinase), PLC (Phospholipase C).

The objectives of present study is to design anti-cancer agents by using molecular docking study. Docking can be used to discover novel ligands for a target by screening large virtual compound libraries. Docking can also provide a useful starting point for structure-based ligand optimization or for investigating a ligand's mechanism of action.

2. MATERIALS AND METHODS

Structure Based Designing of Novel Compounds

Some reported indazole-based Trk inhibitors were identified from the literature possessing anticancer activity. The designing strategy using docking simulations of these compounds in ATP-binding sites is discussed to analyze the binding mode in detail. To design new TrK inhibitors, advantage was taken of the availability of the structure of Entrectinib in complexation with TrK enzyme (PDB id: 5KVT), GNF-5837 in complexation with TrK enzyme (PDB id: 3V5Q) and PF-06273340 in complexation with TrK enzyme (PDB id: 5JFX) (see Figure 1, 2, 3). Analysis of this structure suggests the binding orientation of the compound with VAL524, ASP596, LEU516, MET592, ASP 668, LEU657 residues.

Design strategy: [8-18]

Binding orientation of the reported compounds in the docking mode identified common residues of TrK like VAL524, LEU516, ASP596, LEU657, and PHE669. Taking the structures of GNF-5837, PF-06273340 and Entrectinib into consideration, molecular hybridization approach was utilized to design a new scaffold possessing indazole ring system bound to pyrazine and pyridine through an amide linker. As far as the nature of the group R to the phenyl ring is concerned, various functional groups like -F, -Cl, -Br, 1-methylpiperazine, -piperazine, $-CF_3$, p-NO_2, $-CH_3$, $-OCH_3$ were observed to exhibit good interactions with Tropomyosin receptor kinase.

In Silico Evaluation of Physicochemical Properties

Swiss ADME:

This website allows you to compute physicochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties, drug like nature and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery.

Drug Likeness and ADMET Properties

The drug-likeness of the compounds was analysed on the Swiss ADME website (http://swissadme.ch) to calculate different ADMET properties including the number of Lipinski's rule violations.[19]

Lipinski's Rule

Lipinski's rule of five, also known as Pfizer's rule of five, was developed by Christopher A. Lipinski in 1997. It is a traditional method to evaluate the drug-likeness of a compound.[20-22]

The rule basically estimates the pharmacokinetic properties of the drug without predicting its biological activity. "According to Lipinski's rule, a drug-like compound should not violate more than one of the following criteria:

- The molecular weight of less than 500 g/mol,
- A log P value of less than 5 represents its hydrophobicity,
- No more than 5 hydrogen bond donors (HBD), and
- No more than 10 hydrogen bond acceptor (HBA) sites

If more than one parameter violates the rule, the compound may produce poor absorption or permeability.

Molecular Docking Studies:

It is done by Autodock Vina. Molecular docking is a frequently used method in structure based on drug design. In docking, we search for an appropriate binding of the ligand that fits energetically and geometrically to the protein binding site.

Steps in Autodock Vina:
1. Choose the protein target and ligand molecule.
2. Carry out protein and ligand preparation.
3. Select the docking site in protein.
4. Carry out Protein- ligand docking using docking tool.
5. Different ligand poses are generated.

Procedure of Autodock Vina:
PDB (Protein Data Bank) of the template protein (5JFX) used for homology modeling was retrieved from Protein Data Bank powered by RCSB.[23-25]

I Preparation of protein:
During this step, we will create a PDBQT file of our receptor containing only the polar hydrogen atoms as well as partial charges.

II Preparation of Ligands:
ChemSketch (http://www.acdlabs.com/resources/freeware/chemsketch/) was used to draw the two-dimensional structures of the Indazole derivatives. Then, the 2D structure is converted into a 3D structure using the chem3D 18.1 application by performing energy minimization (perform MMFF94 minimization) and saving in PDBQT format.

III Preparation of Grid:
Drag and drop the ligand and protein in autodock → go to the grid → macromolecule → choose protein → select molecules → click on No and ok. Than open grid → go to thr grid box. There are three dimensions (x, y and z) → click on file → output grid dimension file → go to the folder when save protein and ligand PDBQT file → Save the grid file like grid.txt. Now using this grid box, docking will be done. Grid dimensions can move which is depending on where the pocket region. But if you are unsure about the pocket region or docking site is for protein than keep the default dimensions which the auto clear that has predicted.

IV: Configuration File:
For convenience, some command line options can be placed into a configuration file. For example:
receptor =protein.pdbqt
ligand = ligand.pdbqt
center_x = add by using grid file
center_y = add by using grid file
center_z = add by using grid file
size_x = add by using grid file
size_y = add by using grid file
size_z = add by using grid file
energy_range = 4
Exhaustiveness = 8

Exhaustiveness:

With the default (or any given) setting of exhaustiveness, the time spent on the search is already varied heuristically depending on the number of atoms, flexibility, etc. Normally, it does not make sense to spend extra time searching to reduce the probability of not finding the global minimum of the scoring function beyond what is significantly lower than the probability that the minimum is far from the native conformation. However, if you feel that the automatic trade-off made between exhaustiveness and time is inadequate, you can increase the exhaustiveness level. This should increase the time linearly and decrease the probability of not finding the minimum exponentially.

V Output:

By using command prompt, we can generate the log file and output. Poses will be created and also docking score is generated. From that you have to check the affinity of the compounds.

3. RESULT AND DISCUSSION

Structure Based Designing of Novel Compounds

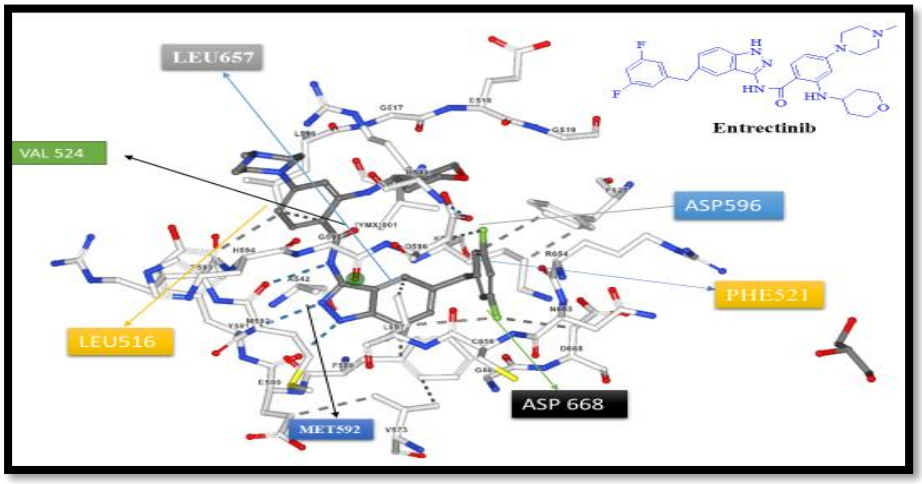


Figure 1. Describes the co-crystal structures of TrKA kinase domain bound to the inhibitor Entrectinib (PDB id: 5KVT). The figure shows the binding orientation of compound with VAL524, ASP596, LEU516, MET592, ASP 668 and LEU657

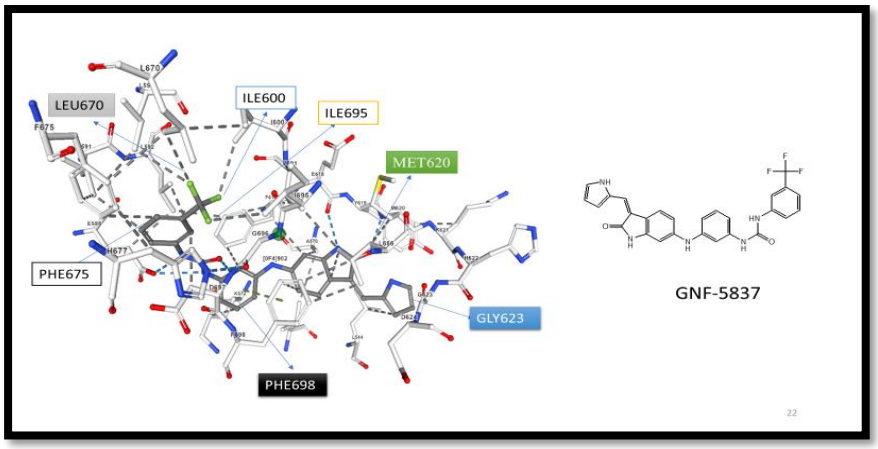


Figure -2. Describes the co-crystal structures of TrKA kinase domain bound to the inhibitor GNF-5837 (PDB id: 3V5Q). The figure shows the binding orientation of compound with GLY623, MET620, ILE695, ILE600, LEU670, PHE675

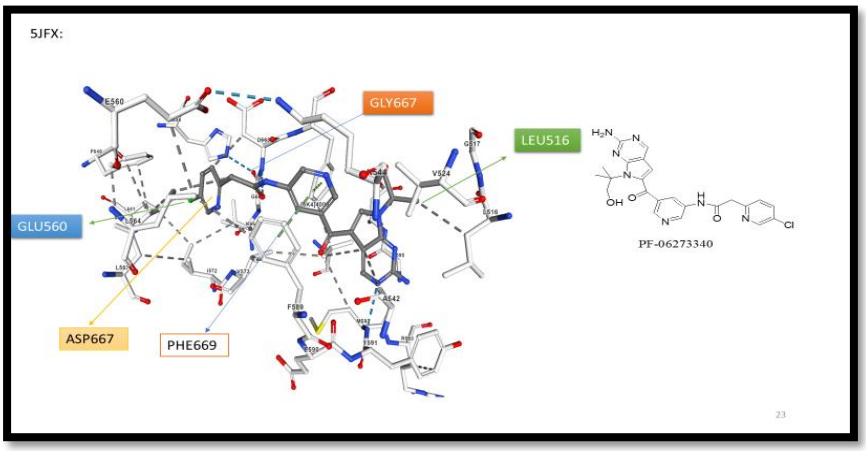


Figure- 3. Describes the Crystal structure of TrkA in complex with PF-06273340 (PDB id: 5JFX). The figure shows the binding orientation of compound with GLY667, PHE669, GLU560, ASP667, have LEU516, GLY667

Design strategy of indazole derivatives:

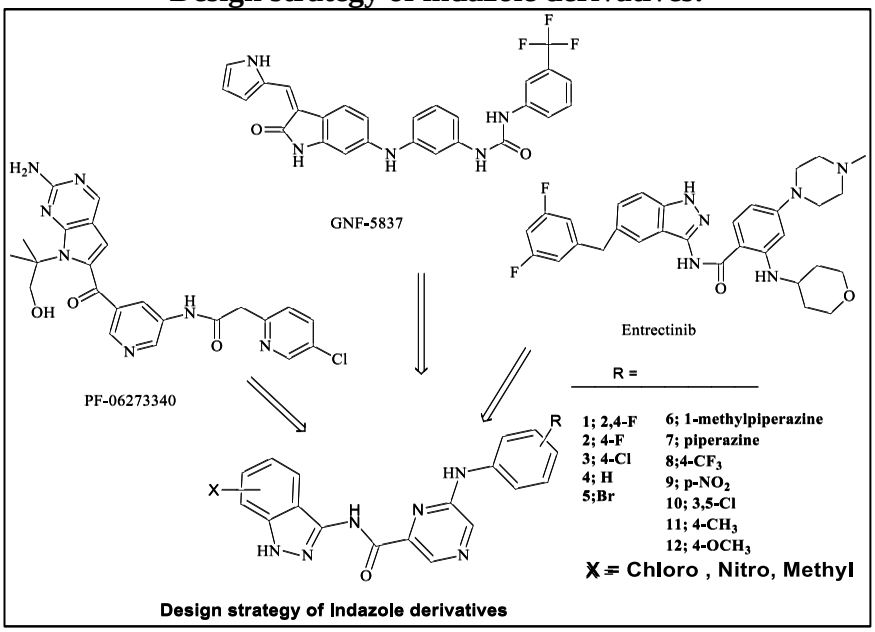
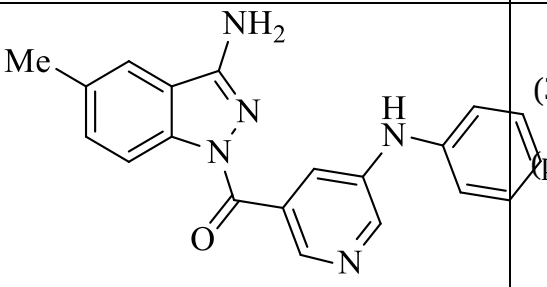
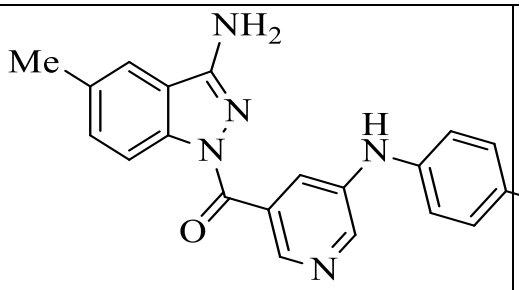
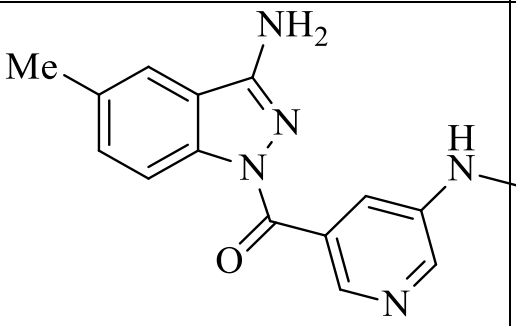
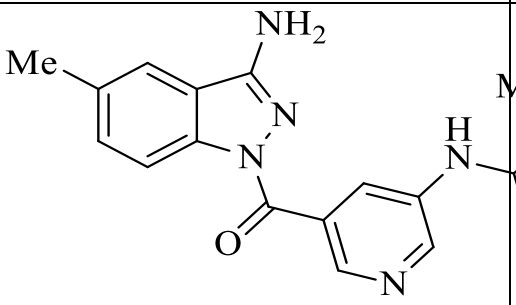
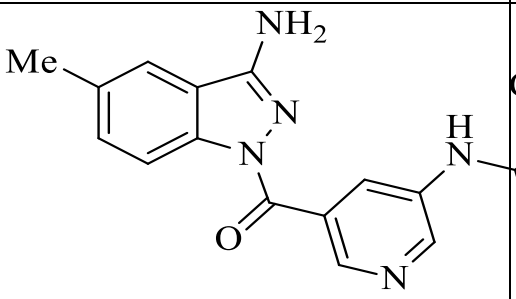
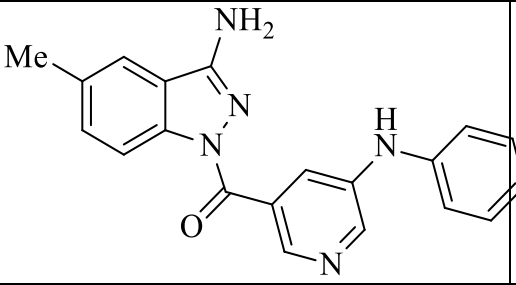
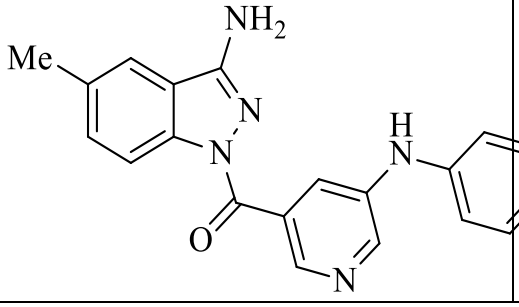


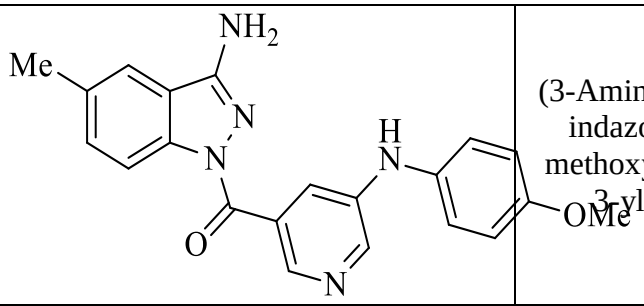
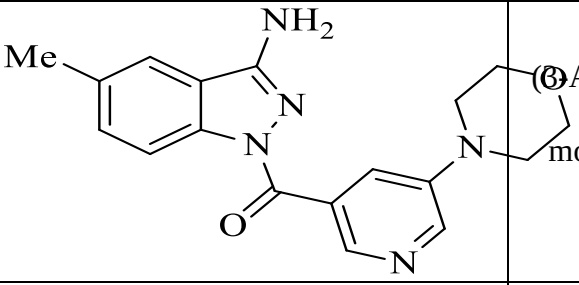
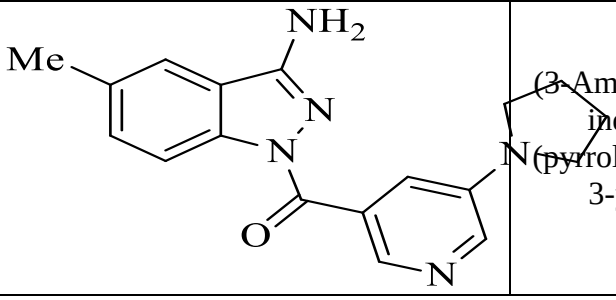
Figure 4. Design strategy of indazole derivatives

Design compounds

Table 1. Design Compounds

Sr. No.	Compound code	Structure	IUPAC name
1	TRK 1		(3-Amino-5-methyl-1*H*-indazol-1-yl)(5-(phenylamino)pyridin-3-yl)methanone

2	TRK 2		(3-Amino-5-methyl-1H-indazol-1-yl)(5-(p-tolylamino)pyridin-3-yl)methanone
3	TRK 3		(3-Amino-5-methyl-1H-indazol-1-yl)(5-(cyclopentylamino) pyridin-3-yl)methanone
4	TRK 4		(3-Amino-5-methyl-1H-indazol-1-yl)(5-(o-tolylamino)pyridin-3-yl)methanone
5	TRK 5		(3-Amino-5-methyl-1H-indazol-1-yl)(5-((2-chlorophenyl)amino)pyridin-3-yl)methanone
6	TRK 6		(3-Amino-5-methyl-1H-indazol-1-yl)(5-((4-(trifluoromethyl)phenyl) amino)pyridin-3-yl)methanone
7	TRK 7		(3-Amino-5-methyl-1H-indazol-1-yl)(5-((4-fluorophenyl)amino)pyridin-3-yl)methanone

8	TRK 8		(3-Amino-5-methyl-1H-indazol-1-yl)(5-((4-methoxyphenyl)amino) 3-yl)methanone
9	TRK 9		(3-Amino-5-methyl-1H-indazol-1-yl)(5-morpholinopyridin-3-yl)methanone
10	TRK 10		(3-Amino-5-methyl-1H-indazol-1-yl)(5-(pyrrolidin-1-yl)pyridin-3-yl)methanone

Swiss ADME: The research work outlined here was conducted with the purpose to forecast the physicochemical attributes, drug likeness, pharmacokinetic/ADME properties, and molecular docking simulations by using in-silico tools. Several physicochemical properties like Molecular weight, H-bond acceptor, H-bond donor, ilogP value and Lipinski rule were listed. These physicochemical properties are given in Table 2 and Pharmacokinetics properties are given in Table 3.

Moreover, through the SWISS ADME web server, the bioavailability radar of some compounds were created as illustrated in Figure: 2 based on six physicochemical characteristics.

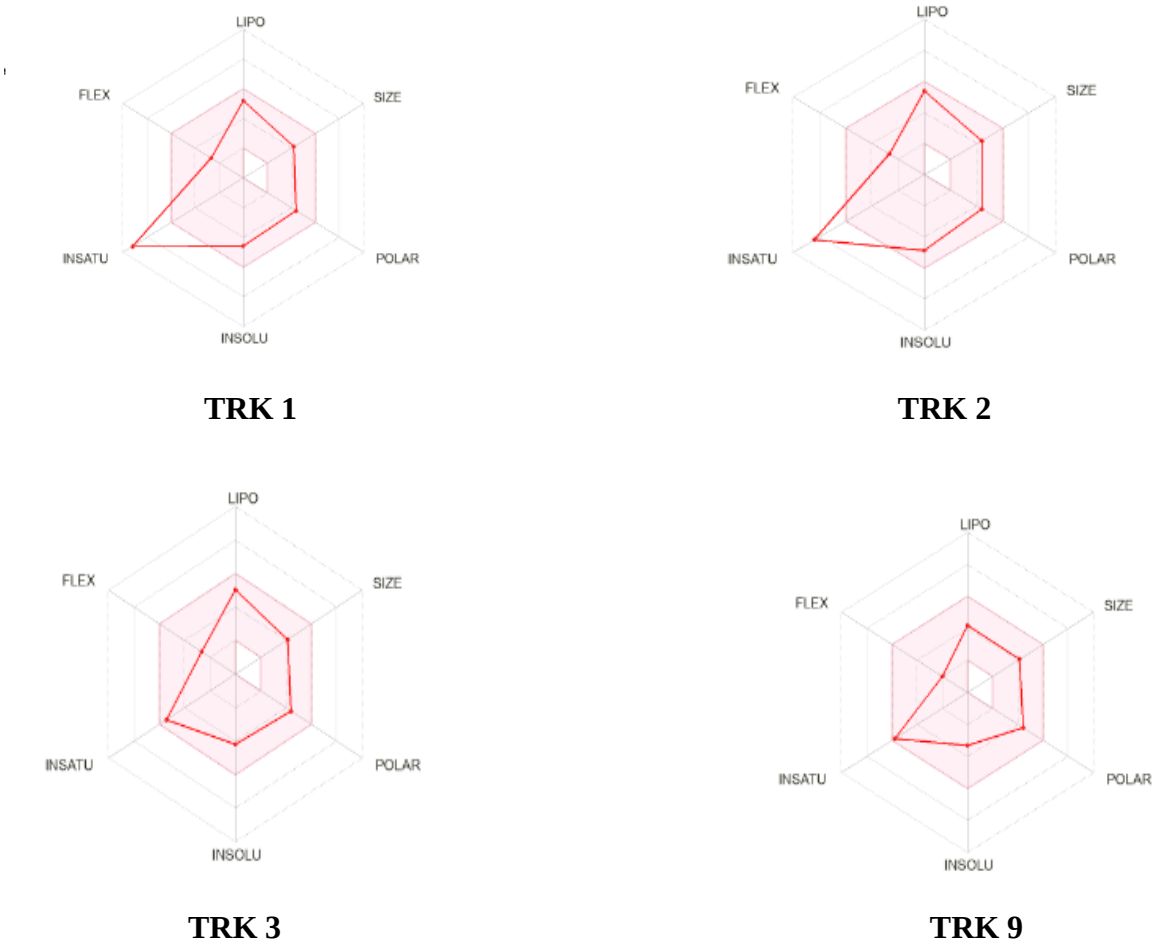


Figure 5. Bioactivity RADAR of some TRK 1,2,3 and 9 (Lipo: Lipophilicity, Flex: Flexibility, Insatu: Instauration, Insol Insolubility, Polar-Polarity, Size)

Table 2. Prediction of Lipinski rule of synthesised compounds

Code	Molecular weight (g/mol)	H-bond acceptor (nON)	H-Bond donor (nOHNH)	iLogP	Lipinski Rule
Standard value	**<500**	**<10**	**<5**	**<5**	**Yes/No**
TRK 1	343.38	3	2	2.59	Yes
TRK 2	357.41	3	2	2.86	Yes
TRK 3	335.40	3	2	2.97	Yes
TRK 4	357.41	3	2	2.95	Yes
TRK 5	377.83	3	2	3.01	Yes
TRK 6	411.38	6	2	2.88	Yes
TRK 7	361.37	4	2	2.71	Yes
TRK 8	373.41	4	2	2.98	Yes
TRK 9	337.38	4	1	2.51	Yes
TRK 10	323.38	3	1	2.70	Yes

Table 3. Pharmacokinetic properties of designed compounds

Code	GI absorption	BBB permeation	Pgp substrate	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
TRK 1	High	No	No	Yes	Yes	Yes	Yes	No
TRK 2	High	No	No	Yes	No	Yes	Yes	No
TRK 3	High	No	Yes	Yes	No	Yes	Yes	No
TRK 4	High	No	No	Yes	No	Yes	Yes	No
TRK 5	High	No	No	Yes	Yes	Yes	Yes	No
TRK 6	High	No	No	Yes	Yes	Yes	Yes	No
TRK 7	High	No	No	Yes	Yes	Yes	Yes	No
TRK 8	High	No	No	Yes	No	Yes	Yes	Yes
TRK 9	High	No	Yes	No	No	Yes	No	No
TRK 10	High	Yes	Yes	Yes	No	Yes	No	No

*Gastro Intestinal absorption, Blood Brain Barrier permeant, P-glycoprotein substrate, CYP1A2: Cytochrome P450 family 1 subfamily A member 2 (PDB:2HI4), CYP2C19: Cytochrome P450 family 2 subfamily C member 19 (PDB:4GQS), CYP2C9: Cytochrome P450 family 2 subfamily C member 9 (PDB:1OG2), CYP2D6: Cytochrome P450 family 2 subfamily D member 6 (PDB:5TFT), h CYP3A4: Cytochrome P450 family 3 subfamily A member 4 (PDB:4K9T)

Molecular Docking Studies: Binding interaction of some selected novel derivatives are mentioned in the figure. The results indicate good binding of the designed molecules with the target enzyme proving their potential for further development.
Describes the binding orientation of TRK compounds with Crystal structure of TrkA. The interaction of PHE 589 (pink), GLU 590 (green), VAL 573 (light pink) , LEU 657 (purple) of receptor(PDB Id: 5JFX). Hydrogen bonds are indicated by green dashed lines to key amino acids and $\pi - \pi$ interaction is indicated by pink dashed lines

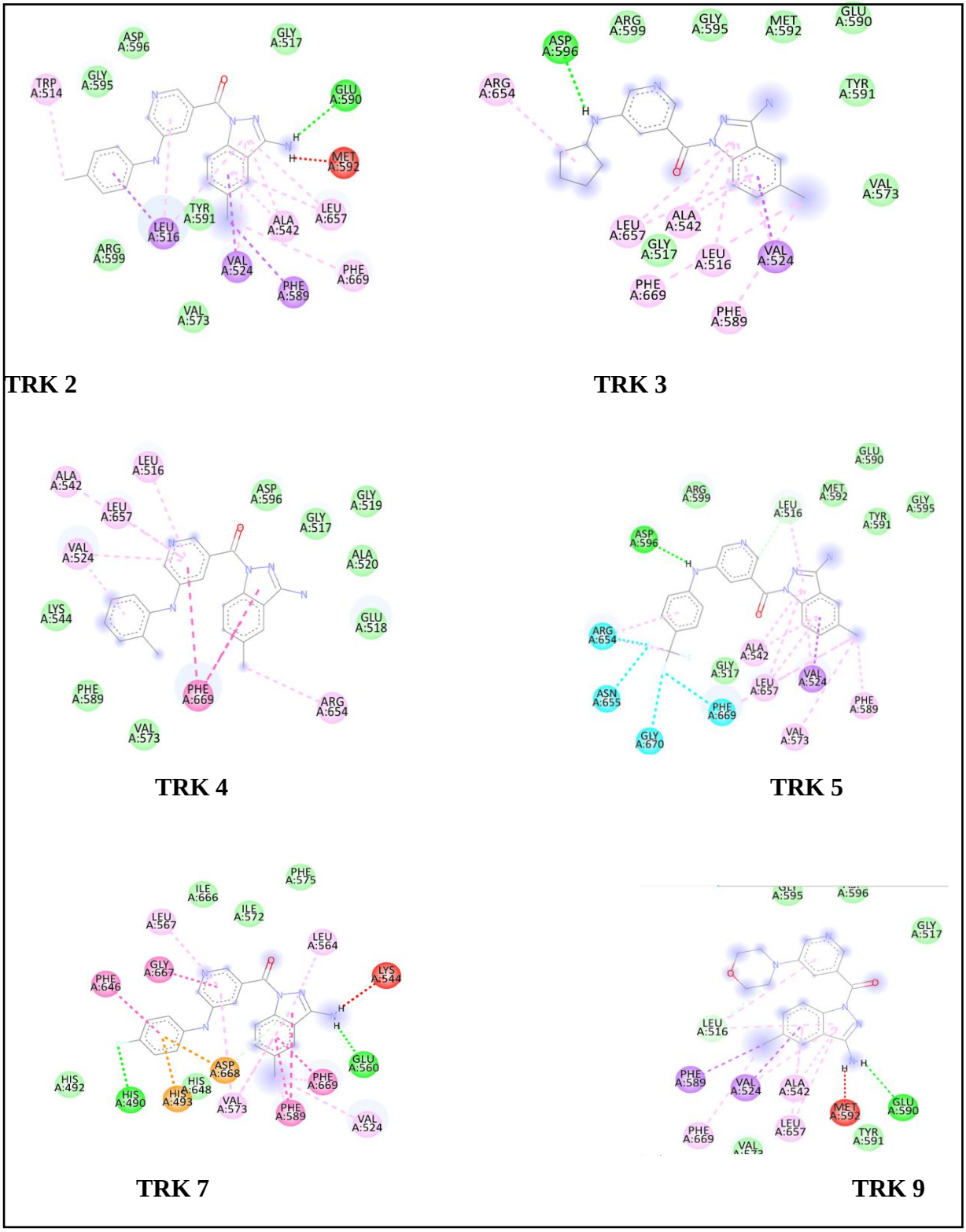


Figure 6. 2D Poses of Ligand Interaction with the Target

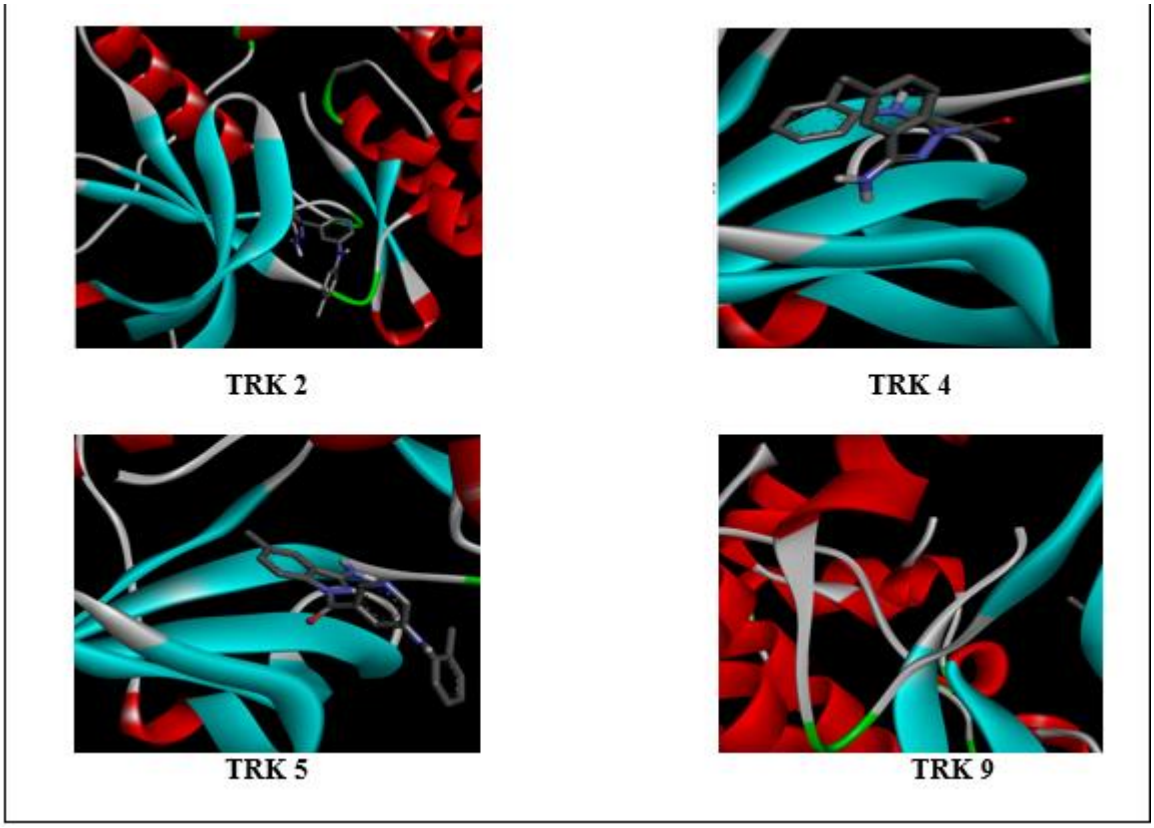


Figure 7. 3D Poses of Ligand Interaction with the Target

Table 4. Binding affinity of designed compounds

Code	Autodock Binding affinity (kcal/mol)
Entrectinib	**-8.4**
TRK 1	-6.3
TRK 2	-7.5
TRK 3	-8.7
TRK 4	-7.3
TRK 5	-8.2
TRK 6	-7.8
TRK 7	-6.8
TRK 8	-6.8
TRK 9	-8.5
TRK 10	-6.5

4. CONCLUSION

Based on docking analysis TRK- 3 and TRK-9 are the best compounds to inhibit Tyrosine Receptor Kinase. Binding affinity of both compounds are similar to the Entrectinib and higher than the other compounds. In order to verify the accuracy of these computer-simulated results, it is essential to conduct experimental studies in a controlled laboratory setting (in vitro) and in living organisms (in vivo).

Acknowledgments:
Parul University provided financial support for this study through the Intramural grant.

Conflict of Interests

The authors declare that there is no conflict of interest.

List of Abbreviations

1. USFDA : United State Food and Drug Administration
2. HER-2 **:** Human Epidermal Growth Factor Receptor
3. EGFR **:** Estimated Glomerular Filtration Rate
4. TRK : Tropomyosin receptor kinase
5. JM : Juxtamembrane
6. NGF : Nerve Growth Factor
7. BDNF : Brain-Derived Neurotrophic Factor
8. MAPK : Mitogen Activated Protein Kinase
9. PI3K : Phosphoinositide 3- Kinase
10. PLC : Phospholipase C
11. ADME : Absorption, Distribution, Metabolism, and Excretion
12. LEU : Leucine
13. VAL : Valine
14. ASP : Aspartic Acid
15. PHE : Phenylalanine
16. LOG P: : Lipophillicity
17. Mol. Wt : Molecular weight
18. HBD : Hydrogen Bond Donors
19. HBA: : Hydrogen Bond Acceptor
20. PDB : Protein Data Bank

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