

Design, Synthesis and Characterization of Novel Sulfonamides Derivatives as Anticancer Agent Targeting EGFR TK, and Development of New Methods of Synthesis by Microwave Irradiation

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Abstract

Some novel sulfonamide-derivatives were designed to develop novel kinase inhibitors. The molecular docking study was performed for the designed compounds against epidermal growth factor kinase receptor T790M/L858R (TMLR) (PDB ID: 5EDQ) to identify new drug candidates for treating cancer. Binding free energy was calculated by Molegro virtual docker (MVD) to select the most promising hits. The corresponding docking score values into EGFR (TMLR) of 4b gave the best energy docking -147.213 Kcal/mol. And some of the designed sulfonamide derivatives have been synthesized by conventional method in addition to a microwave-assisted method of synthesis. The reaction of an amino group-containing drug; sulfamethoxazole and sulfanilamide with carbonyl group in benzoyl chloride and phthalic acid in basic media, generated a series of sulfonamide derivatives. The structures of all the synthesized compounds were well characterized by Mass spectrometry (MS), Infrared spectroscopy (IR), ^1H nuclear magnetic resonance (^1H NMR), ^{13}C nuclear magnetic resonance (^{13}C NMR) and elemental analysis. After obtaining experimental data regarding the yield and the time taken for the synthesis by both the approaches, conventional and microwave-assisted method, it was shown that the microwave-assisted method gave higher yield with shorter time and higher temperature compared to conventional heating methods.

Keywords

Sulfonamide, Anticancer, EGFR, TMLR, 5EDQ, Molegro Virtual Docker, Sulfamethoxazole, Sulfanilamide, Microwave

1. Introduction

Cancer is a worldwide health problem and the most deadly disease in humans [1] [2], and it is considered the second leading cause of mortality after cardiovascular diseases [2]. There are several methods for the treatment of cancer such as Surgery, Chemotherapy, Hormonal therapy, Immunotherapy [3] [4], and Phototherapy [5]. Today, anticancer chemotherapy is still the main method applied in the treatment of cancer [6]. Chemotherapy drugs include antitumor antibiotics, anti-metabolites, mitotic inhibitors, hormonal therapies. Cancer chemotherapy offers a unique advantage: it can treat the entire body, even the cells that may have escaped from the primary tumor [4] [7].

Among the wide range of compounds tested as potential anticancer agents, derivatives of sulfonamide have attracted reasonable attention [8].

The compounds which contain SO_2NH_2 functional group are called sulfonamides. The general formula of sulfonamides is RSO_2NH_2 [9] (Figure 1).

Sulfa drugs are amphoteric, they have pK_a 4.79 to 8.56 and act as weak organic acids. They are weakly soluble in water, their solubility is increased at alkaline pH. The lipophilicity of the N_1 group has the largest effect on protein binding [10]. Sulfonamide derivatives comprise an important class of drugs with diverse biological applications [11]. Over 30 drugs containing this functional group are in clinical use, including antihypertensive, antibacterial, antiprotozoal, antifungal, anti-inflammatory, non-peptidic vasopressin receptor antagonists, translation initiation inhibitors, rheumatoid arthritis, antimalarial, anti-leishmanial, anti-thyroid, Antidepressant [10] [11] [12], hypoglycemics, anticonvulsants [13], diuretic, receptor tyrosine kinase inhibitors, and antipsychotics [1]. They are also used to treat ulcerative colitis, urinary, intestinal, and ophthalmic infections [14]. Recently, sulfonamides have been used as anti-cancer, anti-viral, and anti-HIV [15] [16], and in Alzheimer's disease [13].

Epidermal growth factor receptor (EGFR) is a member of the tyrosine kinase family and is usually overexpressed in several types of cancer, such as non-small-cell lung cancer, breast, esophageal, head, cervical, and neck cancer [17] [18]. The TMLR (T790M/L858R) mutation, the L858R mutation is located in the tyrosine kinase domain of EGFR in exon 2 and deletions within exon 19, and T790M is located in exon 20 of *EGFR* within the kinase domain where the threonine gatekeeper residue Thr790 is exchanged for methionine T790M [19] [20] [21].

Recently, some sulfonamide derivatives carrying pyrrole and pyrrolopyrimidine cycles have been synthesized by Ghorab *et al.* in 2014 and have antitumor activity. Most of the synthesized compounds showed good activity as cytotoxic

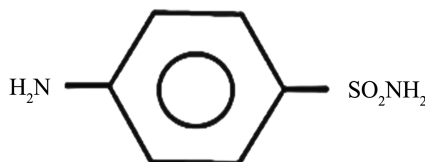


Figure 1. The structure of sulfanilamide.

agents with better IC₅₀ than doxorubicin as a reference drug. The molecular docking study was performed on the human c-Src for all synthesized compounds [22].

Ihmaid *et al.* in 2018 designed and synthesized some novel anthranilate sulfonamide derivatives having anticancer activity. The molecular docking study was performed against EGFR [6].

In the past few years, the use of microwave as an energy resource to induce organic reactions has been a useful technique in organic synthesis. Microwave irradiation has been shown to dramatically reduce reaction times, increase product yields and enhance product purities by reducing unwanted side reactions compared to conventional heating methods [23] [24] [25].

Due to our interest in the development of novel anticancer agents, in this study, we report the design and synthesis of some novel sulfonamide derivatives and we expect that these new compounds might show significant anticancer activity.

2. Materials and Methods

Protein Data Bank (PDB), PubMed and software like ChemSketch version 14.01, Marvin sketch version 21.2, and Molegro Virtual Docker (MVD) version 2011.4.3 were implemented within the current study.

A Monowave 300 microwave oven was used for microwave synthesis (Anton Paar, Austria) and we used reaction vial G30 equipped with snap caps and silicon septa. Melting points were determined in open capillary on a BÜCHI Melting Point B-540 apparatus (BÜCHI Labortechnik, Switzerland). Analytical thin-layer chromatography (TLC) was performed with silica gel 60 F254 aluminum sheets (Macherey-Nagel, Germany). IR spectra (KBr disc) were recorded using an ATR-FTIR Bruker spectrophotometer (Bruker, Billerica, Massachusetts). ¹H-NMR spectra were scanned on JEOL-ECA NMR spectrophotometer (Joel, Tokyo, Japan), operating at 400 MHz for ¹H and ¹³C. Chemical shifts are expressed in δ -values (ppm), using DMSO-d₆ as a solvent. Mass spectra (MS) were scanned by the triple quadrupole mass spectrometer with positive ionization (Sciex, Framingham, USA). The m/z values of the more intense peaks are mentioned.

EGFR structure

The 3D crystal structure of EGFR Kinase T790M/L858R (TMLR) (PDB ID: 5EDQ) domain was retrieved from PDB and has a resolution of 2.8 Å. The kinase domain consists of 327 residues between 695 - 1022 residues. The resolved EGFR structure was co-crystallized as holo-form with a known kinase inhibitor (*N*-(7-Chloro-1*H*-indazol-3-yl)-7,7-dimethyl-2-(1*H*-pyrazol-4-yl)-5*H*-furo[3,4-*d*]pyrimidin-4-amine) (5N3) (Figure 2).

Define the binding pocket:

The binding pocket was defined using the co-crystallized ligand as a center of the pocket, which had a volume of 312.32 Å³, and fitted to the polar surface area for designed compounds (Figure 3).

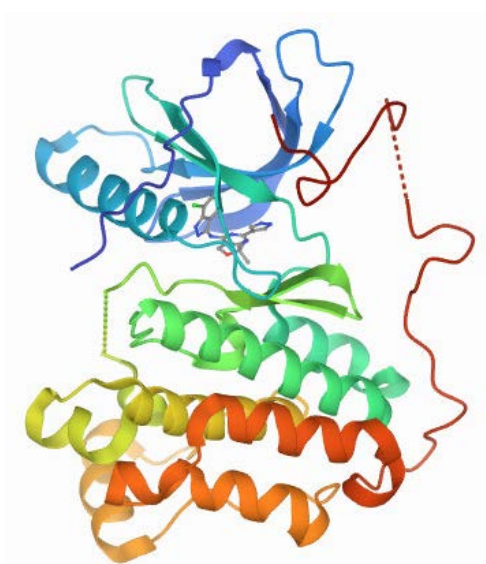


Figure 2. Co-crystal structure of the EGFR TMLR (PDB ID: 5EDQ) kinase domain in complex with 5N3.

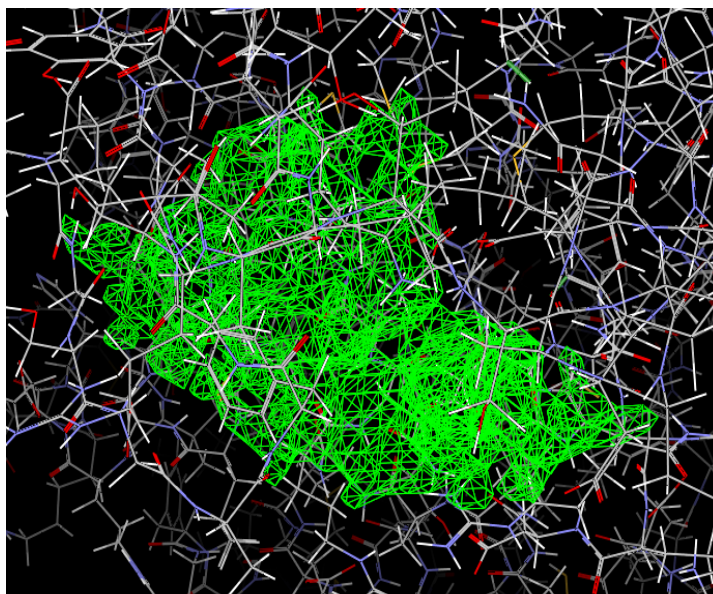


Figure 3. EGFR (TMLR) binding pocket (PDB ID: 5EDQ) used to dock the designed compounds.

Compounds preparation:

Structures of designed compounds were drawn and optimized by using Marvin Sketch and saved as mol2. The preparation of compounds was performed using the default setting to assign bonds, create explicit hydrogens, assign bond orders and hybridization, assign charges (calculated by MVD), assign tripos atom types, and detect flexible torsions in ligands.

Molecular Docking:

The Molecular Docking was performed in MVD. The following parameters were used for docking in the EGFR kinase (**Table 1**).

Table 1. Parameters of Molegro Virtual Docker.

Parameters	Value
Scoring function	Plants score (GRID)
Grid resolution (Å)	0.30
Binding site radius (Å)	15
Searching algorithm	MolDock SE
Number of runs	10
Max iterations	1500
Max population size	50
Energy threshold	100
Simplex evaluation (max steps)	300
Neighbor distance factor	1

The results of docking compounds with the receptors were compared with Osimertinib and Rociletinib which are in use to treat non-small cell lung cancer (NSCLC). Osimertinib docked with EGFR with mutation T790M, L858R, and exon 19 deletions, and Rociletinib docked with EGFR including T790M mutation.

General procedures for the synthesis of compounds:

• Synthesis of compound 1b

Pyridine (10 ml) was placed in a 100 ml round flask fitted with a magnetic stirrer and heated to 70°C. Sulfamethoxazole (5 mmol) was added to the warmed liquid in several portions, creating a homogeneous yellow solution. The warm solution was stirred while benzoyl chloride (5 mmol) was added dropwise. The mixture was stirred for 30 minutes with maintaining the temperature at 70°C. The mixture was cooled in a cold water bath, then poured into chipped ice to produce a white precipitate, then, filtration, drying at 100°C and recrystallizing from ethanol.

• Synthesis of compound 1c

(11.6 mmol) of sulfanilamide dissolved in 20 ml of 4% NaOH and placed in a 100 ml conical flask. Then, (23.2 mmol) of benzoyl chloride was added slowly with vigorous shaking. The flask was closed and shaken for further 30 min or till the odor of benzoyl chloride can no longer be detected. When the reaction finished, a white precipitate was formed, then, filtration, drying and recrystallizing from hot methanol.

• Synthesis of compound 3b

A mixture of sulfamethoxazole (8 mmol) and triethylamine (2 ml) in dry DMF (10 mL) were stirred under reflux for 1 h. A solution of phthalic acid (8 mmol) was then added dropwise. The reaction mixture was heated under reflux at 200°C in oil bath for 3 h. After cooling, the reaction mixture was poured into ice water and the formed solid was collected by filtration and recrystallized from

acetonitrile.

- **Synthesis of compound 3d**

Sulfanilamide (11.6 mmol) and triethylamine (2 ml) in dry DMF (10 mL) were stirred under reflux for 1 h. Phthalic acid (11.6 mmol) was then added dropwise. The reaction mixture was heated under reflux at 200°C in oil bath for 2.5 h. After cooling, the reaction mixture was poured into ice water and the product was collected by filtration and recrystallized from DMF.

Synthesis of compounds by microwave irradiation:

- **Synthesis of compound 1b**

A mixture of sulfamethoxazole (5 mmol) and benzoyl chloride (5 mmol) was dissolved in 5 ml pyridine and placed in a 30-mL sealed vial. Then, it is put in the microwave and heated to 120°C, and maintained at this temperature for 10 min. Finally, the reaction was cooled to 55°C. After completion of the reaction, the mixture of reaction was poured into cold water to produce a white precipitate which was filtered, washed with water and methanol, and dried.

- **Synthesis of compound 1c**

(5.8 mmol) of sulfanilamide dissolved in 5 ml pyridine and placed in a 30-mL sealed vial, then, (11.6 mmol) of benzoyl chloride was added and put in the microwave, then, was heated to 100°C and was maintained at this temperature for 5 min. Then, the reaction was cooled to 55°C. When the reaction finished, a white precipitate was formed, which was filtered, washed with water and methanol, and dried.

- **Synthesis of compound 3b**

Sulfamethoxazole (4 mmol) and phthalic acid (4 mmol) were dissolved in 5 ml pyridine and placed in a 30-mL sealed vial. The reaction mixture was heated to 200°C and was kept at this temperature for 20 min. Then, the reaction was cooled to 55°C. After cooling, the reaction mixture was poured into ice water and the formed solid was collected by filtration, washing with water and methanol, and drying.

- **Synthesis of compound 3d**

A mixture of Sulfanilamide (5.8 mmol) and phthalic acid (5.8 mmol) was dissolved in 10 ml pyridine and placed in a 30-mL sealed vial. The reaction mixture was heated to 200°C and was kept at this temperature for 20 min. Then, the reaction was cooled to 55°C. After cooling, a white precipitate was formed which was filtered, washed with water and methanol, and dried.

3. Results and Discussion

Sulfonamide derivatives

The core scaffold of the sulfonamide shown in (**Figure 4**) was used to design several derivatives in the current study. The structure of designed sulfonamide derivatives is shown in (**Table 2**).

Molecular Docking results

The list of compounds was docked into the EGFR (TMLR) receptor binding pocket using the validated docking methods. Docking results tabulated between

Table 2. Structure of sulfonamide derivatives considered in the study.

	Structure		Structure
1a		5a	
1b		5b	
1c		5c	
2a		6a	
2b		6b	
2c		6c	
3a		7a	
3b		7b	

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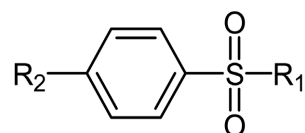
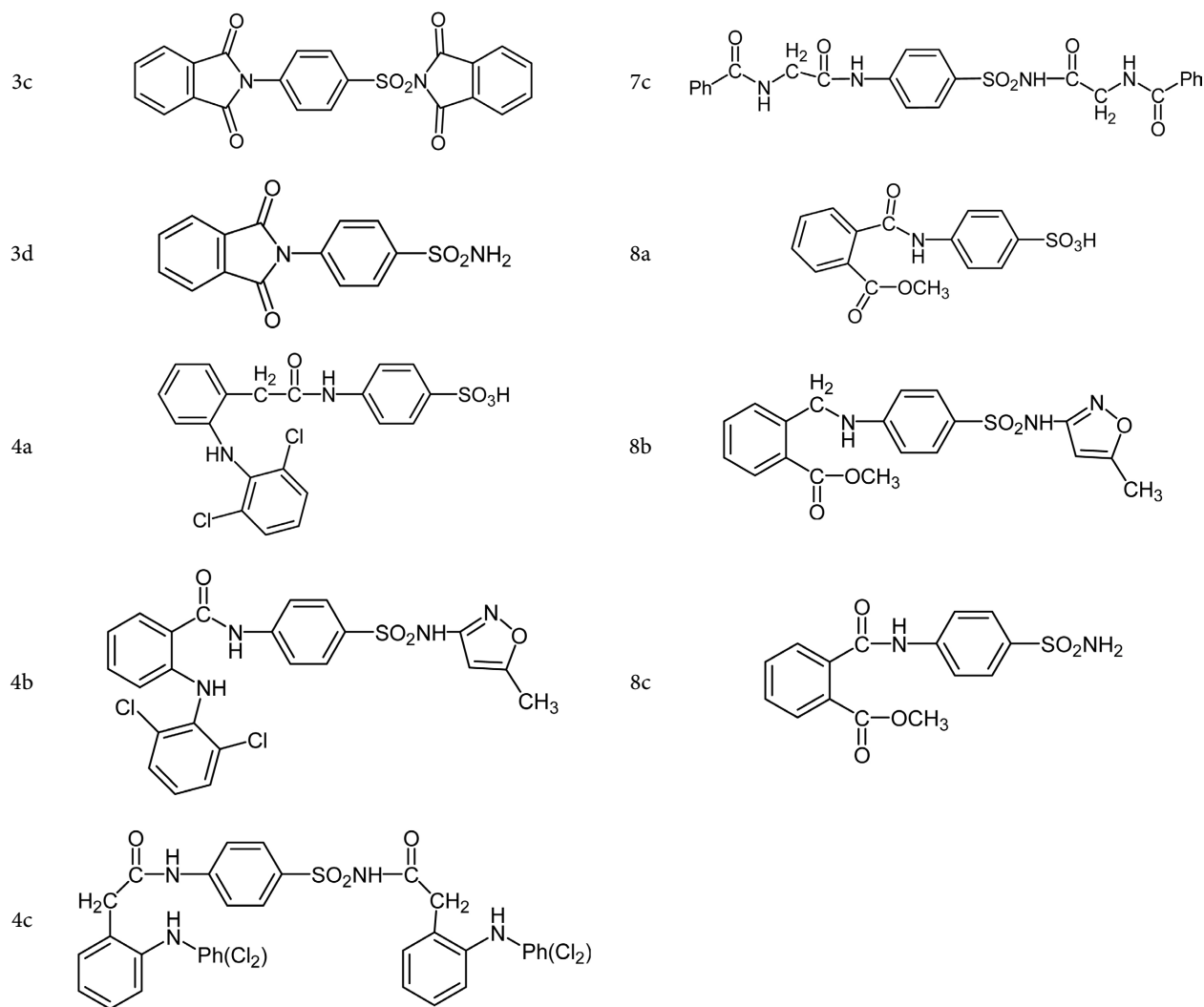


Figure 4. Scaffold of sulfonamide.

the EGFR (TMLR) binding pocket and the derivatives of sulfonamide are shown (Table 3).

Validation docking method

Validation of docking was used to ensure the orientation and position of ligand binding obtained from docking studies by MVD program. Therefore, the parameters must be validated by re-dock the native ligand into the crystal structure (PDB ID: 5EDQ). After that, it was extracted and re-docked into the binding pocket to generate the X-ray binding mode. The ability of the docking algorithm to generate the active binding mode of the ligands was evaluated by

Table 3. The energy docking of ligands with receptors.

Ligand	Energy docking with 2ITY (Kcal/mol)
4b	-147.213
5N3	-140.856
6c	-132.14
5b	-129.55
4c	-118.092
Osimertinib	-115.633
5c	-113.682
6b	-112.186
3b	-111.724
7b	-108.864
6a	-106.005
Rociletinib	-104.706
4a	-104.538
2b	-101.977
3c	-95.6706
3a	-91.0155
2c	-90.485
1b	-90.3229
5a	-88.3473
8b	-88.1895
3d	-86.0797
1c	-84.7441
7c	-83.6132
8a	-81.1525
2a	-80.798
7a	-78.8822
1a	-73.2927
8c	-70.8233

calculating the RMSD (Root Mean Square Deviation) between the docking solutions and the X-ray binding mode. $\text{RMSD} < 2 \text{ \AA}$ considered as a threshold in the computer-aided drug design.

The RMSD value for the top-ranked docking solution of ligand was 0.565382. Thus, the applied docking methods were able to generate the X-ray binding mode of the ligand.

The interactions between the binding pocket residues of the EGFR (TMLR) receptor and the sulfonamide derivatives together with energy docking are shown in **Table 4**.

Synthesis of compounds

• Mechanism of synthesis

Compound 1b

The reaction of sulfamethoxazole (1) with benzoyl chloride (2) in pyridine with refluxing, results in the desired *N*-{4-[(5-methyl-1,2-oxazol-3-yl)sulfamoyl]phenyl}benzamide (1b) in 84% yield, and in 89% yield by microwave synthesis (**Figure 5**).

Compound 1c

The reaction of sulfanilamide (3) with benzoyl chloride (2) in NaOH with shaking, results in the desired *N*-{4 (benzoylamino)phenyl}sulfonyl}benzamide (1c) in 50% yield, and in 82% yield by microwave synthesis (**Figure 6**).

Compound 3b

The reaction of sulfamethoxazole (1) with phthalic acid (4) in DMF with refluxing, results in the desired 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-(5-methyl-1,2-oxazol-3-yl) benzenesulfonamide (3b) in 50% yield, and in 88% yield by microwave synthesis (**Figure 7**).

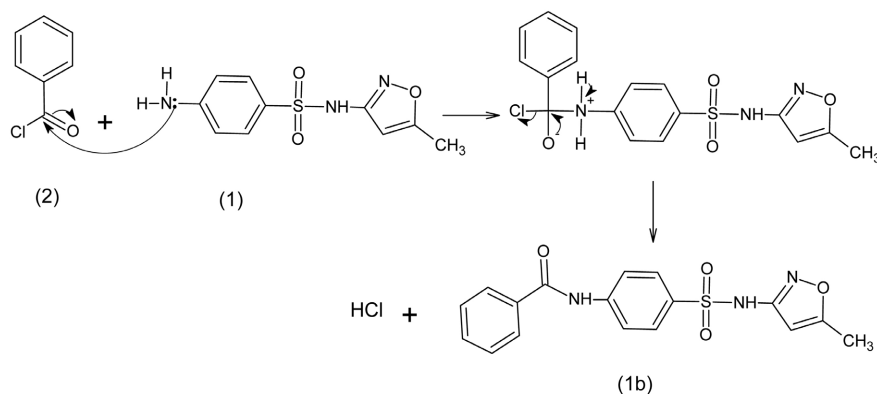


Figure 5. The mechanism of synthesis of *N*-{4-[(5-methyl-1,2-oxazol-3-yl) sulfa-moyl]phenyl}benzamide (1b).

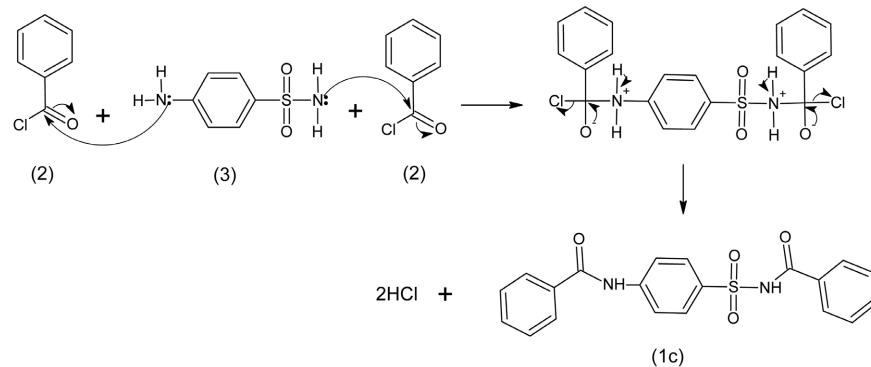


Figure 6. The mechanism of synthesis of *N*-{4 (benzoylamino)phenyl}sulfonyl} benza-mide (1c).

Table 4. Interaction of the amino acids in 5EDQ with ligands.

ligand	Residue	Interaction	Distance (Å)	Energy	Van der Waals interaction
1a	Met793	O-H	3.1	-2.5	-
	Asp855	O-H	3.04	-2.5	
	Asp855	O-H	2.91	-1.55509	
	Thr854	O-H	2.9	-2.5	
1b	Met793	O-H	2.66	-2.5	Pro794
	Pro794	N-H	3.057	-0.701673	
1c	Asp855	N-H	2.94	-2.5	Asp855, Pro794
2a	Met793	O-H	2.8	-2.5	-
	Asp855	O-H	3.05	-1.90438	
	Thr854	O-H	2.9	-2.5	
	Lys745	O-H	3	-2.5	
2b	Gln791	O-H	2.6	-2.5	-
	Met793	O-H	2.98	-1.3688	
	Met793	O-H	2.9	-2.5	
2c	Ser720	O-H	3.04	-0.750539	-
	Gly719	O-H	2.86	-2.5	
3a	Met793	O-H	2.8	-2.5	-
	Thr854	O-H	3.03	-2.5	
	Asp855	O-H	2.92	-1.56838	
	Lys745	O-H	3	-2.5	
3b	Gly724	N-H	3.16	-1.74979	Gly719
	Gly724	O-H	2.85	-1.35909	
	Ser720	N-H	2.8	-2.5	
3c	Lys745	O-H	3.1	-2.5	Val726, Arg841, Gly721
	Val726	O-H	3.3	-0.757545	
	Phe723	O-H	3	-2.5	
	Gly724	O-H	2.65	-2.5	
	Asp855	O-H	2.7	-2.5	
3d	Met793	O-H	2.8	-2.5	-
	Thr854	O-H	2.95	-2.5	
	Lys745	O-H	2.8	-2.5	
4a	Phe795	O-H	3	-2.5	-
4b	Lys745	O-H	2.77	-2.5	Leu788, Thr790, Lys745, Asp855
	Cys797	N-H	3.1	-2.5	

Continued

4c	Lys745	O-H	3.1	-0.195019	Glu762, Lys745, Gly719, Asp855
5a	Thr854	O-H	3.17	-2.10359	Leu844
	Ser720	N-H	2.7	-2.5	
5b	Lys745	N-H	2.7	-1.08594	Glu762
	Lys745	O-H	2.8	-2.08639	
5c	Met793	O-H	3	-2.38289	Val726, Leu747
6a	Thr854	O-H	3	-1.74103	Leu792, Gly796, Pro794, Leu844
6b	Leu718	N-H	3	-2.5	-
6c	Thr854	O-H	2.7	-2.5	Lys745, Met793, Val726
	Gly719	N-H	2.85	-1.99386	
7a	Lys745	O-H	3.2	-1.95469	Phe723
	Cys797	O-H	3.3	-1.34206	
	Asp800	O-H	3.06	-2.49205	
	Gly719	N-H	3.06	-0.808774	
	Gly721	N-H	3.1	-0.651249	
7b	Ser720	N-H	2.6	-2.42948	Ser720
	Gly724	N-H	3.17	-1.54498	
	Gly724	O-H	3	-1.1997	
7c	Met793	O-H	2.93	-2.36929	Lys745, Ala743
	Asp855	N-H	3.1	-0.133077	
	Met793	O-H	3.3	-1.22411	
8a	Thr854	O-H	2.8	-2.5	-
	Lys745	O-H	3	-2.5	
8b	Ser720	N-H	2.6	-1.84127	Met793
8c	Thr854	O-H	2.84	-2.5	-
	Lys745	O-H	2.94	-2.5	

Compound 3d

The reaction of sulfanilamide (3) with phthalic acid (4) in DMF with refluxing, results in the desired 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) benzene-sulfonamide (**3d**) in 91% yield, and in 97% yield by microwave synthesis (**Figure 8**).

- Analytical data

N-{4-[(5-methyl-1,2-oxazol-3-yl)sulfamoyl]phenyl}benzamide (**1b**)

White powder, mp 278°C - 280°C. TLC; the mobile phase was used acetate

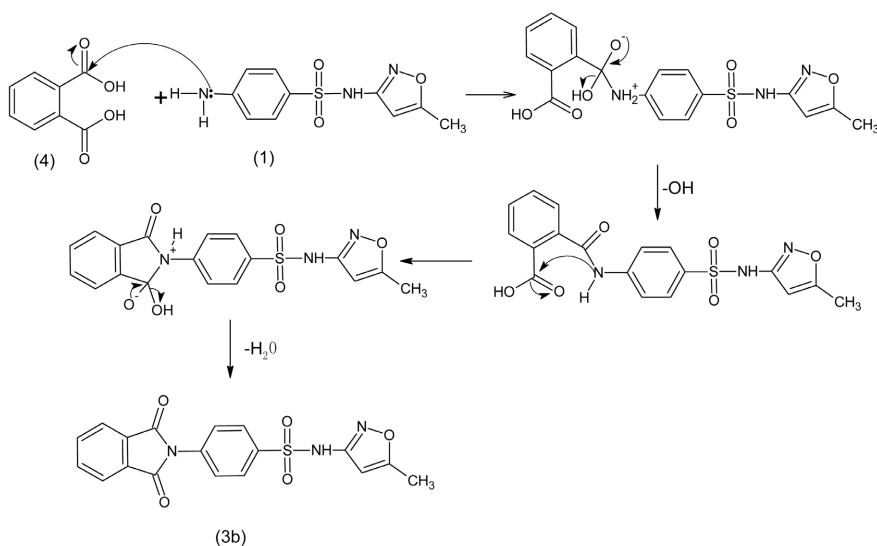


Figure 7. The mechanism of synthesis of 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide (**3b**).

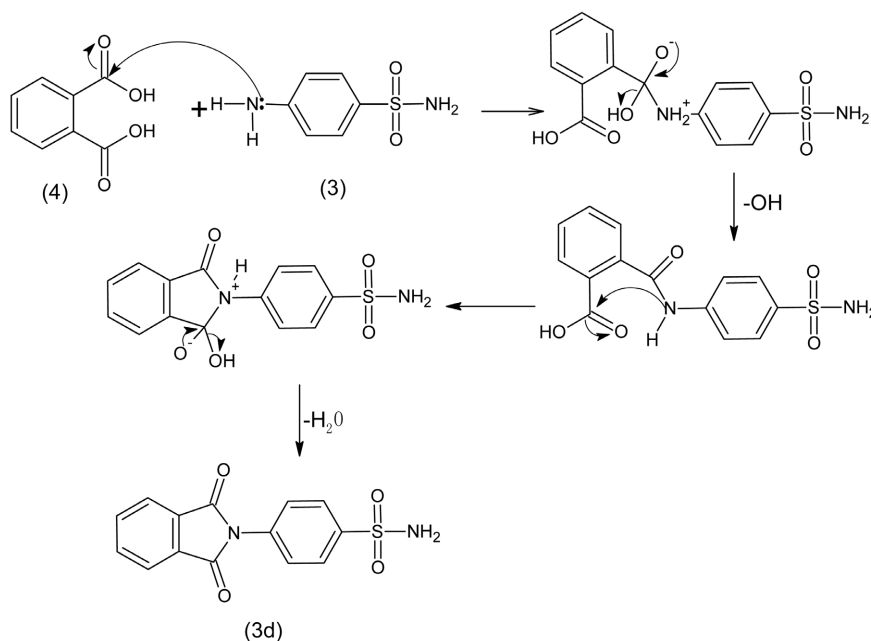
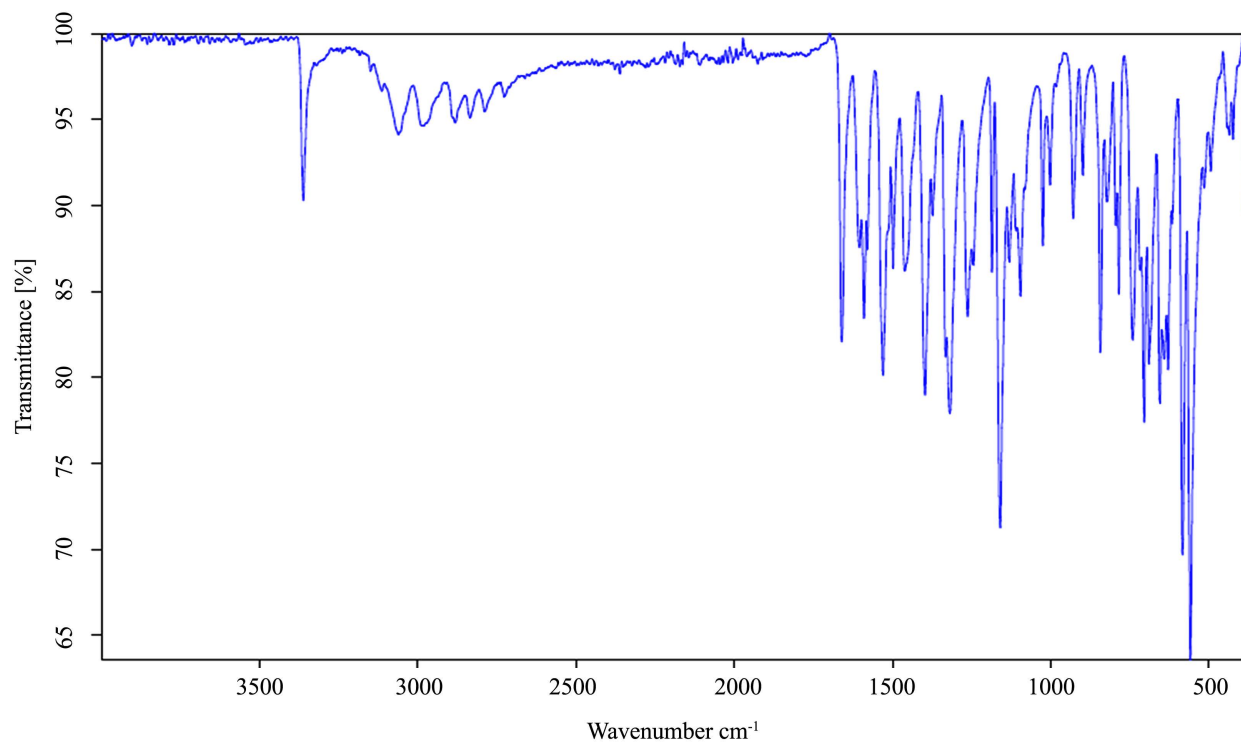
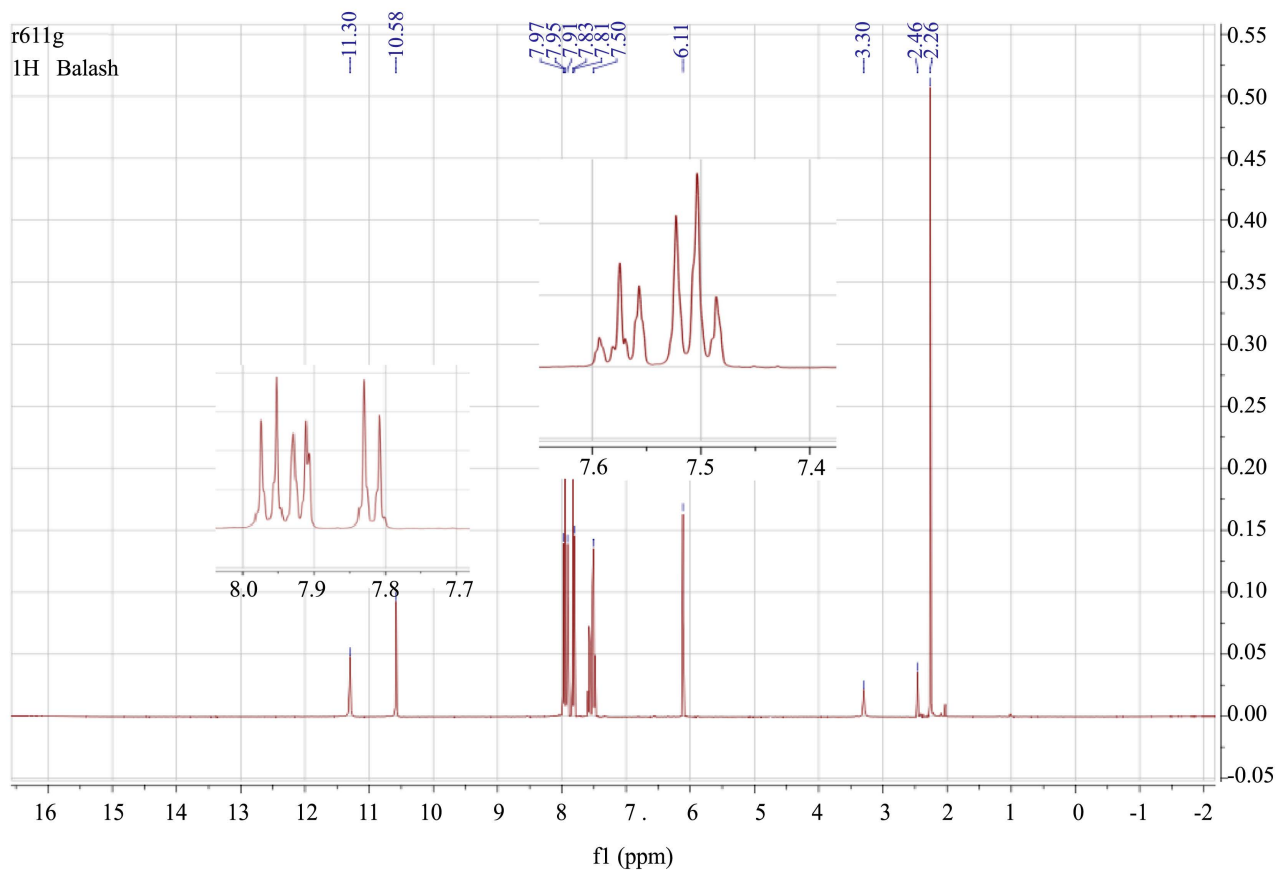


Figure 8. The mechanism of synthesis of 4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)benzenesulfonamide (**3d**).

ethyl: petrolatum ether 9:1, which the R_f for sulfamethoxazole 0.8 and the R_f for compound (**1b**) 0.88. IR spectrum (ν_{\max} , cm^{-1}): 3362 (N–H), 3061 (C–H arom), 2987, 2882 (C–H), 1660 (C=O), 1604, 1589, 1578 (C=C), 1529 (N–O), 1372, 1332 (S=O), 1318 (C–N) (**Figure 9**) [26]. ^1H -NMR spectrum (DMSO- d_6 , δ , ppm): 2.3 (s, 3H, CH_3), 6.1 (s, 1H, =CH–), 7.5 (t, 1H, Ar–H), 7.58 (t, 1H, Ar–H), 7.8 (d, 1H, Ar–H), 7.9 (d, 1H, Ar–H), 7.97 (d, 1H, Ar–H), 10.6 (s, 1H, NH), 11.3 (s, 1H, $\text{SO}_2\text{–NH}$) (**Figure 10**). ^{13}C -NMR (DMSO- d_6 , δ , ppm): 12.5, 96, 120.5, 128.3, 128.4, 129, 132.5, 135, 144, 158, 166.6, 170.8 (**Figure 11**) [27]. Mass spectrum

**Figure 9.** IR spectrum for 1b compound.**Figure 10.** ¹H-NMR spectrum for 1b compound.

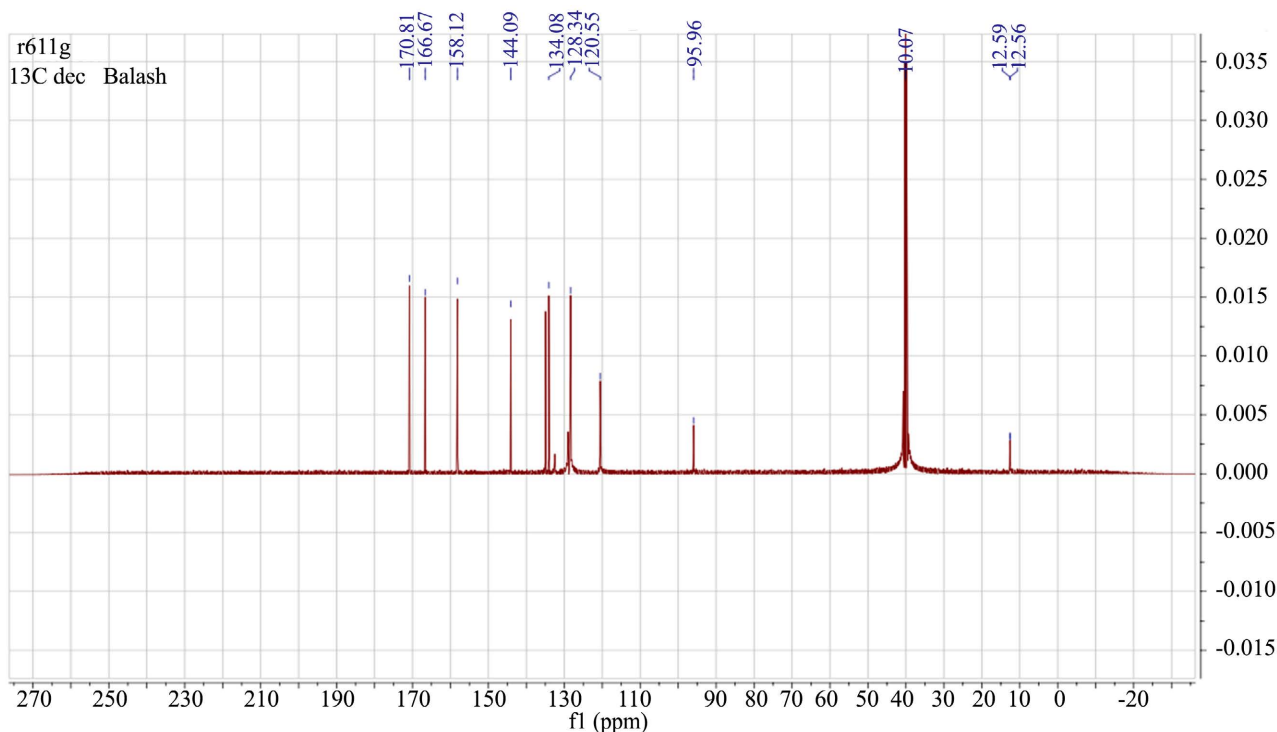


Figure 11. ^{13}C -NMR spectrum for 1b compound.

(m/z , ESI): showed molecular ion $[\text{M} + \text{H}]^+$ peak at 358.12 corresponding to the molecular formula of $\text{C}_{17}\text{H}_{15}\text{N}_3\text{O}_4\text{S}$, and $[\text{M} + \text{Na}]^+$ peak at 380.07.

N-{[4 (benzoylamino)phenyl]sulfonyl}benzamide (**1c**)

White powder, mp 296°C - 300°C . TLC; the mobile phase was used acetate ethyl: petrolatum ether 9:1, which the R_f for sulfanilamide 0.69 and the R_f for compound (**1c**) 0.79. IR spectrum (ν_{max} , cm^{-1}): 3359, 3264 (N-H), 1650 (C=O), 1590, 1513, 1484 (C=C), 1396, 1294 (S=O), 1266 (C-N) (**Figure 12**) [26]. ^1H -NMR spectrum (DMSO- d_6 , δ , ppm): 7.2 (t, 1H, Ar-H), 7.5 (t, 1H, Ar-H), 7.55 (t, 1H, Ar-H), 7.75 (d, 1H, Ar-H), 7.8 (d, 1H, Ar-H), 7.95 (d, 1H, Ar-H), 10.5 (s, 1H, NH) (**Figure 13**). ^{13}C -NMR (DMSO- d_6 , δ , ppm): 120.3, 127.05, 127.1, 128.3, 128.29, 128.31, 129, 132.4, 135.02, 135.04, 139.25, 139.3, 142.7, 166.5 (**Figure 14**) [27]. MS (m/z , ESI): The major peak for compound showed molecular ion $[\text{M} + \text{H}]^+$ peak at 381.15 corresponding to the molecular formula of $\text{C}_{22}\text{H}_{20}\text{N}_2\text{O}_2\text{S}$, and $[\text{M} + \text{Na}]^+$ peak at 403.21.

4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide (**3b**)

Pale white powder, mp 255°C - 258°C . TLC; the mobile phase was used acetate ethyl: petrolatum ether 7:3, which the R_f for sulfamethoxazole 0.67 and the R_f for compound (**3b**) 0.8. IR spectrum (ν_{max} , cm^{-1}): 3476 (N-H), 3107 (C-H arom), 3026, 3001 (C-H), 1739, 1713 (C=O), 1610, 1594, 1500 (C=C), 1413, 1380 (S=O), 1333 (C-N) (**Figure 15**) [26]. ^1H -NMR spectrum (DMSO- d_6 , δ , ppm): 2.3 (s, 3H, CH_3), 6.1 (s, 1H, =CH-), 7.7 (d, 1H, Ar-H), 7.9 (d, 1H, Ar-H), 7.95 (d, 1H, Ar-H), 8 (d, 1H, Ar-H), 11.5 (s, 1H, $\text{SO}_2\text{-NH}$) (**Figure 16**). ^{13}C -NMR

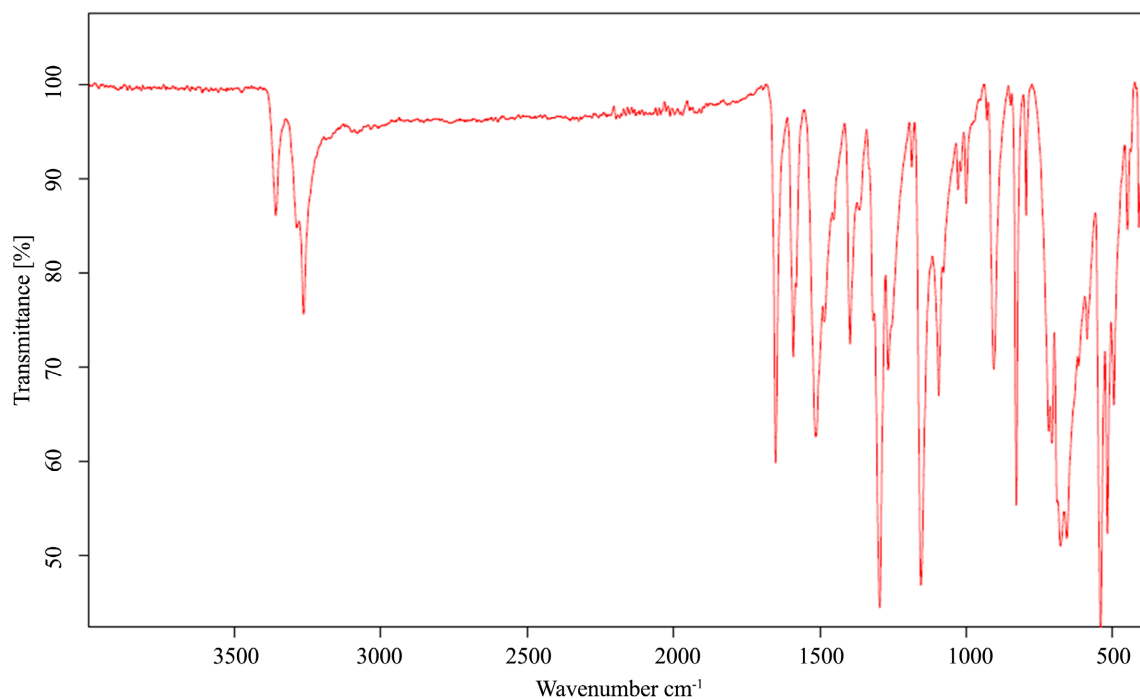


Figure 12. IR spectrum for 1c compound.

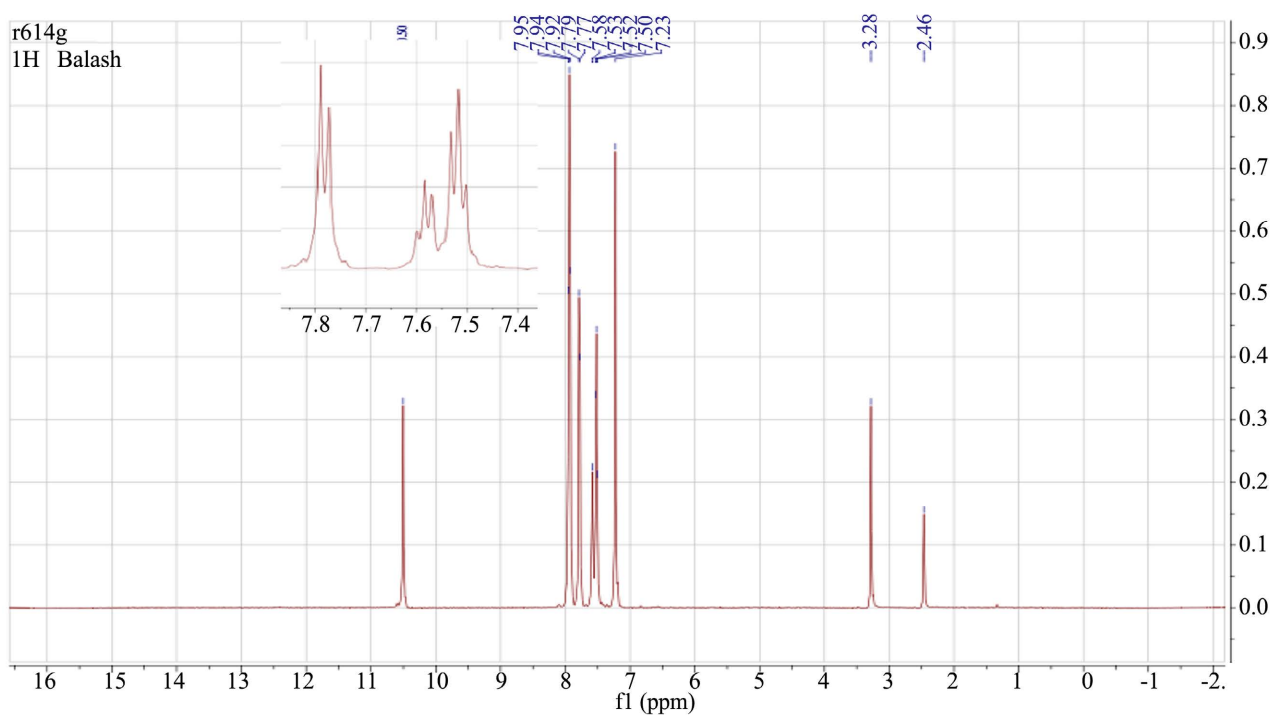


Figure 13. ^1H -NMR spectrum for 1c compound.

(DMSO- d_6 , δ ppm): 12.5, 9.6, 124.1, 128, 128.2, 132, 135.5, 136.7, 138.9, 158, 167, 171 (**Figure 17**) [27]. MS (m/z , ESI): showed $[\text{M} + \text{H}]^+$ peak at 384.05 corresponding to the molecular formula of $\text{C}_{18}\text{H}_{13}\text{N}_3\text{O}_5\text{S}$, and $[\text{M} + \text{Na}]^+$ peak at 406.03.

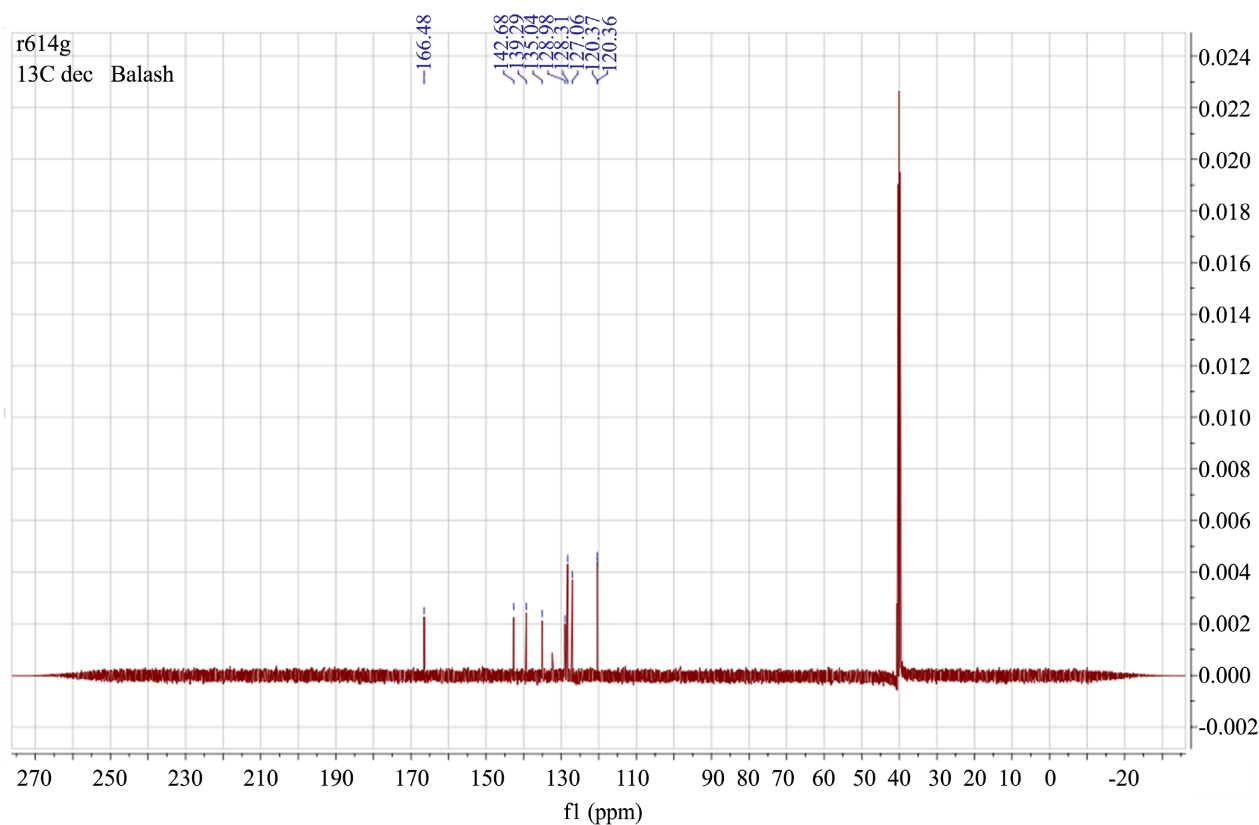


Figure 14. ^{13}C -NMR spectrum for 1c compound.

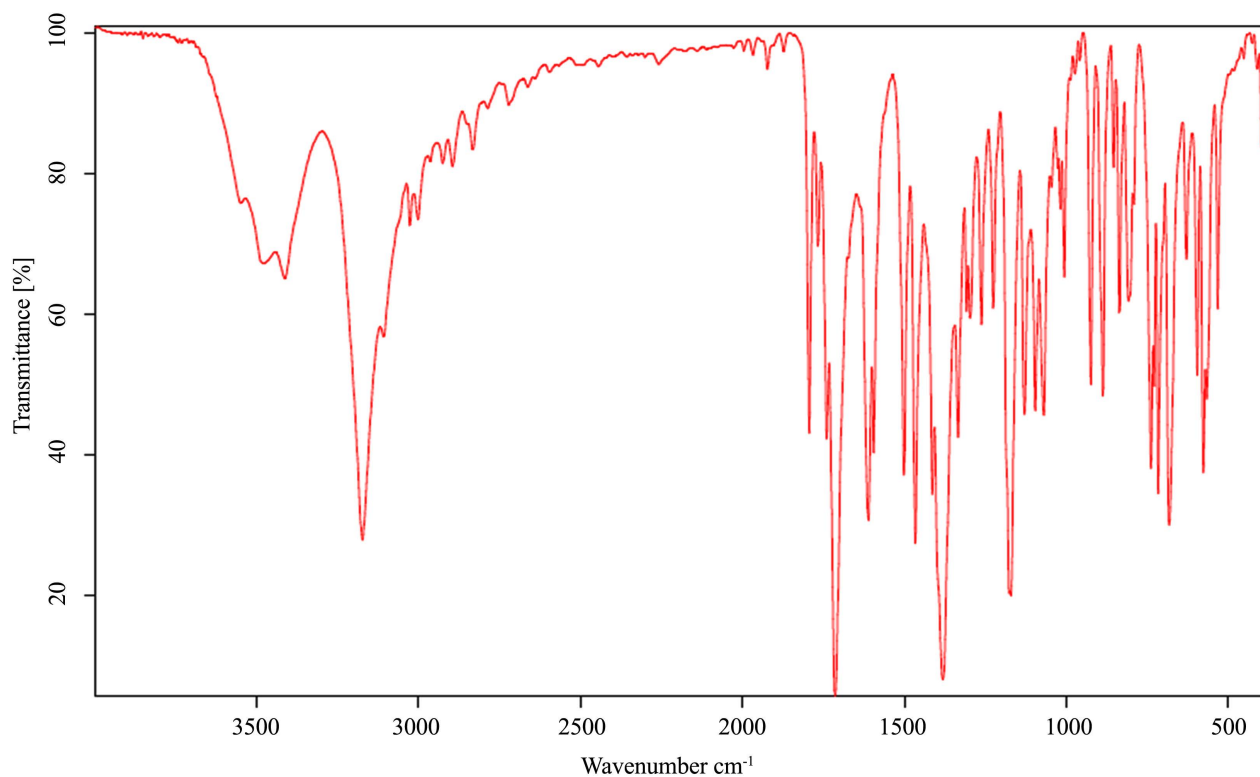


Figure 15. IR spectrum for 3b compound.

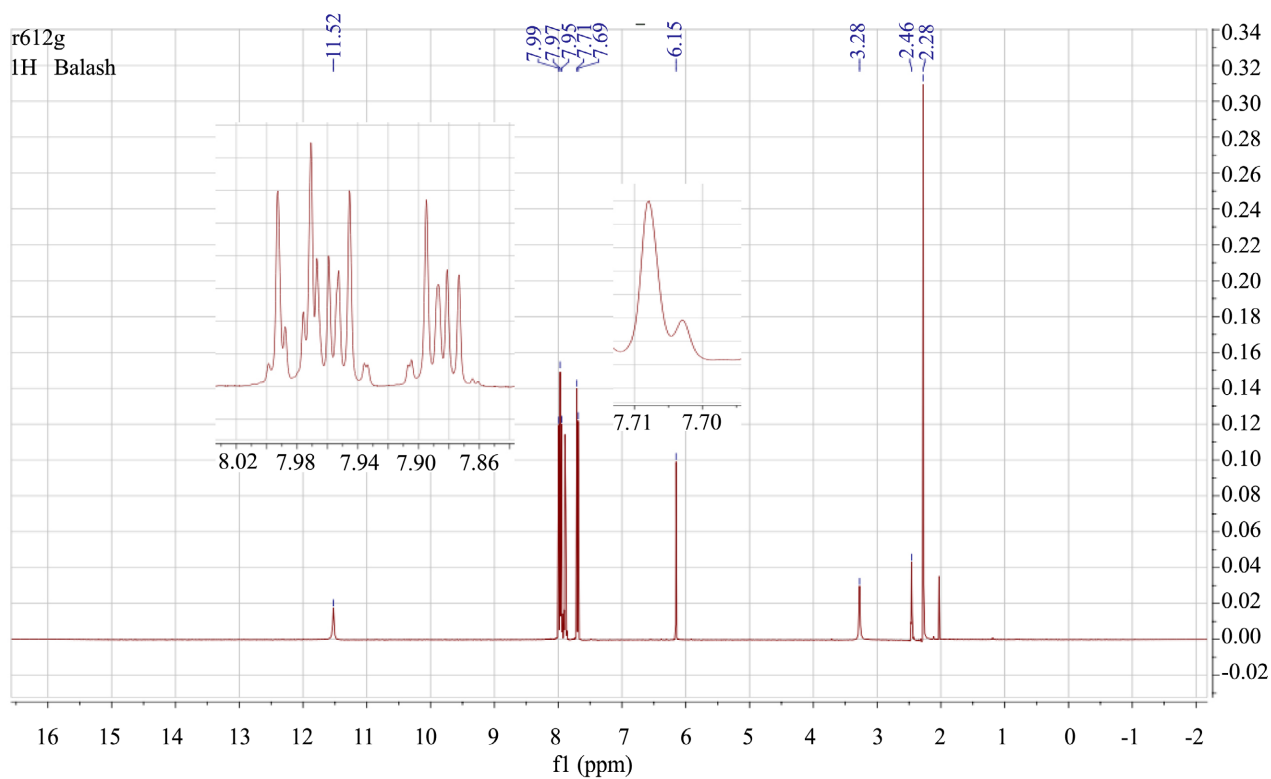


Figure 16. ^1H -NMR spectrum for 3b compound.

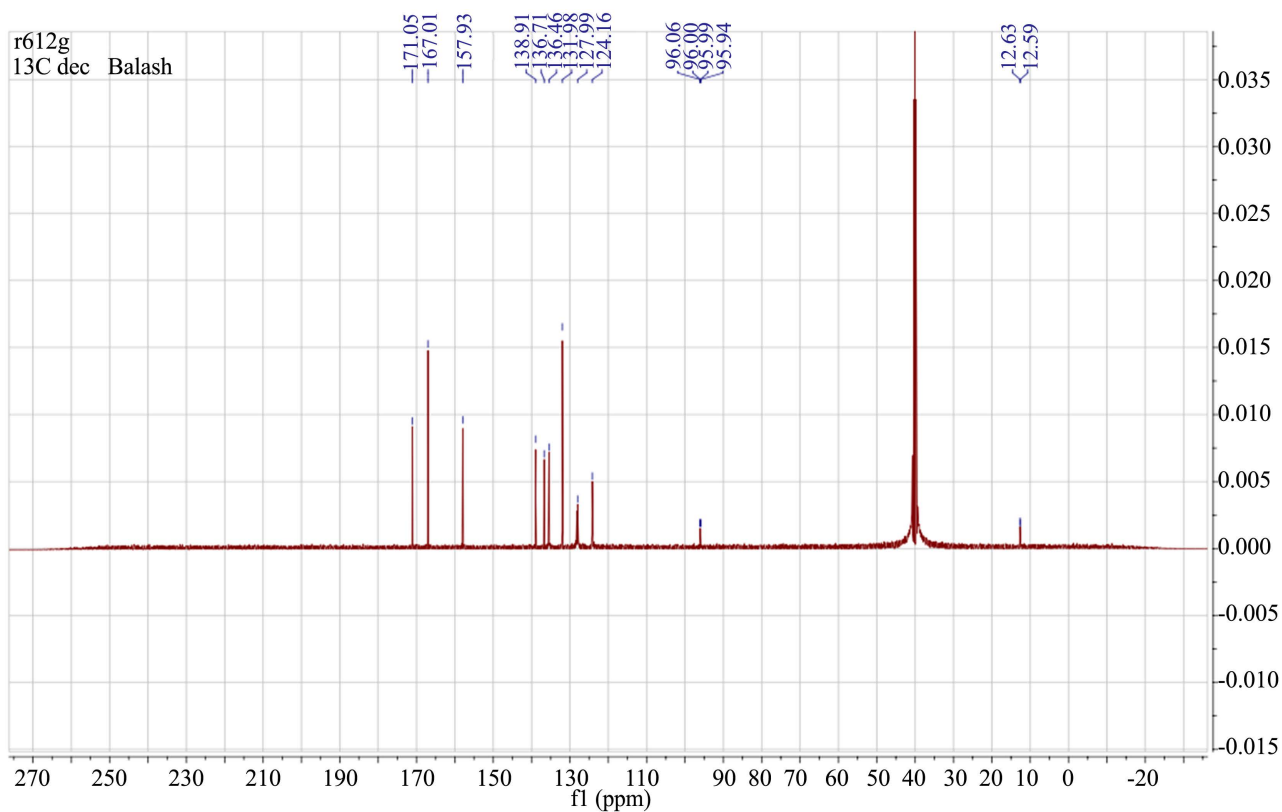


Figure 17. ^{13}C -NMR spectrum for 3b compound.

4-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)benzenesulfonamide (3d)

White powder, mp 364°C - 368°C. TLC; the mobile phase was used acetate ethyl: petrolatum ether 6:4, which the R_f for sulfanilamide 0.44 and the R_f for compound (**3d**) 0.62. IR spectrum (ν_{\max} , cm^{-1}): 3362, 3258 (N–H), 1703 (C=O), 1615, 1594, 1498 (C=C), 1393, 1340 (S=O), 1299, 1286 (C–N), 1224 (C–O) (**Figure 18**) [26]. ^1H -NMR spectrum (DMSO-d_6 , δ , ppm): 7.4 (s, 1H, $\text{SO}_2\text{-NH}$), 7.66 (d, 1H, Ar–H), 7.88 (d, 1H, Ar–H), 7.93 (t, 1H, Ar–H), 7.95 (d, 1H, Ar–H) (**Figure 19**). ^{13}C -NMR (DMSO-d_6 , δ , ppm): 124, 127, 128, 132, 135.3, 135.5, 143.8, 167.18 (**Figure 20**) [27]. MS (m/z , ESI): showed $[\text{M} + 2\text{H}]^+$ peak at 304.26 corresponding to the molecular formula of $\text{C}_{14}\text{H}_{10}\text{N}_2\text{O}_4\text{S}$, and $[\text{M} + \text{Na}]^+$ peak at 325.03.

All the designed compounds were bound to receptors and given energy docking. Only one compound showed higher docking scores toward the receptor than the reference ligand, **Table 3**. The compounds 4b, 6c, and 5b were shown higher energy binding with EGFR (TMLR) binding pocket than other compounds.

Most of the compounds were given hydrogen bonds and van der Waals bonds to receptors with different bond lengths and binding energies. The ligand displayed hydrogen bonds with 9 amino acid residues of 5EDQ: Thr854, Lys745, Asn842, Asp855, Met793, Arg858, Arg841. To stabilize the compounds in the binding pocket; most compounds displayed van der Waals interactions between the amino acid residues and the ligands.

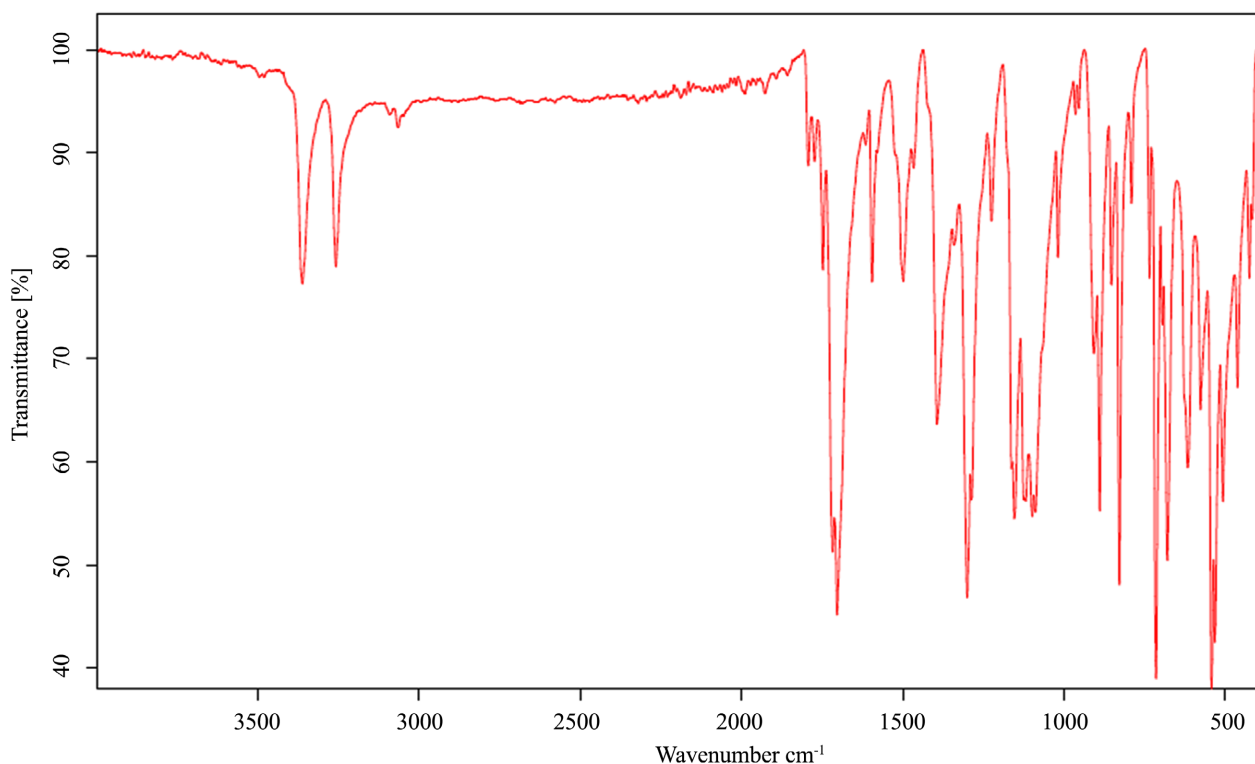


Figure 18. IR spectrum for 3d compound.

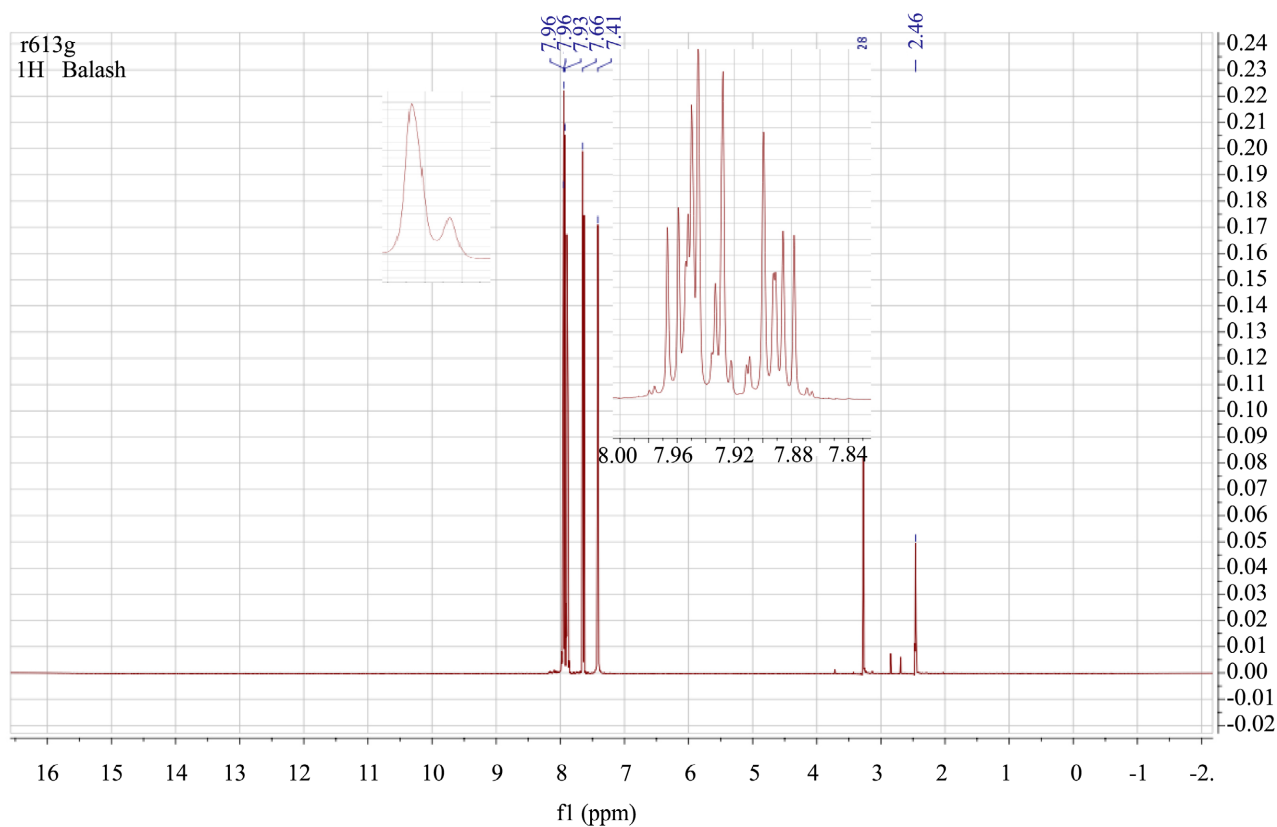


Figure 19. ^1H -NMR spectrum for 3d compound.

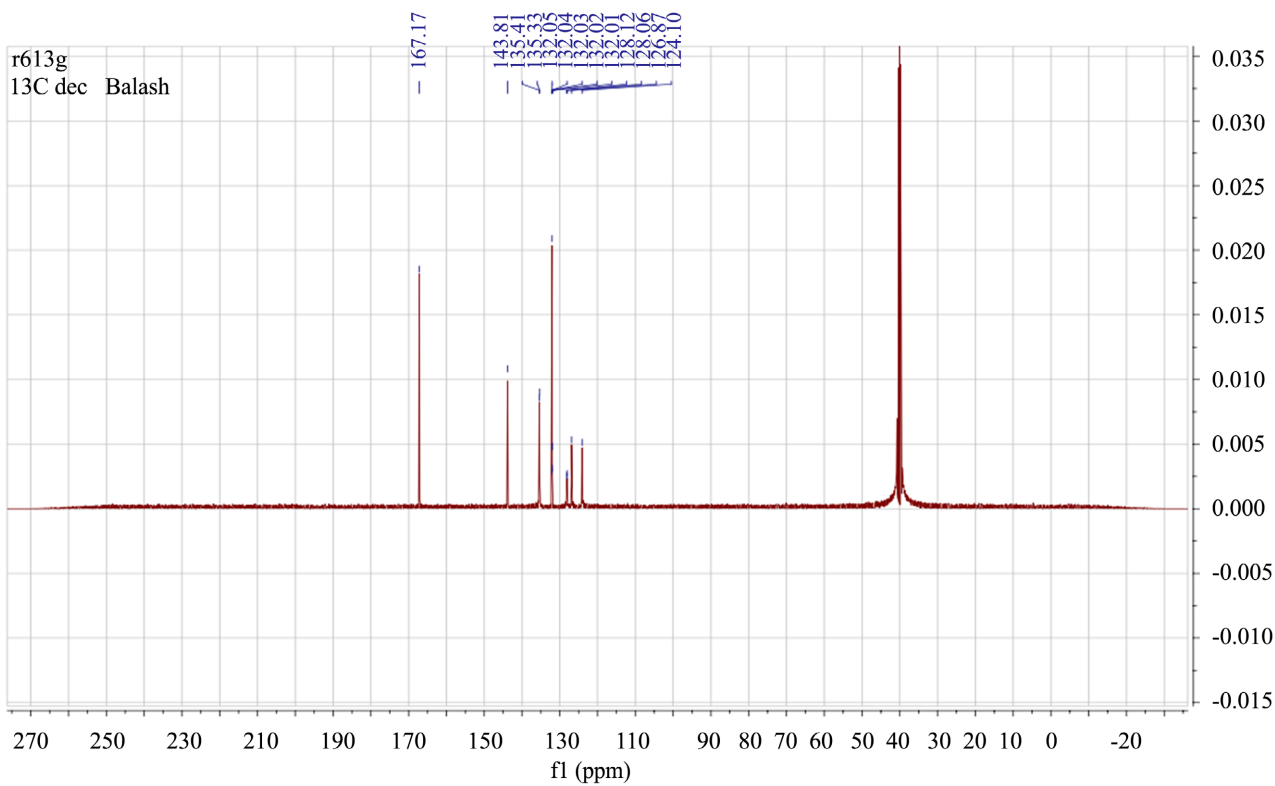


Figure 20. ^{13}C -NMR spectrum for 3d compound.

The predicted binding modes of 4b and 6c and their interactions with the residues in the EGFR (TMLR) binding pocket are shown in (Figure 21).

A series of four sulfonamides were synthesized in basic media by simple reactions of amino-group-containing drug; sulfamethoxazole and sulfanilamide with carbonyl group in benzoyl chloride and phthalic acid. The compounds were obtained in good to excellent yield (50% - 91%), and the yield by microwave synthesis was excellent (82% - 97%). The synthesized compounds were characterized by FT-IR; the characteristics band at 3258 - 3476 cm^{-1} for (N-H) and 1650 - 1739 cm^{-1} for (C=O) and 1413 - 1294 cm^{-1} of S=O stretching and 1266 - 1333 cm^{-1} for (C-N) for all compounds reveals the formation of sulfonamides. Mass spectral data of all synthesized compounds was obtained by ESI-MS. The major peak for compounds was showed good agreement with the calculated molecular masses of the concerned compound. The structures of all the compounds were also confirmed by ^1H NMR by dissolving in DMSO. ^1H NMR spectra of compounds,

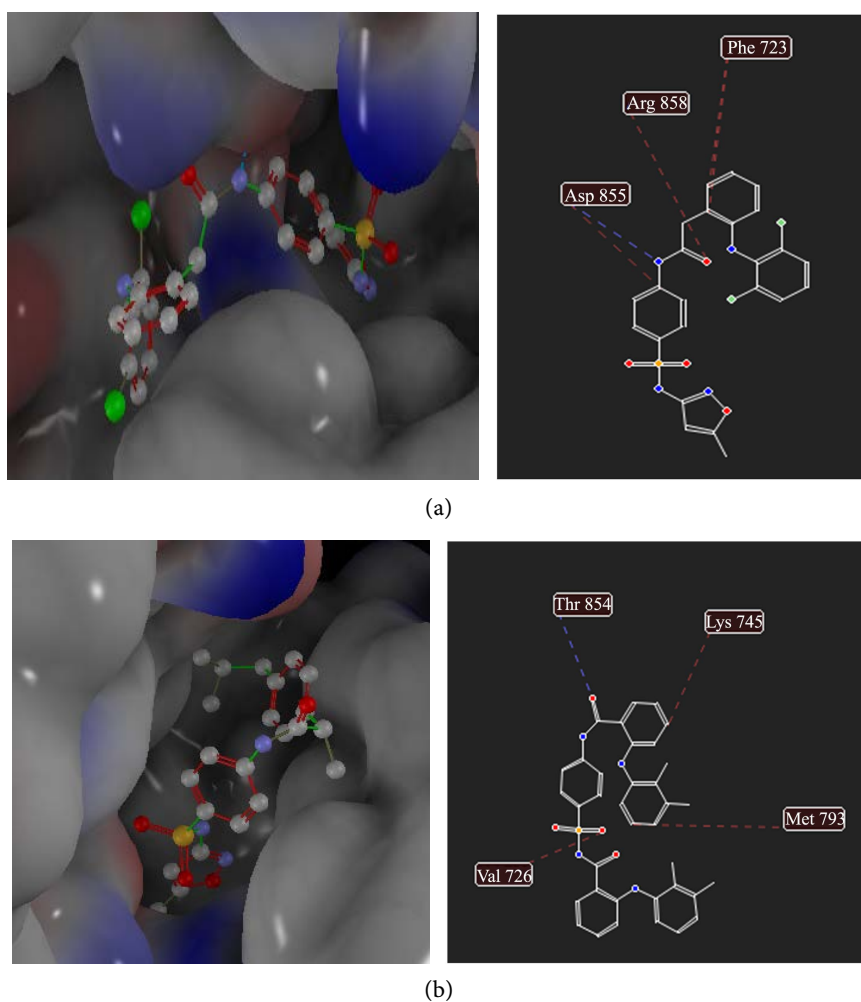


Figure 21. (a) Docking solution of compound (4b) and the interactions with residues of amino acid in the TMLR binding pocket; (b) docking solution of compound (6c) and the interactions with residues of amino acid in the TMLR binding pocket. Blue bonds indicate hydrogen bonds, and red bonds indicate van der Waals.

Table 5. Comparison of conventional heating method and microwave synthesis.

Conditions	Compound 1b		Compound 1c		Compound 3b		Compound 3d	
	C	M	C	M	C	M	C	M
Temperature (°C)	70	120	-	100	200	200	200	200
Time (min)	30	10	30	5	240	20	210	20
Yield (%)	84	89	50	82	50	88	91	97

*C: conventional heating, *M: microwave heating.

there were two signals for solvent at 2.5 and 3.3 ppm. The protons for the benzene ring appeared at 7.2 - 8 ppm. In **1b** and **3b** compounds, there were signals in 2.3 and 6.1 for methyl and CH in oxazole, respectively. In the ^{13}C NMR spectra for compounds, there was a signal for solvent at 40 ppm. Aromatic carbons of benzene ring appeared at chemical shift 120.3 - 144 ppm. The chemical shift value of C=O was observed at 166.5 - 167.18 ppm. In **1b** and **3b** compounds, three carbons were visible at 96, 158 and 171 ppm, and the carbon for methyl group was noted at chemical shift 12.5 ppm.

From the results, all the newly synthesized compounds are in agreement with the spectroscopic analysis. And we noted that the microwave synthesis gave a higher yield than the conventional heating method with less time and higher temperature (**Table 5**).

4. Conclusion

Several sulfonamide derivatives were docked into the EGFR (TMLR) binding pocket using the Molegro Virtual Docker software. The binding free energy was calculated to predict their affinity toward EGFR kinase TMLR to select novel candidates as EGFR inhibitors for treating cancer. The results showed that **4b**, and **6c** gave the highest energy docking -147.213 , -132.14 Kcal/mol toward the EGFR (TMLR) receptor. Their corresponding binding modes were predicted. The obtained results suggested that these compounds may be novel candidates in lung cancer treatment by targeting EGFR tyrosine kinase T790M/L858R. Then, some of new sulfonamide derivatives were synthesized with a simple procedure in one step starting from sulfanilamide and sulfamethoxazole with benzoyl chloride and phthalic acid in basic media. The reactions conditions were easy and excellent yields of compounds were obtained and progress of reaction was monitored by TLC and their structures were confirmed by spectral and elemental analysis. These novel sulfonamide derivatives were also synthesized in rationally good yields by the microwave-assisted method. This is environmentally compassionate technique which gives higher yields and lesser reaction time and easy work-up method. Finally, more studies are still needed to identify the mechanism of action of these derivatives and we are hoping that it will show significant anticancer activity.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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