DESIGN, SYNTHESIS AND IN-SILICO STUDIES OF NEWER 2-(SUBSTITUTED BENZYLIDENE/ETHYLIDENE)-N-(SUBSTITUTED-PHENYL) HYDRAZINE CARBOXAMIDES AND MOLECULAR DOCKING AGAINST EGFR KINASE DOMAIN T790M/L858R AS POTENTIAL ANTICANCER AGENTS.

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Abstract:
Design and studies of newer Hydrazine-carboxamides is reported here. Hydrazine-carboxamides has shown a promising anticancer activity. A series of novel 2-(Substituted Benzylidene/Ethylidene)-N-(Substituted-Phenyl) Hydrazine Carboxamides derivatives were synthesised and were identified by TCL method using suitable solvent system, also they are confirmed by determination of their melting point further their structure were confirmed by Spectral analysis. Novel series of Hydrazine-carboxamides is synthesised using water-ethanol (2:1) solvent system. New Hydrazine-carboxamides analogues were designed using the scaffold hoping technique. All the Hydrazine-carboxamides analogues were studied for their ADME profiles and toxicity studies. All the compounds were found to follow the Lipinski's rule of 5 with safe toxicity profile (Class IV compound) against immunotoxicity, mutagenicity and toxicity. All of the compounds were designed, followed by their molecular docking against EGFR Kinase Domain T790m/L858r (PDB code: 3W2R). One of the appealing cancer targets demonstrated an efficient binding within the binding site of EGFR Kinase. Analogue 4a with docking score = −7.51 kcal/mol shown good binding with the site & can be further explore and evaluate for significant anticancer activity.

Keywords: Anticancer; Hydrazine Carboxamides; Molecular docking; Cell lines studies; EGFR Kinase; T790m/L858r, Anticancer Agents.

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1. Introduction:

Cancer is a genetic disorder. Errors in the process of cells division is one of the major cause of genetic changes causing cancer (1). According to the Global Cancer Observatory (GLOBOCAN) estimates, there were 19.3 million incident cancer cases worldwide for the year 2020 (2). 1,392,179 cases of malignancy were reported in India in year 2020 (3) which is 42% higher than 979,786 cases in the year 2010 (4). The current cancer drug therapy has narrow margin of safety and most of them produces resistance during therapy due to long time therapy and non specific drugs. The statistical and clinical data obtained has drawn the attention of medicinal chemists towards the development of newer target-specific anticancer agents (5, 6). Hydrazine-carboxamide are organic compounds containing the unsaturated group (=C:N.NH.CO.NH_2). When semi carbazide (H_2N.NH.CO.NH_2) reacts with carbonyl groups (C=O) containing aldehydes or ketones Hydrazine-carboxamide are formed (7). Hydrazine-carboxamide have shown anticancer effects and have been classified as protein kinase inhibitors in certain research [8] .Anticonvulsant [9], Antimicrobial [10], cytotoxic [11] etc.

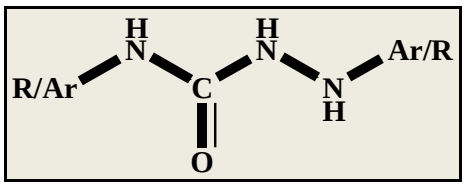


Figure 1: Hydrazine-carboxamide moiety.

2. ADME and Toxicity prediction studies:

In-silico model to estimate oral bioavailability can be used early in the drug discovery process to select the most promising analogue for further Insilico studies, and later on to identify potential molecules for additional clinical development [12]. The Hydrazine-carboxamide derivatives were gone through pre-software studies, drug-likeness, and solubility parameters of drugs and lipophilic character by ALOGPS 2.1 programme and the prediction of ADME was computed from Swiss ADME software [13]. The toxicity studies were carried out online by Pro Tox II software [14].

3. Molecular Docking Studies:

The ligands (4a-d) were investigated for molecular docking against EGFR Kinase domain T790M/L858R mutant with compound 4. The protein data bank (PDB) provided the X-ray crystallographic structure of EGFR Kinase domain T790M/L858R (PDB: 3W2R) with a resolution of 2.05 Å; R value 0.220 (observed) [15]. The ligands (4a-d) saved as mole file, were used to prepare and minimize ligands for docking by using Ligprep, and the docking was carried out in accordance with the procedure outlined elsewhere [16].

4. Hydrazine-carboxamide Scaffold and its substituent's: (Table 1)

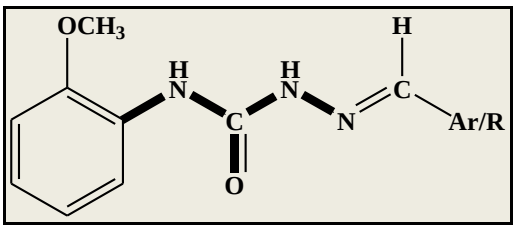


Figure 2: Scaffold of Hydrazine-carboxamide

Table 1: Detail of Hydrazine-carboxamide analogues, substituent's and their IUPAC names.

Compounds	Ar/R	IUPAC Name of benzthiazole
4a	2-hydroxyphenyl-	(E)-1-(2-hydroxybenzylidene)-4-(2-methoxyphenyl) Hydrazine carboxamide
4b	4-hydroxy-3-methoxyphenyl-	(E)-1-(4-hydroxy-3-methoxybenzylidene)-4-(2-methoxyphenyl)hydrazine carboxamide
4c	4-chlorophenyl-	(E)-1-(4-chlorobenzylidene)-4-(2-methoxyphenyl)hydrazine carboxamide
4d	3,4-dimethoxyphenyl-	(E)-1-(3,4-dimethoxybenzylidene)-4-(2-methoxyphenyl)hydrazine carboxamide

5. Result and discussion:

5.1ADME and toxicity prediction for Hydrazine-carboxamide analogues:

The prediction of ADME was computed from Swiss ADME software and their results are given in **Table 2** [8]. Calculated Partition Coefficients and solubility of Hydrazine-carboxamide analogues investigated are as per shown in **Table 3,** whereas biological activity prediction of the Hydrazine-carboxamide analogues by PASS ONLINE software as discussed in **Table 4.** Apart from all the prediction's the toxicity prediction was

also studied for the Hydrazine-carboxamide (4a-d) and their results are given in the **Table 5**. All the compounds were predicted to be class IV compounds in terms of toxicity and poses slight carcinogenicity.

Table 2: ADME studies calculated for good oral response of title HYDRAZINE-CARBOXAMIDE Compounds:

Compound	Volume (A^3)	TPSA (A^2)	NROTB	HBA (<10)	HBD (<5)	Log *P* (iLOGP) (≤5)	MW (<500)	Lipinski's Violations (≤1)
4a	256.06	82.95	6	4	3	2.38	285.3	0
4b	281.61	92.18	7	5	3	2.86	315.32	0
4c	261.58	62.72	6	3	2	2.85	303.74	0
4d	299.14	81.18	8	5	2	3.01	329.35	0

Table 3: Calculated Partition Coefficients and solubility of HYDRAZINE-CARBOXAMIDE analogues investigated.

Compound	ALOGPs	XLOGP2
4a	2.38	2.18
4b	2.86	2.15
4c	2.85	2.86
4d	3.01	2.47

Table 4: Biological activity prediction of the HYDRAZINE-CARBOXAMIDE compounds by PASS ONLINE software:

Compound	Pa	Pi	Biological profile
4a	0,874	0,003	HMGCS2 expression enhancer
	0,746	0,052	Ubiquinol-cytochrome-c reductase inhibitor
	0,606	0,028	Cytoprotectant
4b	0,810	0,005	HMGCS2 expression enhancer
	0,731	0,003	NADPH oxidase inhibitor
	0,722	0,060	Ubiquinol-cytochrome-c reductase inhibitor
4c	0,842	0,004	HMGCS2 expression enhancer
	0,662	0,004	Neuropeptide Y2 antagonist
	0,688	0,073	Ubiquinol-cytochrome-c reductase inhibitor
4d	0,836	0,004	HMGCS2 expression enhancer
	0,630	0,004	Neuropeptide Y2 antagonist
	0,685	0,074	Ubiquinol-cytochrome-c reductase inhibitor

Table 5: Calculated Toxicity Risk Assessment of the HYDRAZINE-CARBOXAMIDE investigated by Pro Tox II Property Explorer.

Compound	Carcinogenicity	Mutagenicity	Cytotoxicity	LD50 (mg/Kg)
4a	-	-	-	1002
4b	-	-	-	1002
4c	-	-	-	1002
4d	-	-	-	1002

(-) INACTIVE, (+) LOW RISK, (++) MEDIUM RISK, (+++) HIGH RISK

5.2Molecular Docking Studies of Hydrazine-Carboxamide Analogues:

We conducted molecular docking experiments for the ligands (4a-d) against EGFR Kinase domain T790M/L858R mutant with compound 4 (PDB code: 3W2R) and their results are shown in **Table 6.**

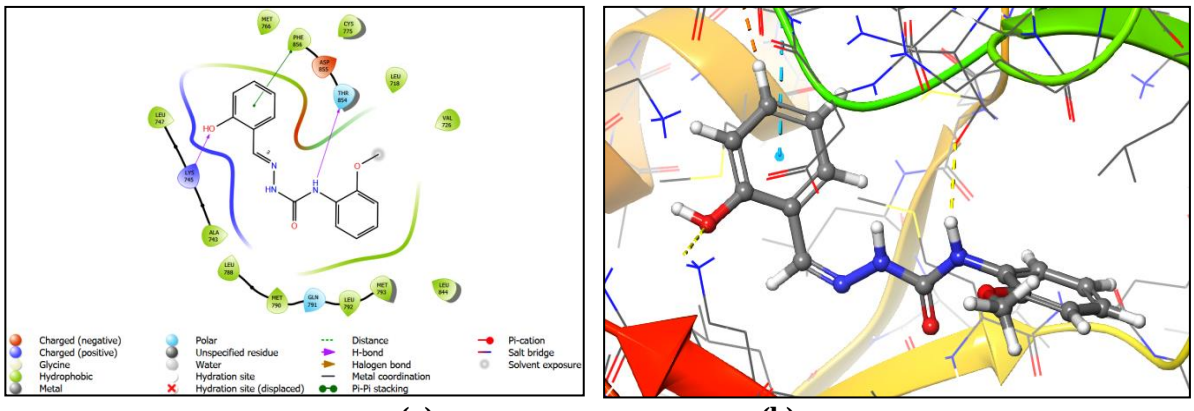
♦ **Table 6: The molecular docking results of Hydrozine-Carboxamide analogues against EGFR (PDB: 3W2R).**

Compound	Docking score	Glide emodel	Types of interactions
4a	-7.51	-64.896	π-π stacking with (PHE856) and H-bond with (THR854 and LYS745)
4b	-6.507	-62.051	π-π stacking and H-bond with (PHE856)
4c	-7.138	-66.029	H-bond with (Asp855) & (Cys775)
4d	-6.611	-58.661	H-bond with (Thr854 through H2O)
Compound 4 (W2R)	-12.699	-152.522	π-π stacking with (PHE856), halogen bond with (LEU788) and H-bond with (GLN791 and LYS745)

• OBSERVATIONS:

The docking was completed in accordance with the procedure with ligands stored as mol files. Molecular docking experiments revealed a variety of interactions, including halogen bonds, π-π stacking, and Hydrogen bonds. The findings of these investigations are as shown in **Table 6.**

- The native ligand **Compound 4 (W2R)** was redocked and with **docking score = −12.699 kcal/mol compound 4** demonstrated **a strong π-π stacking** with the amino acid residue **(PHE856),** an additional **halogen bond** through amino acid residue **(LEU788)**, **Hydrogen bond** through amino acid residue **(GLN791 and LYS745).**
- The ligands 4a to 4d demonstrated an efficient binding within the binding site of EGFR with docking scores ranging from **−6.611** to **−7.51 kcal/mol**.
- The ligands **4a** with highest docking score = **−7.51 kcal/mol** and ligands **4b** (docking score = **−6.507 kcal/mol**) demonstrated ***π-π* stacking** of amino acid residue **(PHE856), 4a** shown H-bond with the amino acid residue **(THR854 and LYS745)** where as **4b** shown H-bond with the amino acid residue **(PHE856)** as shown in Figure 6.3 (a) & 6.4 (a).
- The ligand **4c** (docking score = **−7.138 kcal/mol**) and **4d** (docking score = **−5.914 kcal/mol**) demonstrated interactions with binding region in EGFR through Hydrogen bonding by amino acid residue (**ASP855 & CYS775)** for 4c and with **(THR854)** through H_2O for 4d as shown in Figure 6.4 (b & c).
- The binding pattern of native ligand **compound 4 (W2R)** and our screened derivatives **(4a-d)** during docking demonstrate that the screened derivatives **4a** and **4b** are well accommodated, along with the native ligands **compound 4 (W2R)** within the binding pockets of EGFR through similar type of ***π-π* stacking** and **Hydrogen bonding** by amino acid **(PHE856)** also similar type of **Hydrogen bonding** by amino acid **(LYS745).** From all these types of docking interactions the highlighted analogues **4a** & **4b** are excellent leads for detection of promising anticancer agent through the EGFR kinases suppression.



(a) **(b)**

Figure 6.3: The 2D and 3D Interactions of the native ligand 4a (a) & (b) within the binding site of EGFR Kinase.

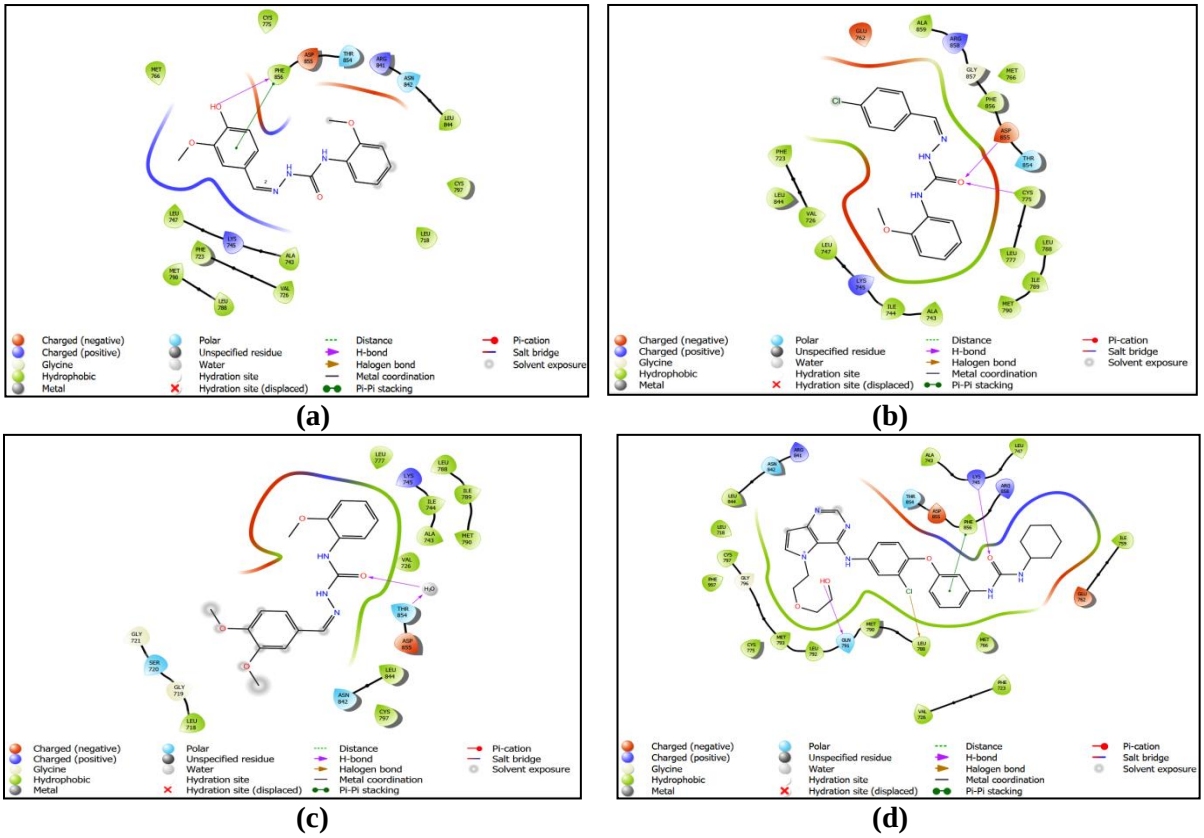


Figure 6.4: The 2D Interactions of the native ligand (a) **4b** (b) **4c** (c) **4d** (d) **native ligand Compound 4** within the binding site of EGFR Kinase.

6. Conclusion:

A series of four new hydrazine carboxamide analogues (4a-d) were prepared. The ADME and toxicity studies were performed. All four analogues followed Lipinski's rule of five with low toxicity. Docking scores for the hydrazine carboxamide ligands ranged from −6.611 to −7.51 kcal/mol. The binding pattern of native ligand **compound 4 (W2R)** and our screened derivatives **(4a-d)** during docking demonstrate that the screened derivatives **4a** and **4b** are well accommodated, along with the native ligands **compound 4 (W2R)** within the binding pockets of EGFR through similar type of **π-π stacking** and **Hydrogen bonding** by amino acid **(PHE856)** also similar type of **Hydrogen bonding** by amino acid **(LYS745).** From all these types of docking interactions the highlighted analogues **4a** & **4b** are excellent leads for detection of promising anticancer agent through the EGFR kinases suppression.

The current study on the anticancer activity of hydrazine carboxamide and their in-silico studies may add therapeutic value to hydrazine carboxamide. Also, the reported analogues can be modified further to increase their anticancer potentials.

Ethics Approval and Consent to Participate
Not applicable

Human and Animal rights
Not applicable

Consent for Publication
Not applicable

Conflicts of interest
The author confirms that this article content has no conflicts of interest.

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