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# Synthesis, cytotoxicity and molecular docking study of novel acid amine coupling derivatives

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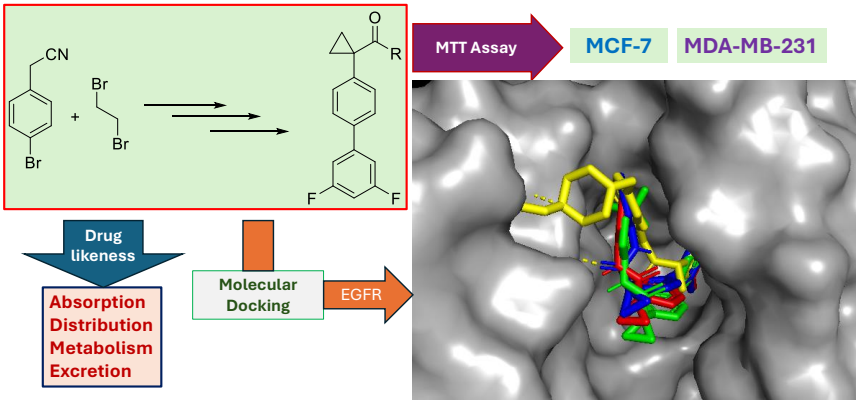
## Abstract

A library of novel acid amine coupling products was synthesized and the components tested for their *in vitro* cytotoxicity against human breast cancer cell lines viz. MCF-7 and MDA-MB-231. One of the compounds displayed superior activity against both cell lines, with lower $IC_{50}$ values compared to standard reference *Doxorubicin*. Two other compounds showed promising activities with encouraging $IC_{50}$ values against MCF-7, and MDA-MB-231. A molecular docking study of these molecules against EGFR gave their docking scores and binding interactions, and predicted their ADME properties.




**Keywords:** Acid-amine coupling, Suzuki cross-coupling, cytotoxicity, molecular docking

## Introduction

Amides are chemically neutral and stable compounds that possess both hydrogen-bond accepting and donating qualities. These characteristics make them well-suited for the development of novel therapeutic molecules.[1] The advanced aspects of synthetic medicinal chemistry focus on incorporating amide linkages, which are then subjected to various transformations.[2] Biomolecules containing amide bonds display essential biological functions, including anticancer, antimalarial, antifungal, antibacterial, anti-inflammatory, and antitubercular actions.[3–6] Analysis of extensive medicinal chemistry databases indicated that 25% of known medications contain at least one amide unit.[7] Valsartan has an amide link, it is an orally administered medication that acts as an antagonist of the Angiotensin II receptor type 1, effectively lowers blood pressure and is commonly prescribed for the treatment of hypertension.[8] Diltiazem is a calcium channel blocker that contains an amide linkage, it has been proven to be an effective and well-tolerated treatment for stable angina and angina caused by coronary artery spasm.[9] Captopril containing an amide link, is a powerful and competitive inhibitor of angiotensin-converting enzyme (ACE), which is responsible for converting angiotensin I into angiotensin II which governs blood pressure and serves as a crucial component of the renin-angiotensin-aldosterone system (RAAS).[10] Bupivacaine is classified as an amide local anesthetic, exerts its physiological impact through the mechanism of local anesthesia.[11] Acetazolamide is a sulfonamide compound that belongs to the group of thiadiazoles and is classified as a monocarboxylic acid amide. It functions as a diuretic, an anticonvulsant, and a carbonic anhydrase inhibitor. It can be beneficial as a supplementary treatment for tonic-clonic, myoclonic, and atonic seizures, especially in women whose seizures happen or worsen at specified times during their menstrual cycle.[12] The design rationale for new molecules is presented in Figure 1.

The acid amine coupling is a reliable and widely utilized reaction commonly employed in chemical synthesis. The reaction combines an amine and a carboxylic acid to produce an amide. From a physicochemical perspective, the transformation combines a hydrophilic basic component (amine) with two hydrogen bond donors, with a hydrophilic acidic component that has one hydrogen bond donor and two hydrogen bond acceptors, resulting in the production of a neutral product.[13] The resulting amide exhibits more lipophilicity compared to the initial reactants, and possesses one hydrogen bond donor and one hydrogen bond acceptor.[14] Chemoinformatic studies have established a connection between physicochemical properties and functions such as toxicity and market success.[15,16] Therefore, the ability to control the quantities of hydrogen bond donors, hydrogen bond acceptors, the partition coefficient logP, the molecular weight, and other properties of a molecule through chemical synthesis is highly significant.[17–19]

Cancer is a grave global health concern that claims the lives of individuals across all age groups worldwide.[20,21] The global death toll in 2020 reached 10 million, and projections indicate that by 2030, it is to rise to 13 million.[22,23] The EGFR, a member of the ErbB family of RTKs, plays a crucial role in the physiological processes of epithelial cells. It is commonly mutated and/or overexpressed in several forms of human malignancies and is the focus of multiple cancer treatments now used in clinical practice.[24,25] Although there are multiple treatments and chemotherapeutics currently accessible, this ailment nonetheless presents a significant life-threatening danger.[26] Therefore, it is imperative to persist in searching for new anticancer medications. There are numerous reports in the scientific literature that discuss the anticancer effects of amide derivatives.[27–30]

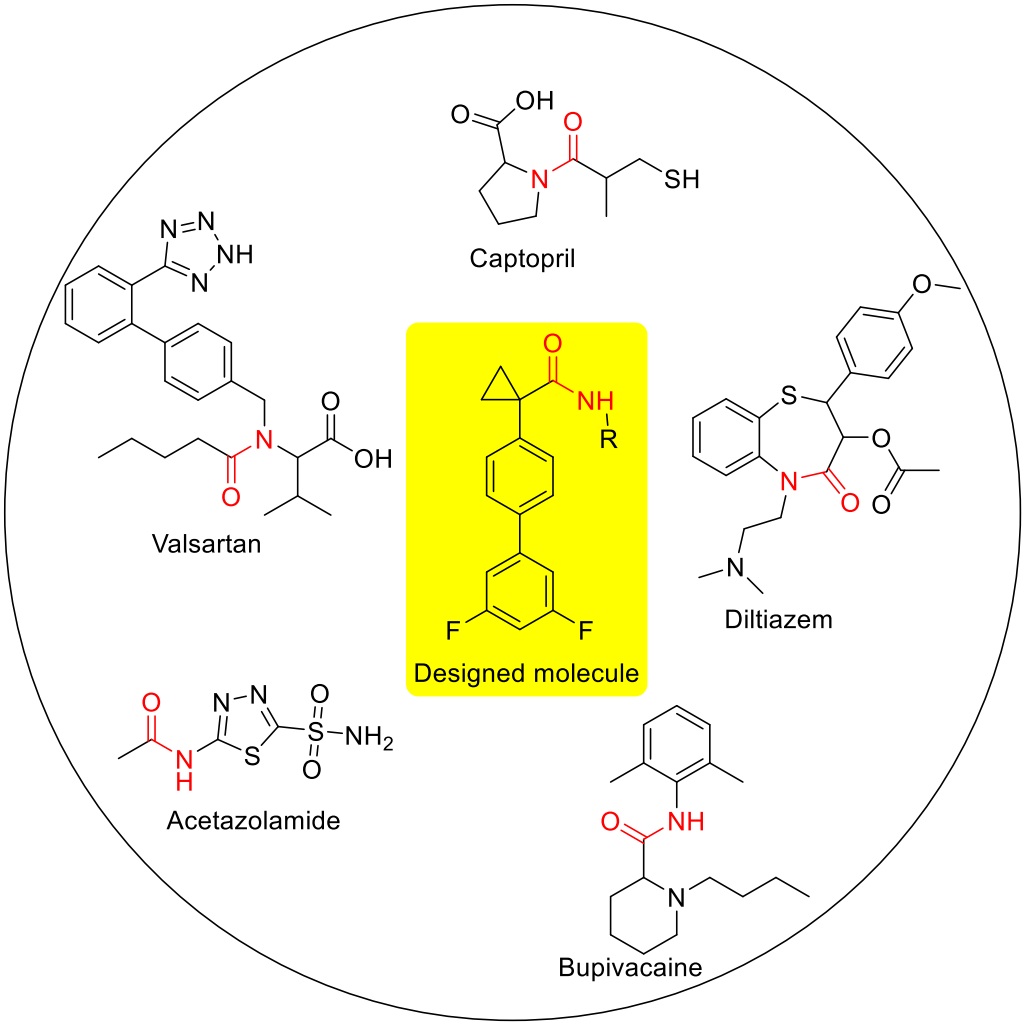



**Figure 1.** Design rationale of new acid amine coupling molecules, i.e. amides.

These reports motivated us to develop new amide derivatives using a straightforward synthetic method. We subsequently investigated the anticancer properties of these derivatives in laboratory tests using human breast adenocarcinoma cells (MCF-7 and MDA-MB-231). Additionally, we conducted molecular docking studies and predicted of their drug-like properties.

## Results and Discussion

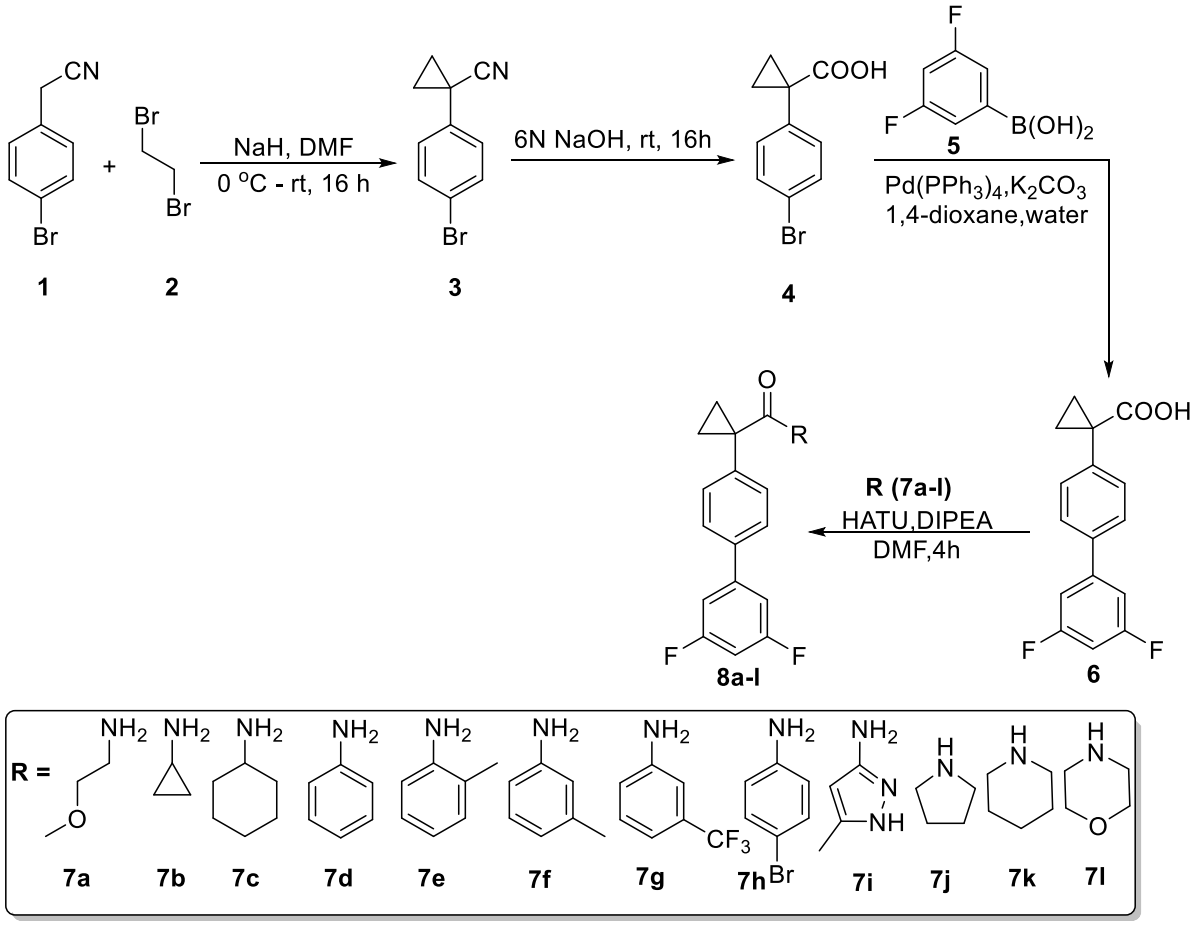
### Chemistry

The syntheses of new acid amine coupling derivatives (**8a-l**) were accomplished as presented in Scheme 1. To a mixture of 2-(4-bromophenyl)acetonitrile **1** and 1,2-dibromoethane **2**, was added NaH dropwise at 0 °C and the mixture stirred the mixture at room temperature for 16 h to obtain 1-(4-bromophenyl)cyclopropane-1-carbonitrile **3**. The cyano function of compound **3** was converted into carboxylic acid by stirring with 6N NaOH and thus we obtained 1-(4-bromophenyl)cyclopropane-1-carboxylic acid **4**. The Suzuki-Mayura coupling reaction of compound **4** and (3,5-difluorophenyl)boronic acid **5** in presence of $Pd(PPh_3)_4$ and $K_2CO_3$ yielded 1-(3',5'-difluoro-[1,1'-biphenyl]-4-yl)cyclopropane-1-carboxylic acid **6**, evidenced by the appearance of typical cyclopropane protons as two multiplets at δ 1.72 – 1.69 ppm and δ 1.31 – 1.28 ppm in $^1$H NMR spectrum. The

seven aromatic protons appeared in the range δ 7.49 to 6.74 ppm. The $^{13}$C NMR spectrum of intermediate **6** confirmed the carboxylic acid carbon signal at δ 180.7 ppm and cyclopropane carbons appeared at δ 28.5 ppm and δ 17.4 ppm, the aromatic carbons appeared in the range δ 164.6 to 102.5 ppm.

Treating nitrile **6** with various amines, **7a-l**, in the presence of hexafluorophosphate azabenzotriazole tetramethyluronium (HATU), along with Hunig's base (DIPEA), we then prepared the title amides, **8a-l**.




**Scheme 1.** Synthetic route for the preparation of new acid amine coupling derivatives (**8a-l**).

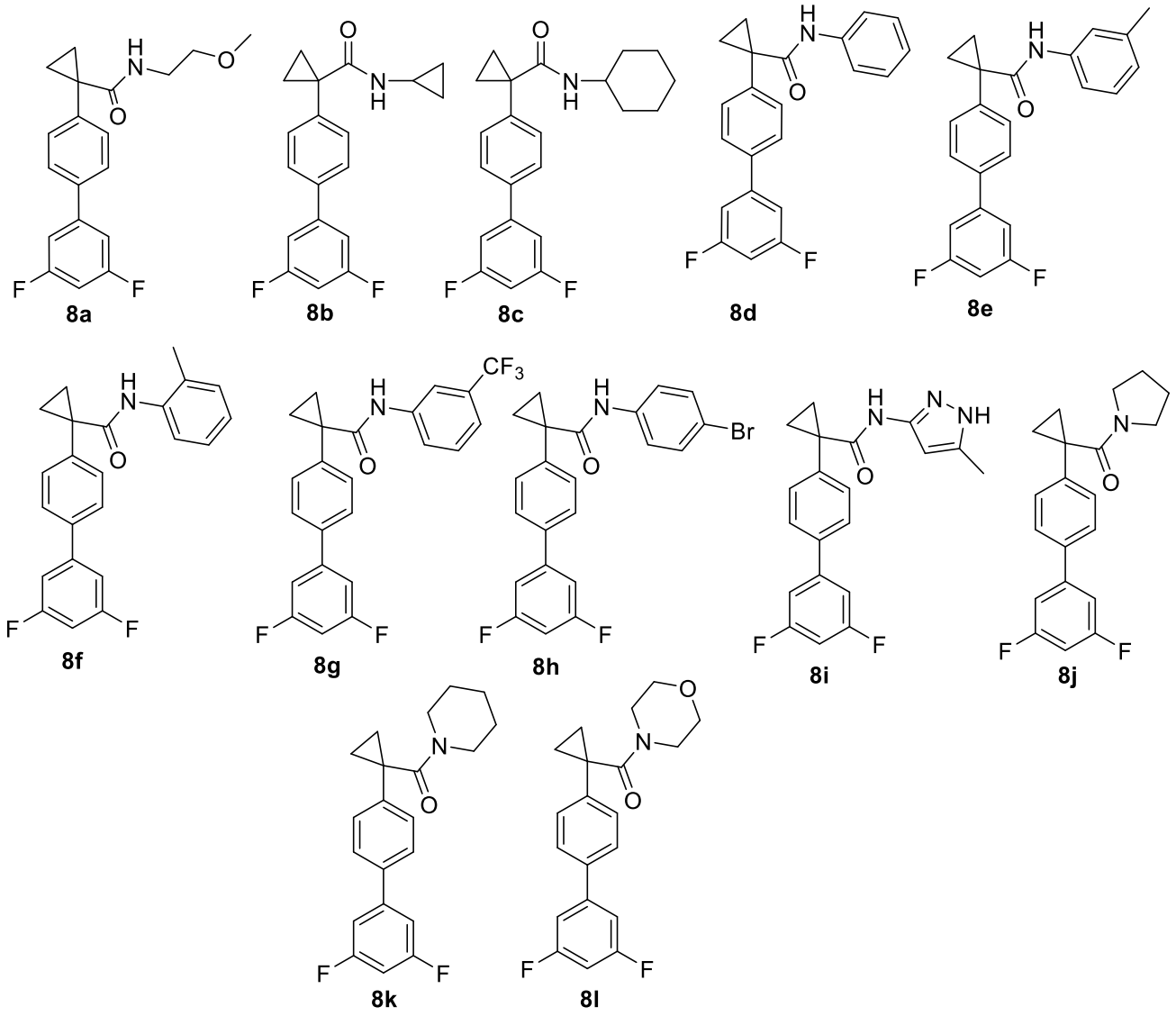



**Figure 2.** Structure of target amides (**8a-l**).

The structure of newly synthesized compounds (Figure 2) was characterized by interpretation of $^{1}$H NMR, $^{13}$C NMR and mass spectral data. For instance, in $^{1}$H NMR spectrum of compound **8a**, the NH- proton of amide group appeared as a singlet at δ 5.69 ppm, the protons of cyclopropane ring appeared as two doublets near δ 1.63 ppm and δ 1.08 ppm, the ethylene protons appeared as a singlet at δ 3.37 ppm, methoxy protons appeared as a singlet at δ 3.25 ppm, and the integration of aromatic protons were convincing with the number of protons in the downfield region. In the $^{13}$C NMR spectrum, the signal of carbonyl carbon of amide bond appeared at δ 173.5 ppm, the two sets of cyclopropane carbon signals appeared at δ 30.1 ppm and δ 15.6 ppm, the signal of ipso carbon signal attached to the F atoms was at δ 164.6 ppm. The absorption stretching frequency peaks of N-H and C=O were confirmed near 3330 cm$^{-1}$ and 1650 cm$^{-1}$ respectively in IR spectrum. The ESI-MS spectrum of compound **8a** confirmed its *m/z* 332.12 $[M+H]^{+}$ peak.

**Cytotoxicity**

The newly synthesized acid amine coupling derivatives were screened for their *in vitro* anticancer activities against human breast adenocarcinoma cell lines MCF-7 (ER positive) and MDA-MB-231 (Triple negative) via MTT

assay using *Doxorubicin* as standard reference. The $IC_{50}$ values of all compounds are presented in Table 1. Significantly, compound **8g**, with (trifluoromethyl)phenyl substitution, displayed spectacular activity against both cell lines MCF-7 and MDA-MB-231 with $IC_{50}$ value of 8.92±0.91 µM and 7.54±0.95 µM respectively, compared to the *Doxorubicin* $IC_{50}$ value of 9.29±1.02 µM and 7.68±5.36 µM. this may be due to the presence of electron-withdrawing function $CF_3$, which makes the molecule bind with electron-rich biological target. Moreover, fluorine atoms could form H-bond and halogen bond interactions with active sites present on biological targets, essential for their efficacy. The other *meta* methyl substituted phenyl group compound, **8f**, also showed promising activities with $IC_{50}$ values of 9.14±0.92 µM and 8.78±0.58 µM against MCF-7 and MDA-MB-231 respectively. Changing position of the methyl group from *para* to *ortho* in compound **8e** altered its activity slightly with $IC_{50}$ value of 12.19±1.12 µM and 12.19±1.11 µM against MCF-7 and MDA-MB-231 respectively. Compounds **8b** and **8l** had shown very poor results against MCF-7 cells, whereas the activities of all other compounds are good-to-moderate against both the cell lines. Attachment of aliphatic rings in place of aromatic phenyl ring in all other compounds reduced their activity. Furthermore, the effect of these molecules tested on normal cell lines MCF-10A did not show any significant impact.

**Table 1.** Anticancer activity of acid amine derivatives (**8a-l)** against human breast cancer cell line

| Entry | $IC_{50}$ (µM±SD) | | |
|---|---|---|---|
| | MCF-7 | MDA-MB-231 | MCF-10A |
| **8a** | 62.54 ± 5.33 | 69.53 ± 5.25 | 87.01 ± 5.89 |
| **8b** | >100 | 82.33 ± 7.14 | 82.72 ± 4.12 |
| **8c** | 62.05 ± 7.24 | 55.20 ± 5.69 | 90.40 ± 6.41 |
| **8d** | 83.39 ± 7.92 | 53.25 ± 5.27 | 85.22 ± 5.80 |
| **8e** | 12.19 ± 1.12 | 12.19 ± 1.11 | 87.26 ± 5.37 |
| **8f** | 9.14 ± 0.92 | 8.78 ± 0.58 | 89.88 ± 6.36 |
| **8g** | 8.92 ± 0.91 | 7.54 ± 0.95 | 91.07 ± 6.51 |
| **8h** | 68.24 ± 7.05 | 61.32 ± 5.33 | 90.45 ± 6.92 |
| **8i** | 39.12 ± 3.34 | 55.32 ± 4.98 | 89.41 ± 5.58 |
| **8j** | 57.18 ± 6.35 | 19.98 ± 1.24 | 88.25 ± 6.21 |
| **8k** | 48.73 ± 4.16 | 54.47 ± 4.22 | 85.63 ± 6.15 |
| **8l** | >100 | 59.25 ± 5.67 | 87.54 ± 6.01 |
| *Doxorubicin* | 9.29 ± 1.02 | 7.68 ± 5.36 | 89.27 ± 6.23 |

**Molecular docking studies**

Molecular docking is a computer method employed to forecast the most favorable configuration of a ligand and its macromolecular target (receptor) when they are joined together to form a stable complex.[31] The epidermal growth factor receptor (EGFR) plays a vital role in the advancement of several malignancies, making it an important focus for cancer therapy.[32–34] The most convincing molecules **8e, 8f** and **8g** were docked into the active site pocket of the crystal structure of EGFR (PDB ID: 2J6M) and the results validated by redocking the co-crystalized ligand AEE788, which presented an RMSD of 1.08 Å. The docking scores of the ligands **8e, 8f** and **8g** were -9.2 kcal/mol, -9.3 kcal/mol and -9.5 kcal/mol respectively, on a par with docking score of AEE788, value -9.3 kcal/mol. The compound **8e**, displayed a key interaction with amino acid site Lys745 of EGFR with a bond distance of 2.93 Å. There were four halogen bond interactions[35] with amino acids Ala743, Ile744, Glu762 and Leu788, a π-anion interaction with Asp855 and other hydrophobic interactions were reported against Leu718,

Val726, Leu792 and Gly796 of EGFR (Figure 3). For compound **8f**, two key interactions were observed against Lys745 and Cys797 of EGFR with a bond distance of 2.85 Å and 2.95 Å correspondingly. The halogen and other hydrophobic interactions were reported as the same as for compound **8e** (Figure 4). The compound **8e**, showed an H-bond interaction against Met793 of EGFR with a bond distance of 2.55 Å. The halogen and other hydrophobic interactions could be seen to be the same as earlier compounds (Figure 5), whereas the co-crystalized ligand AEE788 displayed two H-bond interactions with Gln791 and Met793, and hydrophobic interactions with Leu718, Val726, Ala743, Lys745, Leu788, Thr790, Asp800 and Leu844 of EGFR (Figure 6). The docking pose of ligands **8e**, **8f**, **8g** and AEE788 are shown in Figure 7.

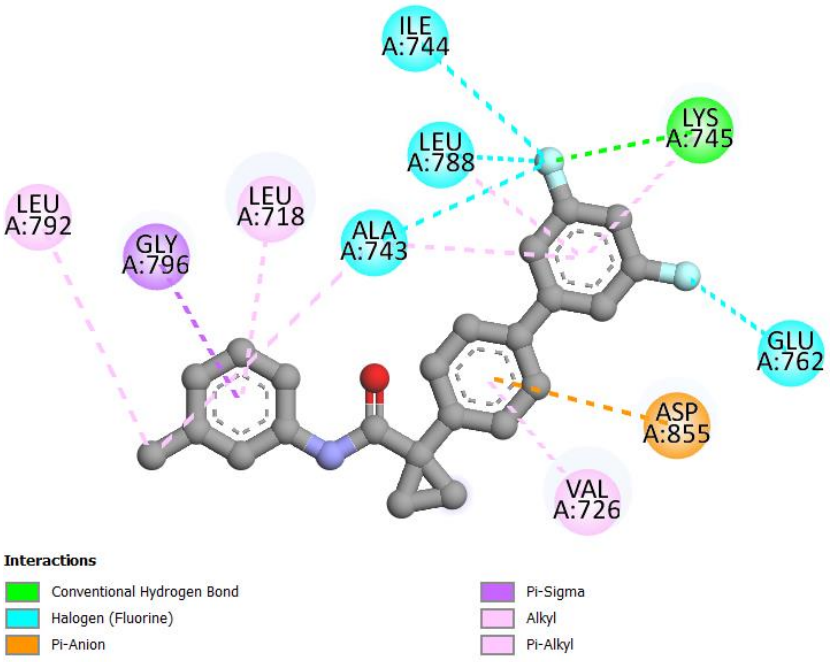



**Figure 3.** Binding interactions of compound **8e** in cavity of EGFR.

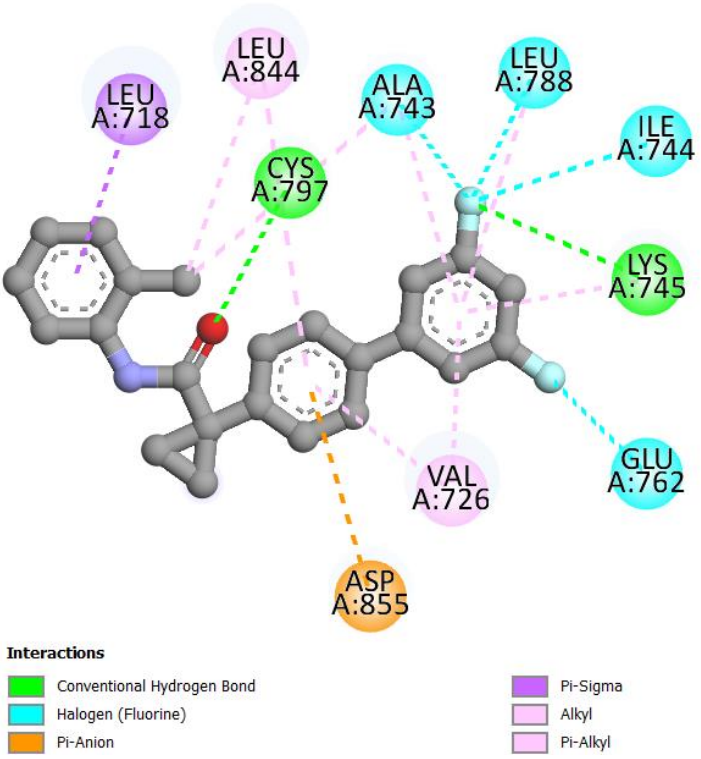



**Figure 4.** Binding interactions of compound **8f** in cavity of EGFR.

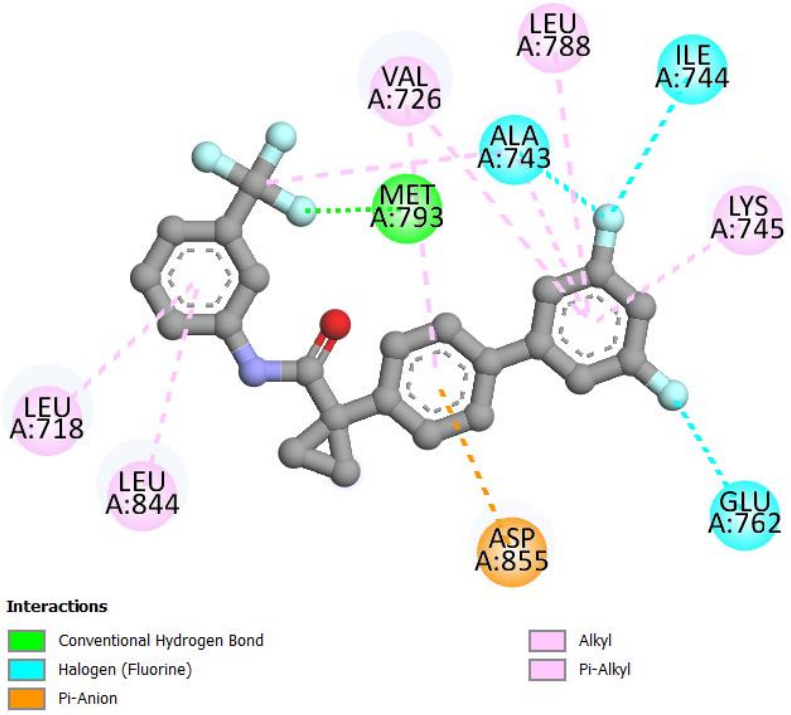



**Figure 5.** Binding interactions of compound **8g** in cavity of EGFR.

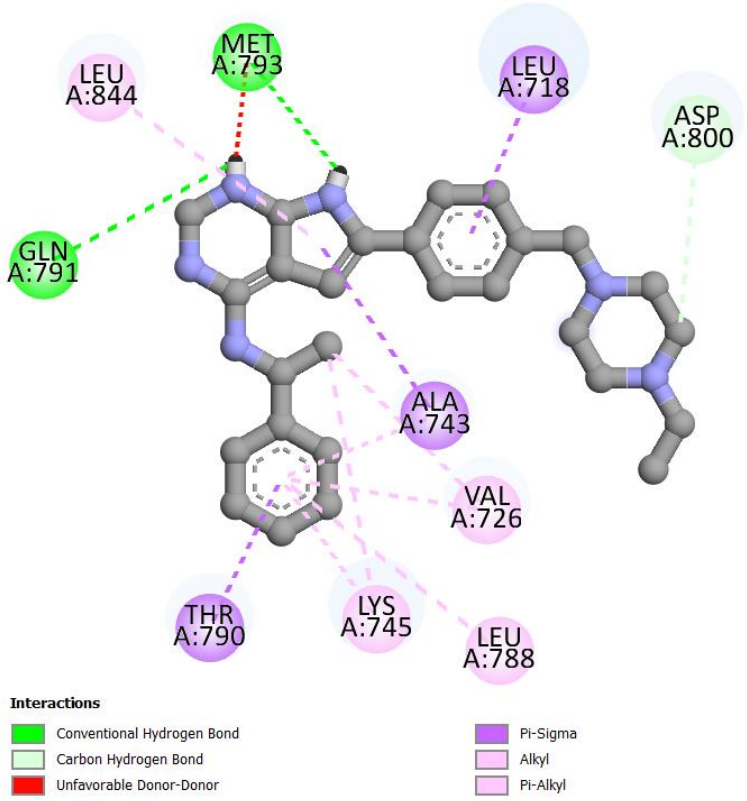



**Figure 6.** Binding interactions of **AEE788** in cavity of EGFR.

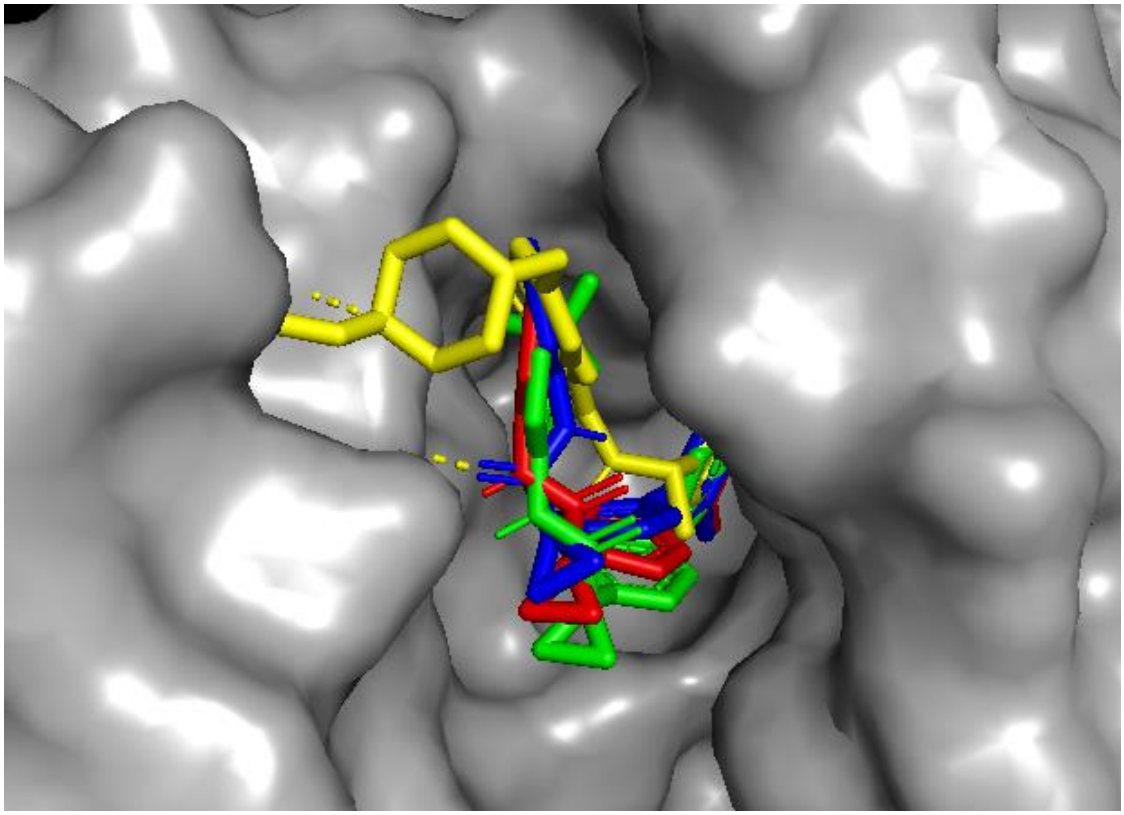


**Figure 7.** Docking pose of ligands **8e** (red), **8f** (blue), **8g** (green) and AEE788(yellow) in cavity of EGFR.

**ADME prediction**

Absorption, distribution, metabolism and excretion properties of synthesized compounds **8a-l** were determined by using SwissADME web tool,[36,37] and presented in Table 2. The molecular weights of compounds were <500, according to Lipinski's rule of five these could be absorbed and distributed in the body for effective metabolism and there were no violations of it.[38] The number of rotatable bonds, H-bond acceptors and H-bond donors were in the desirable range. The octanal/water partition coefficient (Log $P_{o/w}$) value is below 5, which is very important for metabolism process of drug.[39] Topological surface area, bioavailability score and synthetic accessibility value describe these molecules as appropriate drug candidates.

**Table 2.** ADME properties of compounds **8a-l**

| Compound | Molecular weight (range ≤ 500) | Rotatable bonds (range 1-10) | H-bond acceptors (range ≤10) | H-bond donors (range ≤5) | TPSA | Log $P_{o/w}$ (range ≤5) | Molar refractivity (Range 40 – 130) | QPLogS (Solubility) | Lipinski violations | Bioavailability Score (range 0.4 – 0.6) | Synthetic Accessibility |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **8a** | 331.36 | 7 | 4 | 1 | 38.33 | 3.30 | 87.46 | -3.79 | 0 | 0.55 | 2.27 |
| **8b** | 313.34 | 5 | 3 | 1 | 29.10 | 3.24 | 84.26 | -4.27 | 0 | 0.55 | 2.09 |
| **8c** | 355.42 | 5 | 3 | 1 | 29.10 | 3.80 | 98.68 | -5.28 | 0 | 0.55 | 2.46 |
| **8d** | 349.37 | 5 | 3 | 1 | 29.10 | 3.45 | 97.98 | -5.26 | 0 | 0.55 | 2.22 |
| **8e** | 363.40 | 5 | 3 | 1 | 29.10 | 3.72 | 102.94 | -5.55 | 0 | 0.55 | 2.38 |
| **8f** | 363.40 | 5 | 3 | 1 | 29.10 | 3.80 | 102.94 | -5.55 | 0 | 0.55 | 2.40 |
| **8g** | 417.37 | 6 | 6 | 1 | 29.10 | 3.73 | 102.98 | -6.10 | 0 | 0.55 | 2.53 |
| **8h** | 428.27 | 5 | 3 | 1 | 29.10 | 3.76 | 105.68 | -6.16 | 0 | 0.55 | 2.36 |
| **8i** | 353.37 | 5 | 4 | 2 | 57.78 | 2.76 | 95.09 | -4.64 | 0 | 0.55 | 2.53 |
| **8j** | 327.37 | 4 | 3 | 0 | 20.31 | 3.52 | 93.07 | -4.48 | 0 | 0.55 | 2.20 |
| **8k** | 341.39 | 4 | 3 | 0 | 20.31 | 3.65 | 97.88 | -4.78 | 0 | 0.55 | 2.30 |
| **8l** | 343.37 | 4 | 4 | 0 | 29.54 | 3.37 | 94.16 | -4.03 | 0 | 0.55 | 2.37 |

## Conclusions

A library of new acid amine coupling products were synthesized via a series of conventional synthetic procedures involving reactions like cyclization coupling, functional group conversion, Suzuki-Mayura cross coupling and acid amine coupling reactions. All newly synthesized compounds were tested for their invitro anticancer activity against human breast cancer cell lines viz. MCF-7 and MDA-MB-231. A (trifluoromethyl)phenyl substitution analogue **8a** displayed superior activity against both cell lines MCF-7 and MDA-MB-231 with $IC_{50}$ value of 8.92±0.91 µM and 7.54±0.95 µM respectively, compared to the *Doxorubicin* $IC_{50}$ value of 9.29±1.02 µM and

7.68±5.36 µM. The methyl substituted phenyl group compounds **8f** and **8e** showed promising activities with $IC_{50}$ values of 9.14±0.92 µM and 12.19±1.12 µM against MCF-7, and 8.78±0.58 µM and 12.19±1.11 µM against MDA-MB-231 respectively. The molecular docking study of these molecules against EGFR proved their binding efficacy with notable docking scores and binding interactions such as H-bond and hydrophobic interactions. The anticipated ADME attributes indicate that they possess desirable drug-like characteristics.

## Experimental Section

**General.** All chemicals were obtained from commercial sources and used without any further purification. Melting points were taken in glass capillary tubes on a Haake Bucher apparatus and are uncorrected. All proton NMR spectra were determined with a Varian 400 MHz spectrometer using deuterated dimethyl sulfoxide (DMSO-$d_6$) and are reported in δ (ppm) units. Thin layer chromatography (TLC) was performed in E. Merck presoaked silica gel plates. Visualization was obtained by exposure to iodine vapors and/or under UV light (254 nm).

**Procedure for the preparation of the 1-(3',5'-difluoro-[1,1'-biphenyl]-4-yl)cyclopropane-1-carboxylic acid (6).** A mixture of 1-(4-bromophenyl)cyclopropane-1-carboxylic acid (1.5 g., 12.18 mmol), in 1,4-dioxane:$H_2O$(4:1) (20 mL) were (3,5-difluorophenyl)boronic acid (1.78 g., 12.18 mmol), $K_2CO_3$ (1.21 g, 12.18 mmol), followed by addition of $Pd(PPh_3)_4$(0.14 mL, 2.44 mmol) the resulting reaction mixture was stirred at 80 °C for 16 h. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with water (10 ml) and extracted with EtOAc (2 x 10 ml). The combined organic layer was washed with water, followed by brine and dried over anhydrous $Na_2SO_4$, filtered and evaporated the solvent under reduced pressure to give crude compound. The crude compound was purified by column chromatography using ethyl acetate/pet ether. Yield: 70%, off white solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.41 (m, 4H), 7.10 – 7.04 (m, 2H), 6.79 – 6.74 (m, 1H), 1.72 – 1.69 (m, 2H), 1.31 – 1.28 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 180.7, 164.6, 162.0, 139.0, 138.0, 131.1, 126.9, 109.8, 102.5, 28.5, 17.4. ESI-MS: *m/z* 275.08 $[M+H]^+$.

**General procedure for the preparation of the 4-(2-azido-1-(2-cyclopropylphenyl)ethyl) morpholine (8a-l).** A mixture of 4-(3',5'-difluoro-[1,1'-biphenyl]-4-yl)cyclopropane-1-carboxylic acid (1 eq.) in DMF (2 mL) were added HATU (2 eq.), and DIPEA (3 eq.), followed by the addition of amine (1.2 mmol) at RT, the resulting reaction mixture was stirred at RT for 4 - 16 h. Reaction was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with water (10 ml) and extracted with EtOAc (2 x 10 ml). The combined organic layer was washed with water, followed by brine and dried over anhydrous $Na_2SO_4$, filtered and evaporated the solvent under reduced pressure to give crude compound. The crude compound was purified by column chromatography using ethyl acetate/pet ether (30:70). Yield: 70 - 80%.

**1-(3',5'-Difluoro-[1,1'-biphenyl]-4-yl)-*N*-(2-methoxyethyl)cyclopropane-1-carboxamide (8a).** White solid, Yield: 72%. mp 156 – 158 °C. IR (KBr in $cm^{-1}$): 3360, 3005, 2933, 2862, 1624, 1448, 831. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.47 (m, 4H), 7.10 (d, *J* ,7.2 Hz, 2H), 6.83 – 6.78 (m, 1H), 5.69 (bs, 1H), 3.37 (s, 4H), 3.25 (s, 3H), 1.63 (d, J = 2.0 Hz, 2H), 1.08 (s, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 173.5, 164.6, 162.21, 140.20, 138.4, 131.6, 127.5, 110.0, 102.7, 71.2, 58.7, 39.9, 30.1, 15.6. ESI-MS: *m/z* 332.20 $[M+H]^+$. Elemental analysis calcd for chemical formula $C_{19}H_{19}F_2NO_2$: C, 68.87; H, 5.78; N, 4.23. Found: C, 68.81; H, 5.73; N, 4.18.

***N*-Cyclopropyl-1-(3',5'-difluoro-[1,1'-biphenyl]-4-yl)cyclopropane-1-carboxamide (8b).** White solid, Yield: 70%. mp 159 – 161 °C. IR (KBr in $cm^{-1}$): 3298, 3014, 2970, 1737, 1367, 983. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.44 (m, 4H), 7.10 (d, *J* 6.8 Hz, 2H), 6.83 – 6.78 (m, 1H), 5.37 (bs, 1H), 2.66 – 2.59 (m, 3H), 1.64 (d, J 2.4 Hz, 2H), 1.05

(d, J 2.4 Hz, 2H), 0.71 – 0.68 (m, 2H), 0.35 (s, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 174.8, 164.6, 164.5, 140.2, 138.4, 131.6, 127.6, 110.0, 102.8, 30.0, 23.1, 15.7, 6.6. ESI-MS: *m/z* 336.25 $[M+Na]^+$. Elemental analysis calcd for chemical formula $C_{19}H_{17}F_2NO$: C, 72.83; H, 5.47; N, 4.47. Found: C, 72.77; H, 5.41; N, 4.43.

***N*-Cyclohexyl-1-(3',5'-difluoro-[1,1'-biphenyl]-4-yl)cyclopropane-1-carboxamide (8c).** White solid, Yield: 78%. mp 167 – 169 °C. IR (KBr in $cm^{-1}$): 3007, 2968, 1739, 1637, 1365, 1209, 1112. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.46 (m, 4H), 7.15 – 7.09 (m, 2H), 6.83 – 6.77 (m, 1H), 5.16 (d, J 7.6 Hz, 1H), 3.77 – 3.68 (m, 1H), 1.81 – 1.77 (m, 2H), 1.63 – 1.61 (m, 2H), 1.59 – 1.51 (m, 4H), 1.32 – 1.35 (m, 2H), 1.05 – 1.03 (m, 2H), 0.99 – 0.92 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.4, 164.4, 162.4, 140.5, 138.3, 131.6, 127.5, 109.9, 102.7, 48.6, 32.9, 30.1, 25.4, 24.7, 15.4. ESI-MS: *m/z* 356.17 $[M+H]^+$. Elemental analysis calcd for chemical formula $C_{22}H_{23}F_2NO$: C, 74.34; H, 6.52; N, 3.94. Found: C, 74.28; H, 6.47; N, 3.88.

**1-(3',5'-Difluoro-[1,1'-biphenyl]-4-yl)-*N*-phenylcyclopropane-1-carboxamide (8d).** White solid, Yield: 78%. mp 177 – 179 °C. IR (KBr in $cm^{-1}$): 3442, 3365, 3008, 2939, 1737, 1365, 1207, 1126. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.65 – 7.57 (m, 5H), 7.53 – 7.50 (m, 1H), 7.38 – 7.30 (m, 2H), 7.18 – 7.12 (m, 3H), 6.99 – 6.80 (m, 2H), 1.78 – 1.76 (m, 2H), 1.23 – 1.21 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.97, 162.50, 139.19, 138.26, 131.82, 129.41, 128.05, 122.83, 120.88, 117.98, 116.45, 110.10, 109.90, 103.07, 30.97, 16.72. ESI-MS: *m/z* 350.13 $[M+H]^+$. Elemental analysis calcd for chemical formula $C_{22}H_{17}F_2NO$: C, 76.63; H, 4.90; N, 4.01. Found: C, 76.58; H, 4.85; N, 3.96.

**1-(3',5'-Difluoro-[1,1'-biphenyl]-4-yl)-*N*-(*m*-tolyl)cyclopropane-1-carboxamide (8e).** White solid, Yield: 81%. mp 172 – 174 °C. IR (KBr in $cm^{-1}$): 3320, 2992, 1652, 1272, 536. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* 8.0 Hz, 1H), 7.62 (s, 4H), 7.19 – 7.15 (m, 1H), 7.14 – 7.10 (m, 2H), 7.06 – 7.04 (m, 1H), 7.00 – 6.96 (m, 2H), 6.85 – 6.79 (m, 1H), 1.82 (s, 3H), 1.78 – 1.75 (m, 2H), 1.21 – 1.18 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.5, 164.7, 139.9, 139.0, 136.0, 131.9, 130.2, 127.8, 127.4, 126.8, 124.6, 121.5, 110.0, 109.8, 102.9, 31.1, 17.0, 16.1. ESI-MS: *m/z* 363.14 $[M+H]^+$. Elemental analysis calcd for chemical formula $C_{23}H_{19}F_2NO$: C, 75.63; H, 4.90; N, 4.01. Found: C, 75.58; H, 4.85; N, 3.97.

**1-(3',5'-Difluoro-[1,1'-biphenyl]-4-yl)-*N*-(*o*-tolyl)cyclopropane-1-carboxamide (8f).** White solid, Yield: 80%. mp 170 – 172 °C. IR (KBr in $cm^{-1}$): 3310, 2927, 1648, 1270, 539. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* 8.4 Hz, 1H), 7.62 (s, 4H), 7.18 – 7.10 (m, 3H), 7.06 – 7.04 (m, 1H), 7.00 – 6.96 (m, 2H), 6.85 – 6.79 (m, 1H), 1.82 (s, 3H), 1.78 – 1.75 (m, 2H), 1.21 – 1.18 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.59, 165.16, 139.94, 139.00, 136.01, 131.93, 130.28, 127.81, 127.47, 126.85, 124.62, 121.51, 110.13, 109.79, 102.99, 31.10, 17.06, 16.14. ESI-MS: *m/z* 363.17 $[M+H]^+$. Elemental analysis calcd for chemical formula $C_{23}H_{19}F_2NO$: C, 75.63; H, 4.90; N, 4.01. Found: C, 75.57; H, 4.86; N, 3.96.

**1-(3',5'-Difluoro-[1,1'-biphenyl]-4-yl)-*N*-(3-(trifluoromethyl)phenyl)cyclopropane-1-carboxamide (8g).** White solid, Yield: 82%. mp 191 – 193 °C. IR (KBr in $cm^{-1}$): 3300, 2965, 1647, 1258, 542. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.66 – 7.58 (m, 5H), 7.52 – 7.48 (m, 1H), 7.39 – 7.29 (m, 2H), 7.17 – 7.13 (m, 2H), 6.98 – 6.80 (m, 2H), 1.78 – 1.76 (m, 2H), 1.23 – 1.21 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.99, 164.37, 162.50, 143.41, 139.20, 138.27, 131.82, 129.41, 128.04, 122.85, 120.87, 116.47, 110.10, 109.89, 103.06, 30.97, 16.71. ESI-MS: *m/z* 418.11 $[M+H]^+$. Elemental analysis calcd for chemical formula $C_{23}H_{16}F_5NO$: C, 66.19; H, 3.86; N, 3.36. Found: C, 66.12; H, 3.80; N, 3.29.

***N*-(4-Bromophenyl)-1-(3',5'-difluoro-[1,1'-biphenyl]-4-yl)cyclopropane-1-carboxamide (8h).** White solid, Yield: 82%. mp 191 – 193 °C. IR (KBr in $cm^{-1}$): 3332, 2968, 1662, 1275, 560. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.63 – 7.56 (m, 4H), 7.51 – 7.39 (m, 1H), 7.37 – 7.33 (m, 2H), 7.25 – 7.22 (m, 2H), 7.16 – 7.11 (m, 2H), 6.85 – 6.79 (m, 1H), 1.76 – 1.73 (m, 2H), 1.21 – 1.18 (m, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$) δ 171.72, 165.16, 139.39, 136.88, 131.83, 131.17, 127.97, 126.82, 121.27, 116.86, 110.15, 109.81, 103.05, 30.97, 16.57. ESI-MS: *m/z* 429.03 $[M+H]^+$. Elemental analysis calcd for chemical formula $C_{22}H_{16}BrF_2NO$: C, 61.70; H, 3.77; N, 3.27. Found: C, 61.66; H, 3.71; N, 3.22.

**1-(3',5'-Difluoro-[1,1'-biphenyl]-4-yl)-*N*-(5-methyl-1*H*-pyrazol-3-yl)cyclopropane-1-carboxamide (8i).** White solid, Yield: 81%. mp 181 – 183 °C. IR (KBr in $cm^{-1}$): 3420, 3332, 1662, 1275, 560. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.57 – 7.42 (m, 6H), 7.12 – 7.05 (m, 2H), 6.84 – 6.78 (m, 1H), 6.40 (bs, 1H), 2.24 (s, 3H), 1.75 – 1.72 (m, 2H), 1.19 – 1.17 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.35, 164.67, 162.07, 143.69, 139.03, 131.84, 131.12, 127.96, 126.88, 110.14, 109.88, 102.90, 29.74, 16.56, 11.53. ESI-MS: *m/z* 354.13 $[M+H]^+$. Elemental analysis calcd for chemical formula $C_{20}H_{17}F_2N_3O$: C, 67.98; H, 4.85; N, 11.89. Found: C, 67.92; H, 4.79; N, 11.81.

**(1-(3',5'-Difluoro-[1,1'-biphenyl]-4-yl)cyclopropyl)(pyrrolidin-1-yl)methanone (8j).** White solid, Yield: 80%. mp 175 – 177 °C. IR (KBr in $cm^{-1}$): 3014, 2968, 1737, 1205, 1105, 983. $^1$H NMR (400 MHz, $CDCl_3$) 7.49 – 7.46 (m, 2H), 7.27 – 7.26 (m, 2H), 7.11 – 7.06 (m, 2H), 6.79 – 6.74 (m, 1H), 3.53 – 3.43 (m, 4H), 1.82 – 1.77 (m, 4H), 1.49 – 1.16 (m, 2H), 1.20 – 1.17 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.50, 141.14, 136.77, 127.21, 126.63, 109.87, 109.53, 102.46, 102.12, 49.50, 40.19, 25.14, 15.11. ESI-MS: *m/z* 328.14 $[M+H]^+$. Elemental analysis calcd for chemical formula $C_{20}H_{19}F_2NO$: C, 73.38; H, 5.85; N, 4.28. Found: C, 73.32; H, 5.80; N, 4.23.

**(1-(3',5'-Difluoro-[1,1'-biphenyl]-4-yl)cyclopropyl)(piperidin-1-yl)methanone (8k).** White solid, Yield: 78%. mp 172 – 174 °C. IR (KBr in $cm^{-1}$): 3336, 2933, 2826, 1624, 1448, 983. $^1$H NMR (400 MHz, $CDCl_3$) 7.50 – 7.46 (m, 2H), 7.25 – 7.23 (m, 2H), 7.11 – 7.06 (m, 2H), 6.79 – 6.73 (m, 1H), 3.59 – 6.41 (m, 4H), 1.64 – 1.55 (m, 4H), 1.47 – 1.44 (m, 2H), 1.36 – 1.25 (m, 2H), 1.22 – 1.19 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.40, 164.51, 162.18, 141.69, 127.18, 125.93, 109.81, 109.56, 102.44, 46.83, 29.38, 25.58, 24.49, 15.60. ESI-MS: *m/z* 342.25 $[M+H]^+$. Elemental analysis calcd for chemical formula $C_{21}H_{21}F_2NO$: C, 73.88; H, 6.20; N, 4.10. Found: C, 73.84; H, 6.15; N, 4.06.

**(1-(3',5'-Difluoro-[1,1'-biphenyl]-4-yl)cyclopropyl)(morpholino)methanone (8l).** White solid, Yield: 78%. mp 172 – 174 °C. IR (KBr in $cm^{-1}$): 3007, 2968, 1739, 1365, 1209, 1112. $^1$H NMR (400 MHz, $CDCl_3$) 7.51 – 7.48 (m, 2H), 7.25 – 7.22 (m, 2H), 7.11 – 7.05 (m, 2H), 6.80 – 6.75 (m, 1H), 3.65 – 3.44 (m, 8H), 1.49 – 1.46 (m, 2H), 1.25 – 1.22 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.81, 164.53, 141.05, 137.02, 127.38, 125.84, 109.85, 109.60, 102.58, 66.49, 42.77, 29.08, 15.54. ESI-MS: *m/z* 344.20 $[M+H]^+$. Elemental analysis calcd for chemical formula $C_{20}H_{19}F_2NO_2$: C, 69.96; H, 5.58; N, 4.08. Found: C, 69.90; H, 5.53; N, 4.02.

**MTT assay protocol** [40]

The Human breast adenocarcinoma cell lines MCF-7 (ER Positive) and MDA-MB-231 (triple negative) were procured from the National Centre for Cell Sciences (NCCS), Pune India, and were sub-cultured in-house at Synteny Life Sciences Pvt. Ltd., Hyderabad, India. The cells were seeded in a 96-well flat-bottom microplate and maintained at 37 ºC in 95% humidity and 5% $CO_2$ overnight. Different concentration (100, 50, 25, 12.5, 6.25, 3.125 µg/ml) of samples were treated. The cells were incubated for another 48 hours. The wells were washed twice with PBS and 20 µL of the MTT staining solution was added to each well, and the plate was incubated at 37 °C. After 4h, 100 µL of DMSO was added to each well to dissolve the formazan crystals, and absorbance was recorded with a 570 nm using microplate reader. All the experiments were carried out in triplicate.

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound}}{\text{Mean OD of Negative control}}\ x\ 100$$

**Molecular docking method**

Autodock Vina integrated PyRx tool was employed for docking simulations[41,42]. The crystal structure of Epidermal Growth Factor Receptor (EGFR) (PDB ID: 2J6M) were retrieved from Protein Data Bank (www.rcsb.org). Initially, water molecules and heteroatoms of protein were removed and added polar hydrogens. The ligands were sketched using ChemDraw Professional 16.0 in MDL file format. Minimized the energies of all ligands after loading into PyRx and converted to PDBQT file format. The 3D grid box was configured with dimensions of center_x = -53.06, center_y = -1.28, center_z = -17.95, size_x = 17.12, size_y =

17.74 and size_z = 27.22, docking simulations were performed after assigning the exhaustiveness value of 8. The docking result were visualized using Pymol and Biovia Discovery Studio Visualizer.

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## Supplementary Material

Copies of all $^{1}H$ and $^{13}C$ NMR spectra of all products are available in the Supplementary material section.

## References

1. Suresh, A. S.; Baburajan, P.; Ahmed, M. *Tetrahedron Lett.* **2015**, *56*, 4864. https://doi.org/10.1016/j.tetlet.2015.06.054
2. Singh, G.; Rani, S.; Saroa, A.; Promila; Arora, A.; Choquesillo-Lazarte, D. *Inorg. Appl. Pharm. Sci. Chim. Acta* **2015**, *433*, 78. https://doi.org/10.1016/j.ica.2015.04.034
3. Shoaib Ahmad Shah, S.; Ashfaq, M.; Najam, T.; Mehboob Ahmed, M.; Shaheen, S.; Tabassum, R.; Abida Ejaz, S. *Curr. Bioact. Compd.* **2013**, *9*, 211.
4. Liu, H.-B.; Tang, H.; Yang, D.; Deng, Q.; Yuan, L.-J.; Ji, Q.-G. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5845. https://doi.org/10.1016/j.bmcl.2012.07.081
5. Nayak, P. S.; Narayana, B.; Sarojini, B. K.; Hegde, K.; Shashidhara, K. S. *Med.Chem.Res.* **2014**, *23*, 4280. https://doi.org/10.1007/s00044-014-1003-3
6. Cui, Y.; Rao, X.; Shang, S.; Song, Z.; Shen, M.; Liu, H. *J. Saudi Chem. Soc.* **2017**, *21*, S258. https://doi.org/10.1016/j.jscs.2014.02.012
7. Thakral, S.; Singh, V. *Curr.. Bioact. Compd.* **2019**, *15*, 316. https://doi.org/10.2174/1573407214666180614121140
8. Siddiqui, N.; Husain, A.; Chaudhry, L.; Alam, M. S.; Mitra, M.; Bhasin, P. S. *J. Appl. Pharm. Sci*. **2011**, 12. Chaffman, M.; Brogden, R. N. *Drugs* **1985**, *29*, 387.
9. Siddiqui, N.; Husain, A.; Chaudhry, L.; Alam, M. S.; Mitra, M.; Bhasin, P. S. *J. Appl. Pharm. Sci*. **2011**, 12. https://doi.org/10.2165/00003495-198529050-00001
10. Heel, R. C.; Brogden, R. N.; Speight, T. M.; Avery, G. S. *Drugs* **1980**, *20*, 409. https://doi.org/10.2165/00003495-198020060-00001
11. Babst, C. R.; Gilling, B. N. *Anesth. Prog.* **1978**, *25*, 87.
12. Reiss, W. G.; Oles, K. S. *Annal. Pharmacotherap*.**1996**, *30*, 514. https://doi.org/10.1177/106002809603000515
13. Mahjour, B.; Shen, Y.; Liu, W.; Cernak, T. *Nature* **2020**, *580*, 71. https://doi.org/10.1038/s41586-020-2142-y
14. Kumari, S.; Carmona, A. V.; Tiwari, A. K.; Trippier, P. C. *J. Med .Chem.* **2020**, *63*, 12290.

 

https://doi.org/10.1021/acs.jmedchem.0c00530 .
15. Gasteiger, J. *Molecules* **2016**, *21*, 151. https://doi.org/10.3390/molecules21020151
16. Wade, R. C.; Goodford, P. J. *Prog. Clin. Biol. Res.* **1989**, *289*, 433.
17. Liu, J.; Patlewicz, G.; Williams, A. J.; Thomas, R. S.; Shah, I. *Chem. Res. Toxicol.* **2017**, *30*, 2046. https://doi.org/10.1021/acs.chemrestox.7b00084
18. Boström, J.; Brown, D. G.; Young, R. J.; Keserü, G. M. *Nat. Rev. Drug Discov.* **2018**, *17*, 709. https://doi.org/10.1038/nrd.2018.116
19. Liu, R.; Li, X.; Lam, K. S. *Curr. Opin. Chem. Bio.l* **2017**, *38*, 117. https://doi.org/10.1016/j.cbpa.2017.03.017
20. Al-Ghorbani, M.; Bushra Begum, A.; Zabiulla, Z.; Mamatha, S. V.; Khanum, S. A. *Res. J. Pharm. Technol*. **2015**, *8*, 611. https://doi.org/10.5958/0974-360X.2015.00100.6
21. Vishnu, T.; Veerabhadraiah, M.; Krishna Chaitanya, V.; Nagamani, M.; Raghavender, M.; Jalapathi, P. *Mol. Divers.* **2023**, *27*, 2695. https://doi.org/10.1007/s11030-022-10575-6
22. Veeranna, D.; Ramdas, L.; Ravi, G.; Bujji, S.; Thumma, V.; Ramchander, J. *ChemistrySelect* **2022**, *7*, e202201758. https://doi.org/10.1002/slct.202201758
23. Ruddarraju, R. R.; Murugulla, A. C.; Kotla, R.; Tirumalasetty, M. C. B.; Wudayagiri, R.; Donthabakthuni, S.; Maroju, R. *Medchemcomm* **2017**, *8*, 176. https://doi.org/10.1039/C6MD00479B
24. Yarden, Y.; Pines, G. *Nat. Rev. Cancer* **2012**, *12*, 553. DOI:10.1038/nrc3309.
25. Schlessinger, J. *Cold Spring Harb. Perspect. Biol.* **2014**, *6*, a008912. https://doi.org/10.1101/cshperspect.a008912
26. Vanga, M. K.; Bhukya, R.; Thumma, V.; Ambadipudi, S. S. S. S. S.; Nayak, V. L.; Andugulapati, S. B.; Manga, V. *RSC Med. Chem.* **2024**, *15*, 1709. https://doi.org/10.1039/D4MD00015C
27. Lu, Y.; Wang, Z.; Li, C.-M.; Chen, J.; Dalton, J. T.; Li, W.; Miller, D. D. *Bioorg. Med. Chem.***2010**, *18*, 477. https://doi.org/10.1016/j.bmc.2009.12.020
28. Huo, H.; Jiang, W.; Sun, F.; Li, J.; Shi, B. St*eroids* **2021**, *176*, 108931. https://doi.org/10.1016/j.steroids.2021.108931
29. Ashraf, Z.; Mahmood, T.; Hassan, M.; Afzal, S.; Rafique, H.; Afzal, K.; Latip, J. *Drug Des. Devel. Ther.* **2019**, *13*, 1643. https://doi.org/10.2147/DDDT.S178595
30. Rejinthala, S.; Endoori, S.; Thumma, V.; Mondal, T. *ChemistrySelect* **2024**, *9*. https://doi.org/10.1002/slct.202303299
31. Nagamani, M.; Vishnu, T.; Jalapathi, P.; Srinivas, M. *J. Iran. Chem. Soc.* **2022**, *19*, 1049. https://doi.org/10.1007/s13738-021-02365-y
32. Padrón, D.; Sato, M.; Shay, J. W.; Gazdar, A. F.; Minna, J. D.; Roth, M. G. *Cancer Res* **2007**, *67*, 7695. https://doi.org/10.1158/0008-5472.CAN-07-0484
33. Rejinthala, S.; Endoori, S.; Thumma, V.; Mondal, T. *Chem. Biodivers.* **2024**. https://doi.org/10.1002/cbdv.202301456
34. Nicholson, R. I.; Gee, J. M. W.; Harper, M. E. *Eur. J. Cancer* **2001**, *37*, 9.

 

https://doi.org/10.1016/S0959-8049(01)00231-3
35. Kurczab, R.; Kucwaj-Brysz, K.; Śliwa, P. *Molecules* **2020**, *25*. https://doi.org/10.3390/molecules25010091
36. Daina, A.; Michielin, O.; Zoete, V. *Sci. Rep.* **2017**, *7*, 42717. https://doi.org/10.1038/srep42717
37. Thumma, V.; Mallikanti, V.; Matta, R.; Dharavath, R.; Jalapathi, P. RSC Med. Chem. **2024**, *15*, 1283. https://doi.org/10.1039/D3MD00479A
38. Lipinski, C. A. *Drug Discov Today Technol* **2004**, *1*, 337. https://doi.org/10.1016/j.ddtec.2004.11.007
39. Daina, A.; Michielin, O.; Zoete, V. J *Chem. Inf. Model* **2014**, *54*, 3284. https://doi.org/10.1021/ci500467k
40. Myakala, N.; Kandula, K.; Rayala, N.; Kuna, S.; Thumma, V.; Durga Bhavani Anagani, K. *Chem. Biodivers.* **2023**, *20*, e202300800. https://doi.org/10.1002/cbdv.202300800
41. Dallakyan, S.; Olson, A. J. In *Methods in Molecular Biology*; Springer, **2015**; Vol. 1263, 243. https://doi.org/10.1007/978-1-4939-2269-7_19
42. Gali, S.; Raghu, D.; Mallikanti, V.; Thumma, V.; Vaddiraju, N. *Mol. Divers.* **2024**, *28*, 1347. https://doi.org/10.1007/s11030-023-10661-3