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Design, synthesis, molecular docking, and anticancer activity of benzoxazole derivatives as VEGFR-2 inhibitors

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Abstract

Novel series of benzoxazoles **4$_{a–f}$–16** were designed, synthesized, and evaluated for anticancer activity against HepG2, HCT-116, and MCF-7 cells. HCT-116 was the most sensitive cell line to the influence of the new derivatives. In particular, compound **5$_e$** was found to be the most potent against HepG2, HCT-116, and MCF-7 with IC_{50} = 4.13 ± 0.2, 6.93 ± 0.3, and 8.67 ± 0.5 μM, respectively. Compounds **5$_c$**, **5$_f$**, **6$_b$**, **5$_d$**, and **6$_c$** showed the highest anticancer activities against HepG2 cells with IC_{50} of 5.93 ± 0.2, 6.58 ± 0.4, 8.10 ± 0.7, 8.75 ± 0.7, and 9.95 ± 0.9 μM, respectively; HCT-116 cells with IC_{50} of 7.14 ± 0.4, 9.10 ± 0.8, 7.91 ± 0.6, 9.52 ± 0.5, and 12.48 ± 1.1 μM, respectively; and MCF-7 cells with IC_{50} of 8.93 ± 0.6, 10.11 ± 0.9, 12.31 ± 1.0, 9.95 ± 0.8, and 15.70 ± 1.4 μM, respectively, compared with sorafenib as a reference drug with IC_{50} of 9.18 ± 0.6, 5.47 ± 0.3, and 7.26 ± 0.3 μM, respectively. The most active compounds **5$_{c–f}$** and **6$_{b,c}$** were further evaluated for their vascular endothelial growth factor receptor-2 (VEGFR-2) inhibition. Compounds **5$_e$** and **5$_c$** potently inhibited VEGFR-2 at lower IC_{50} values of 0.07 ± 0.01 and 0.08 ± 0.01 μM, respectively, compared with sorafenib (IC_{50} = 0.1 ± 0.02 μM). Compound **5$_f$** potently inhibited VEGFR-2 at low IC_{50} value (0.10 ± 0.02 μM) equipotent to sorafenib. Our design was based on the essential pharmacophoric features of the VEGFR-2 inhibitor sorafenib. Molecular docking was performed for all compounds to assess their binding pattern and affinity toward the VEGFR-2 active site.

KEYWORDS
anticancer agents, benzoxazole, molecular docking, VEGFR-2 inhibitors

1 | INTRODUCTION

VEGF signaling pathway plays fundamental roles in regulating tumor angiogenesis. VEGF as a therapeutic target has been validated in various types of human cancers.[1] Vascular endothelial growth factor receptor-2 (VEGFR-2) represents a major target within the angiogenesis-related kinases, hence considered the most important transducer of VEGF-dependent angiogenesis.[2] Thus, inhibition of VEGF/VEGFR signaling pathway is regarded as an attractive therapeutic target for inhibition of tumor angiogenesis and subsequent tumor growth.[3–6]

Sorafenib (Nexavar®) is a potent VEGFR-2 inhibitor and has been approved as antiangiogenic drug.[7–9] Study of the structure–activity relationships (SAR) and common pharmacophoric features shared by sorafenib and various VEGFR-2 inhibitors revealed that most VEGFR-2 inhibitors shared four main features as shown in Figure 1:[10–12] (a) The core structure of most inhibitors consists of a flat heteroaromatic ring system that contains at least one N-atom, which occupied the catalytic ATP-binding domain. (b) A central aryl ring (hydrophobic spacer), occupying the linker region between the ATP-binding domain and the DFG domain of the enzyme.[13] (c) A linker containing a functional group acting as pharmacophore (e.g., amino or

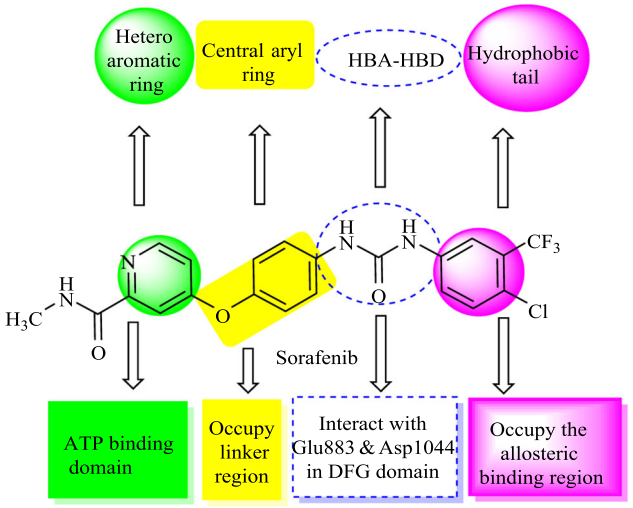


FIGURE 1 The basic structural requirements for sorafenib as reported vascular endothelial growth factor receptor-2 inhibitor

urea) that possesses both H-bond acceptor and donor to bind with two crucial residues (Glu883 and Asp1044) in the DFG (Asp–Phe–Gly) motif, an essential tripeptide sequence in the active kinase domain. The NH motifs of the urea or amide moiety usually form one hydrogen bond with Glu883, whereas the C═O motif forms another hydrogen bond with Asp1044. (d) The terminal hydrophobic moiety of the inhibitors occupies the newly created allosteric hydrophobic pocket, revealed when the phenylalanine residue of the DFG loop flips out of its lipophilic pocket defining DFG-out or inactive conformation. Thus, hydrophobic interactions are usually attained in this allosteric binding region.[14] Furthermore, analysis of the X-ray structure of various inhibitors bound to VEGFR-2 confirmed the sufficient space available for various substituents around the terminal heteroaromatic ring.[15,16]

Benzoxazole is a heterocyclic scaffold in many synthetic compounds, so the chemistry of benzoxazole derivatives became increasingly interesting due to their various biological and pharmacological activities.[17,18] Benzoxazole nucleus is a core structure in many synthetic compounds having different biological activities as anti-inflammatory[19] and anticancer.[20] In addition, the bis(benzoxazole) natural product UK-1 displayed a potent anticancer activity with an IC_{50} value 20 nM against certain solid tumors, leukemia, and lymphoma.[21] VEGFR-2 inhibitory activity of benzoxazole was also reported.[17,22] It is suggested that benzoxazoles act as competitive inhibitors at the ATP-binding site of tyrosine kinases.[17]

Benzoxazole nucleus is a privileged scaffold forming the most promising class of heterocycles, which is well-tolerated in humans and possesses antitumor activity.[17,21,22] Moreover, they are the backbone of many bioactive compounds that show potential activities as VEGFR inhibitors.[17,22,23] In addition, several sulfonamide,[24] sulfonylurea,[25] sulfonylthiourea,[26] thiosemicarbazone,[27] hydrazone,[28] oxime,[29] and pyrazoline[30] moieties were reported to possess anticancer activities.

Depending on ligand-based drug design, particularly a molecular hybridization approach that involves the coupling of two or more groups with relevant biological properties,[31] molecular hybridization of benzoxazole and other effective antitumor moieties was carried out in an attempt to get new molecules with promising antitumor activities having the main pharmacophoric features of VEGFR-2 inhibitors.

The goal of our work was the synthesis of new agents with the same essential pharmacophoric features of the reported and clinically used VEGFR-2 inhibitors (e.g., sorafenib). The main core of our molecular design rationale comprised bioisosteric modification strategies of VEGFR-2 inhibitors at four different positions (Figure 2).

2 | RESULTS AND DISCUSSION

2.1 | Rationale and structure-based design

Our target compounds were designed to have different spacers and different linkers with HBA–HBD, the main pharmacophoric feature in sorafenib, hoping to obtain more potent VEGFR-2 inhibitors. First, bioisosteric approach was adopted in the target benzoxazole to replace pyridine ring. The second strategy is to use 2-sulfanyl-*N*-phenylacetamide and/or 3-sulfanyl-*N*-phenylpropanamide ring systems to replace the central aryl ring of lead structure to increase the space (linkers) between the pyridine ring and the central aryl ring to impart more flexibility aiming to increase VEGFR-2–binding affinity. The third strategy is using HBA–HBD linkers containing functional groups that possess H-bond acceptors and/or donors, such as sulfonamide linker (SO_2NH) in compounds $\mathbf{4}_{a-f}$, sulfonylurea (–SO_2–NH–CO–NH–) in compounds $\mathbf{5}_{a-f}$, and sulfonylthiourea (–SO_2–NH–CS–NH–) in compounds $\mathbf{6}_{a-c}$. Compounds $\mathbf{8}_{a,b}$ contain only carbonyl (CO) group, whereas thiosemicarbazone (═N–NH–CS–NH_2) in compounds $\mathbf{11}_{a,b}$ and hydrazone (═N–NH–CO) in compounds $\mathbf{12}_{a-d}$ and $\mathbf{13}_{a,b}$. Also oxime (═N–OH), chalcone (COCH═CH–), and acetylpyrazoline linkers were used in compound **14**, **15**, and **16**, respectively. Also, the hydrophobic phenyl tail of the reported ligand was replaced in many compounds by other groups such as thiazole in compound $\mathbf{4}_{b}$, pyridine in compound $\mathbf{4}_{c}$, cyclohexyl in compounds $\mathbf{5}_{a,c,e}$, ethyl in compounds $\mathbf{6}_{a-c}$, and methyl in compounds $\mathbf{8}_{a,b}$, $\mathbf{11}_{a,b}$, $\mathbf{13}_{a,b}$, and **14**. The fourth strategy focused on the usage of different substituents on phenyl moiety as in compounds $\mathbf{12}_{b-d}$ and methoxy group in compounds **15** and **16**.

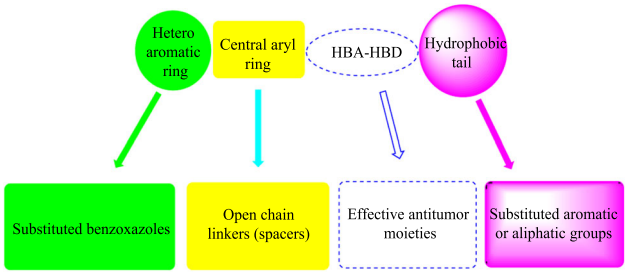


FIGURE 2 Summary for the possible modifications of vascular endothelial growth factor receptor-2 inhibitors

Furthermore, the substitution pattern was selected to ensure different electronic and lipophilic environments, which could influence the activity of the target compounds. On the contrary, the linker in compound **16** was designed in a different way, where it constituted a part of the rigid acetylpyrazoline ring structure to study the effect of the free rotated open-chain linkers and/or the ring structure on SAR. These modifications were performed to carry out further elaboration of the benzoxazole scaffold and to explore a valuable SAR. The designed target benzoxazole derivatives were synthesized and evaluated as potential VEGFR-2 inhibitory and antitumor activities against three human tumor cell lines, namely, hepatocellular carcinoma (HepG2), breast cancer (MCF-7), and colorectal carcinoma (HCT-116).

The essential pharmacophoric features[28,32–35] in the benzoxazole-derived VEGFR-2 inhibitors (Figure 3) include: the presence of five-membered hetero ring, oxazole, fused with benzene ring, as hydrophobic portion, forming aromatic system represented by benzoxazole ring linked to different (un)substituted hydrophobic moieties through different spacers and linkers (HBA–HBD), which interacts as H-bond donors through its NH with the side chain carboxylate of the essential amino acid residue Glutamate883 and through its carbonyl group with Aspartate1044, and also through hydrophobic interaction with its (un)–substituted hydrophobic moieties with the hydrophobic pocket lined with the hydrophobic side chains of Alanine864, Valine865 Lysine866, Valine897, Valine914, Phenylalanine916, Cysteine917, Leucine1033,

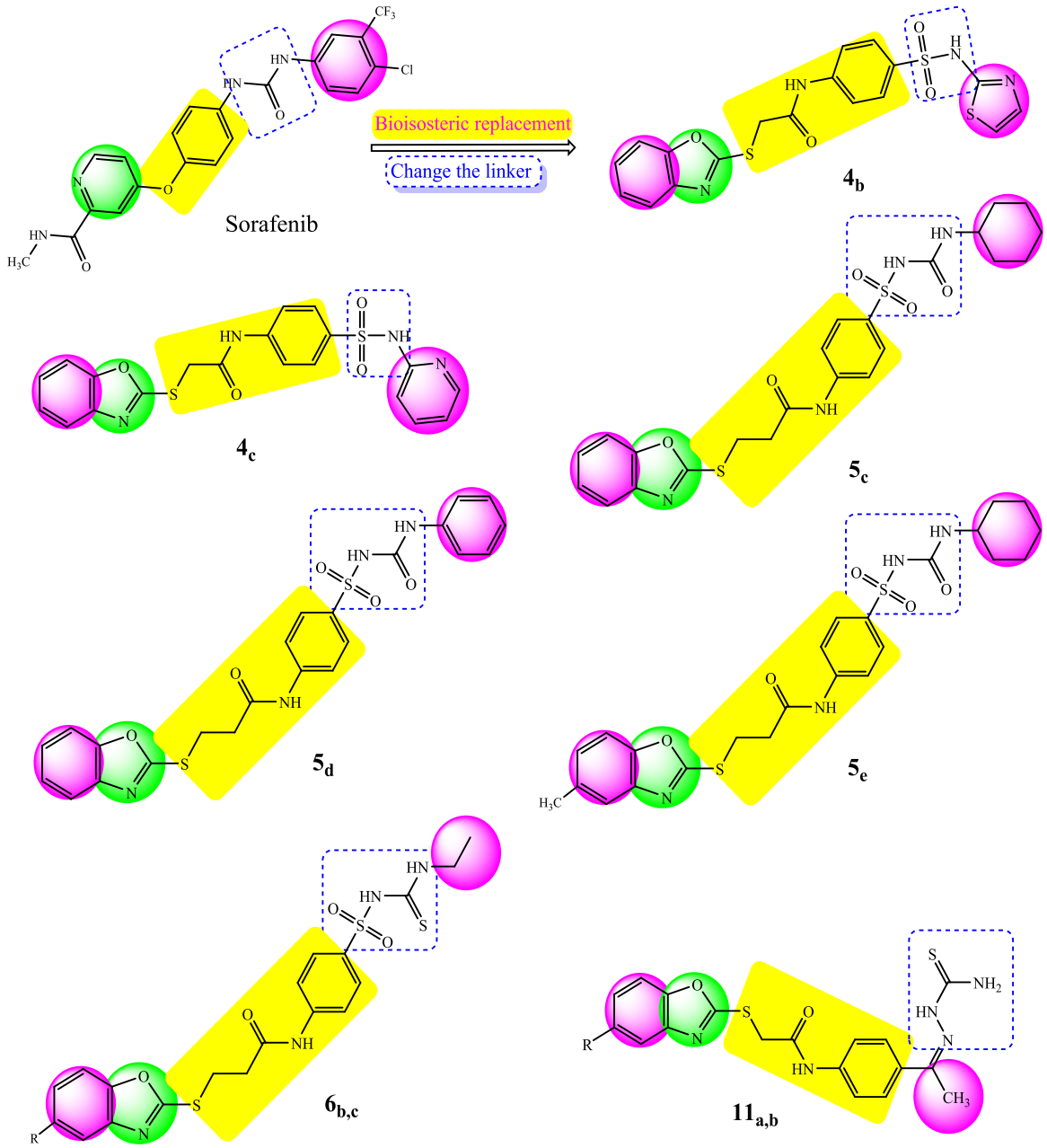


FIGURE 3 Structural similarities and pharmacophoric features of vascular endothelial growth factor receptor-2 inhibitors and selected designed compounds

and Cysteine1043. In addition, oxazole moiety was designed to replace the pyridine moiety of the reference ligand sorafenib. Compounds **5**$_{e,f}$, have $SO_2NHCONH$ while compounds **6**$_{b,c}$ have $SO_2NHCSNH$, which resemble the urea of sorafenib and form H-bond with the essential amino acids Glutamate883 and Aspartate1044. They also have hydrophobic distal cyclohexyl, phenyl, and/or ethyl moieties, which increase the affinity toward VEGFR-2 by hydrophobic interactions. These interactions may explain the high anticancer activities of these compounds.

Our target compounds were designed as hybrid molecules. These molecules formed of benzoxazole ring system joined with different (un)substituted moieties through different linkers offering various electronic and lipophilic environments to study their impact on the activity, hoping to obtain more potent anticancer agents. Molecular docking studies were carried out to study the interaction of the newly synthesized compounds with VEGFR-2, their binding mode and the ability to satisfy the pharmacophoric features required to induce the desired inhibition.

2.2 | Chemistry

The synthetic strategy for preparation of the target compounds (**4–16**) is depicted in Schemes 1–3. Synthesis was initiated by reacting 2-amino-4-substituted phenol with carbon disulfide in the presence of alcoholic potassium hydroxide to provide the corresponding 2-mercaptobenzoxazole derivatives (**1**$_{a,b}$), respectively, which was treated with alcoholic potassium hydroxide to afford the corresponding potassium salts (**2**$_{a,b}$). 4-Amino-*N*-substituted phenylsulfonamides were reacted with chloroacetyl chloride and/or chloropropionyl chloride to afford the corresponding chloroamides (**3**$_{a–d}$). The obtained potassium salts (**2**$_{a,b}$) were refluxed with the appropriate chloroamide derivative (**3**$_{a–c}$) to get the corresponding sulfonamides (**4**$_{a–f}$). The formed sulfanilamide derivatives (**4**$_{a,d–g}$) underwent further reaction with the appropriate isocyanates and/or isothiocyanates, namely, cyclohexyl isocyanate, phenyl isocyanate, and/or ethyl isothiocyanate to furnish the corresponding sulphonyl urea derivatives (**5**$_{a–f}$) and/or sulphonyl thiourea derivatives (**6**$_{a–c}$), respectively (Scheme 1). 4-Aminoacetophenone reacted with chloroacetyl chloride to obtain the corresponding chloroamide derivative (**7**), which underwent reaction with the appropriate potassium salt (**2**$_{a,b}$) to produce the corresponding acetyl derivatives (**8**$_{a,b}$), which underwent further condensation with thiosemicarbazide to furnish the corresponding thiosemicarbazone derivatives (**11**$_{a,b}$). Esterification of (un)substituted benzoic acid with ethanol was carried out in the presence of conc. H_2SO_4 to get the corresponding ethyl benzoate esters (**9**$_{a–d}$), respectively, which was allowed to react with hydrazine hydrate to afford the corresponding benzohydrazide derivatives (**10**$_{a–d}$), respectively. Condensation of the acetyl derivative **8**$_a$ with the appropriate benzohydrazide derivative (**10**$_{a–d}$) resulted in the corresponding benzoylhydrazone derivatives (**12**$_{a–d}$), respectively (Scheme 2). Condensation of the appropriate acetyl derivative **(8**$_{a,b}$**)** with 2-cyanoacetohydrazide, hydroxylamine, and/or 4-methoxybenzaldehyde afforded the corresponding 2-cyanoacetyl)hydrazones (**13**$_{a,b}$), oxime (**14**), and/or chalcone (**15**) derivatives, respectively. Cyclocondensation of the chalcone (**15**) with hydrazine hydrate in the presence of acetic acid glacial produced the corresponding acetylpyrazoline derivative (**16**; Scheme 3).

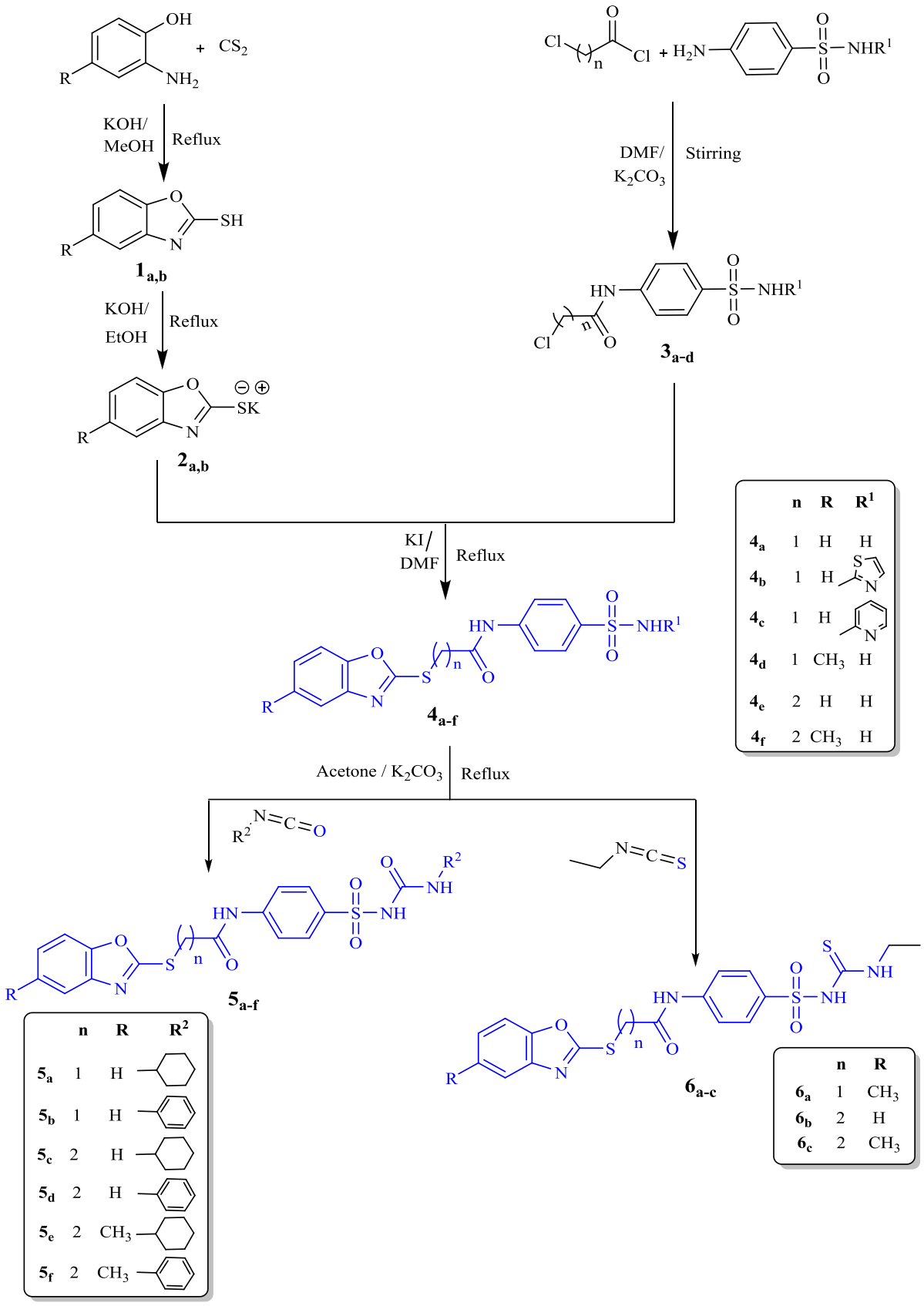
2.3 | Docking studies

In the present work, all modeling experiments were performed using Molsoft software. Each experiment used VEGFR-2 downloaded from the Brookhaven Protein Databank (PDB ID 1YWN).[36]

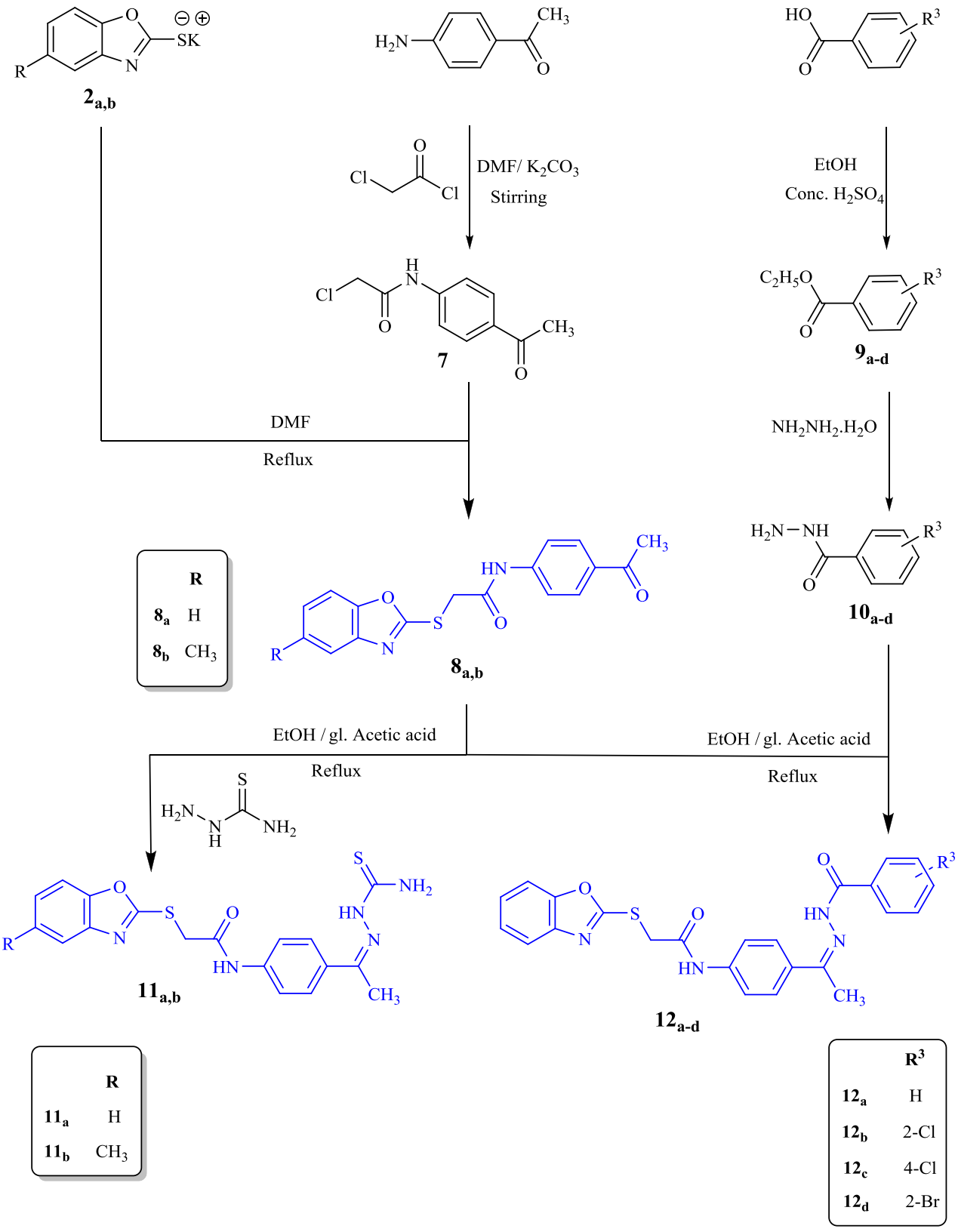
The obtained results indicated that all studied ligands have similar position and orientation inside the putative binding site of VEGFR-2, which revealed a large space bounded by a membrane-binding domain that served as an entry channel for substrate to the active site (Figure 4). In addition, the affinity of any small molecule can be considered as a unique tool in the field of drug design. There is a relationship between the affinity of organic molecules and the free energy of binding.[37–40] This relationship can contribute in prediction and interpretation of the activity of the organic compounds toward the specific target protein. The obtained results of the free energy of binding (ΔG) explained that most of these compounds had good binding affinity toward the receptor and the computed values reflected the overall trend (Table 1).

The proposed binding mode of sorafenib revealed affinity value of −100.87 kcal/mol and four H-bonds. The urea linker formed one H-bond with the key amino acid Glutamate883 (2.13 Å) through its NH group and one H-bond with Aspartate1044 (1.65 Å) through its carbonyl group. The central phenyl ring occupied the hydrophobic pocket formed by Glutamate883, Isoleucine886, Leucine887, Isoleucine1042, Cysteine1043, and Aspartate1044. Moreover, the distal hydrophobic 3-trifluromethyl-4-chlorophenyl moiety attached to the urea linker occupied the hydrophobic pocket formed by Cysteine1043, Leucine1033, Valine897, Valine914, Alanine864, Valine865, and Lysine866. Furthermore, the *N*-methylpicolinamide moiety occupied the hydrophobic groove formed by Arginine1025, Histidine1024, Isoleucine1023, Cysteine1022, Leucine1017, Isoleucine890, Histidine889, and Isoleucine886 while its carbonyl was stabilized by formation of two H-bonds with Arginine1025 (1.90 and 2.12 Å; Figure 5). The urea linker played an important role in the binding affinity toward VEGFR-2 enzyme, where it was responsible for the higher binding affinity of sorafenib. This finding encouraged us to use different linkers that resembled urea of sorafenib to obtain potent VEGFR-2 inhibitors.

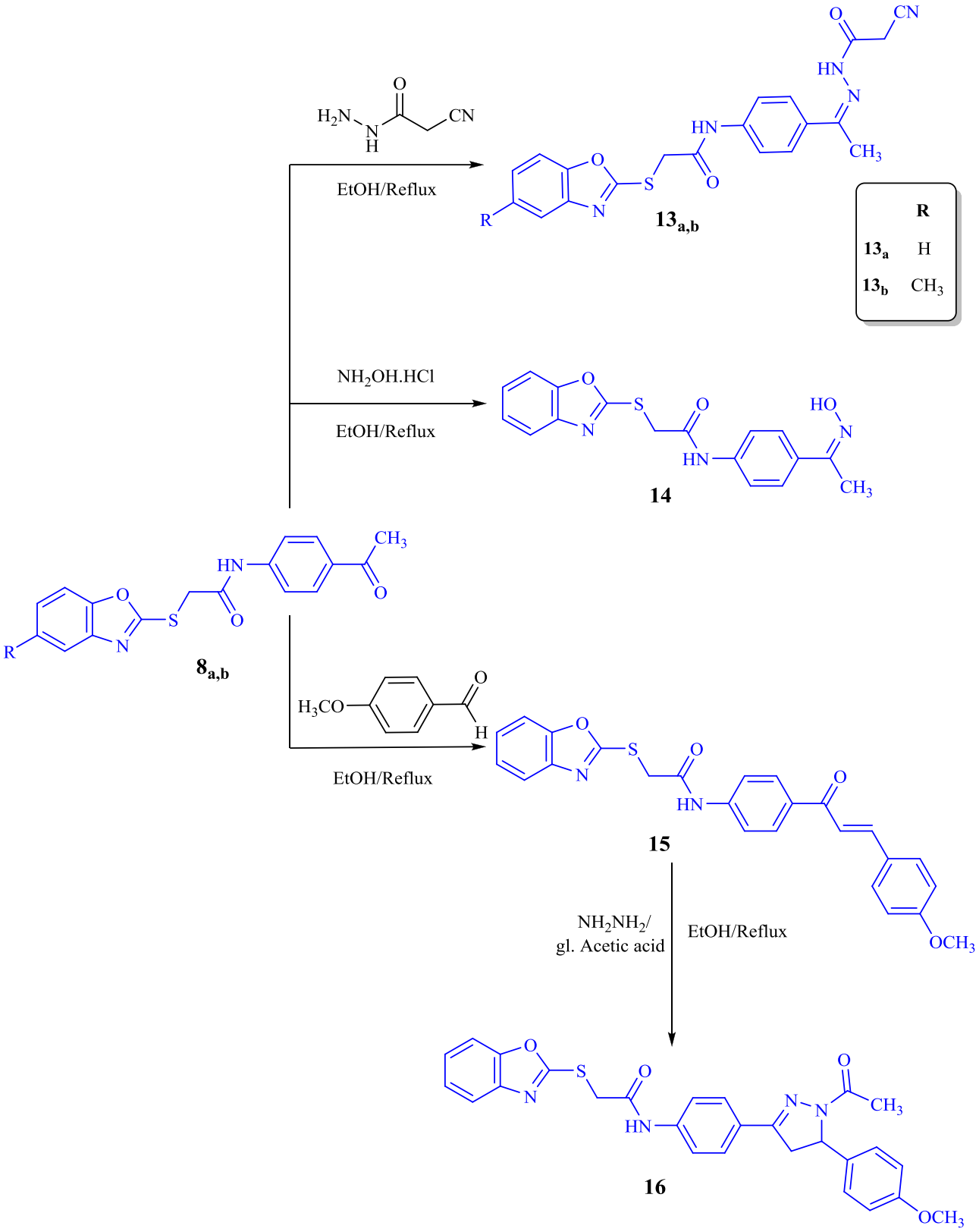
As planned, the proposed binding mode of compound **5**$_e$ is virtually the same as that of sorafenib, which revealed affinity more than that of sorafenib with value of −133.72 kcal/mol and five H-bonds. The NH group of the sulfonylurea linker formed one H-bond with Glutamate883 (2.39 Å) and one H-bond with Aspartate1044 (1.26 Å) through its carbonyl group. The SCH_2CH_2CONH spacer was stabilized by formation of H-bond with Aspartate1044 (2.37 Å) and 2 H-bonds with Arginine1025 (1.67 and 1.99 Å). The central phenyl ring occupied the hydrophobic pocket formed by Glutamate883, Isoleucine886, Leucine887, Leucine1017, Histidine1024, Isoleucine1042, and Aspartate1044. The distal cyclohexyl moiety occupied



SCHEME 1 Synthetic route for preparation of the target compounds 4_{a-f}–6_{a-c}



SCHEME 2 Synthetic route for preparation of the target compounds **8–12**$_{a-d}$



SCHEME 3 Synthetic route for preparation of the target compounds **13–16**

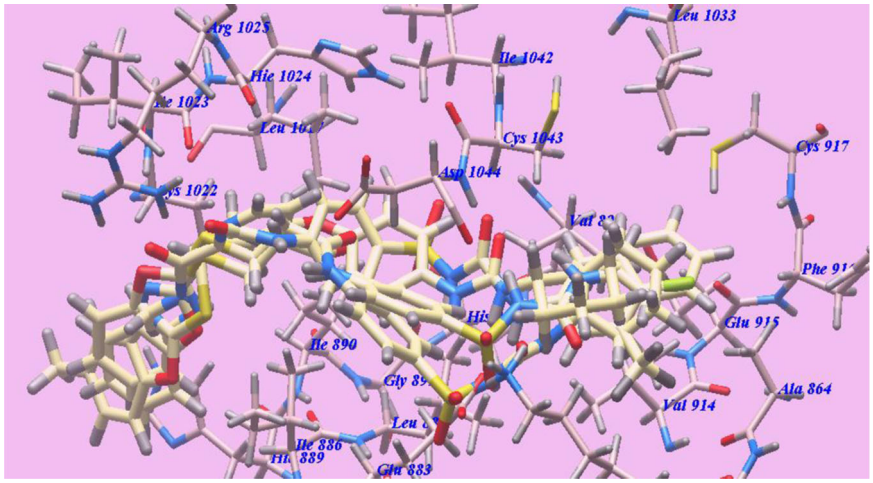


FIGURE 4 Superimposition of some docked compounds inside the binding pocket of 1YWN

the hydrophobic pocket formed by Alanine864, Valine865, Lysine866, Valine897, Valine914, Phenylalanine916, Cysteine917, Leucine1033, and Cysteine1043. Furthermore, the benzoxazole moiety occupied the hydrophobic groove formed by Cysteine1022, Isoleucine890, Histidine889, and Isoleucine886 (Figure 6). These interactions of compound **5e** may explain the highest anticancer activity.

The proposed binding mode of compound **5c** is virtually the same as that of **5e**, which revealed affinity value of −132.88 kcal/mol and five H-bonds. The sulfonylurea linker was stabilized by formation of four H-bonds. The NH group formed one H-bond with Glutamate883 (2.78 Å) and three H-bonds with Isoleucine886 (1.49, 1.70, and 1.99 Å). The SCH_2CH_2CONH spacer formed H-bond with Aspartate1044 (2.30 Å). The central phenyl ring occupied the hydrophobic pocket formed by Glutamate883, Isoleucine886, Leucine887, Leucine1017, Histidine1024, Isoleucine1042, and Aspartate1044. The distal cyclohexyl moiety occupied the hydrophobic pocket formed by Alanine864, Valine865 Lysine866, Valine897, Valine914, Phenylalanine916, Cysteine917, Leucine1033, and Cysteine1043. Furthermore, benzoxazole moiety occupied the hydrophobic groove formed by Cysteine1022, Isoleucine890, Histidine889, and Isoleucine886 (Figure 7). These interactions of compound **5c** may explain the highest anticancer activity.

From the obtained docking results (Table 1), we concluded that sulfonylurea and/or sulfonylthiourea linkers impart higher affinities toward VEGFR-2 enzyme than urea linker. The longer SCH_2CH_2CONH spacer showed higher binding affinities than the SCH_2CONH one. The open-chain free rotating linkers exhibited higher binding affinities than the rigid cyclic ones. Also lipophilicity played an important role in their VEGFR-2 inhibitory activities, which may be due to higher hydrophobic interactions.

TABLE 1 The calculated free energy of binding (ΔG in kcal/mol) for the ligands

Compound	ΔG (kcal/mol)	Compound	ΔG (kcal/mol)
4a	−84.17	**8a**	−74.49
4b	−105.60	**8b**	−73.48
4c	−105.95	**11a**	−105.99
4d	−95.40	**11b**	−111.01
4e	−93.34	**12a**	−78.88
4f	−93.87	**12b**	−72.61
5a	−120.83	**12c**	−82.35
5b	−118.31	**12d**	−71.55
5c	−132.88	**13a**	−86.33
5d	−129.92	**13b**	−85.78
5e	−133.72	**14**	−66.11
5f	−131.03	**15**	−92.35
6a	−116.81	**16**	−104.55
6b	−128.05	Sorafenib	−100.87
6c	−125.05		

2.4 | Biological activity

2.4.1 | In vitro cytotoxic activity

Antiproliferative activity of the newly synthesized benzoxazoles **4a–f–16** was examined against three human tumor cell lines, namely, hepatocellular carcinoma (HepG2), breast cancer (MCF-7), and colorectal carcinoma (HCT-116) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay as described by Mosmann.[41–43] Sorafenib was included in the experiments as a reference cytotoxic drug. The results were expressed as growth inhibitory concentration (IC_{50}) values, which represent the compound concentrations required to produce a 50% inhibition of cell growth after 72 hr of incubation calculated from the concentration–inhibition response curve and summarized in Table 2. From the obtained results, it was explicated that most of the prepared compounds displayed excellent to modest growth inhibitory activity against the tested cancer cell lines. Investigations of the cytotoxic activity against HCT-116 and HepG2 indicated that they were more sensitive cell lines to the influence of the new derivatives, respectively. In particular, compound **5e** was found to be the most potent derivative overall in the tested compounds against HepG2, HCT-116, and MCF-7 cancer cell lines with $IC_{50} = 4.13 \pm 0.2$, 6.93 ± 0.3, and 8.67 ± 0.5 μM, respectively. It has nearly the half

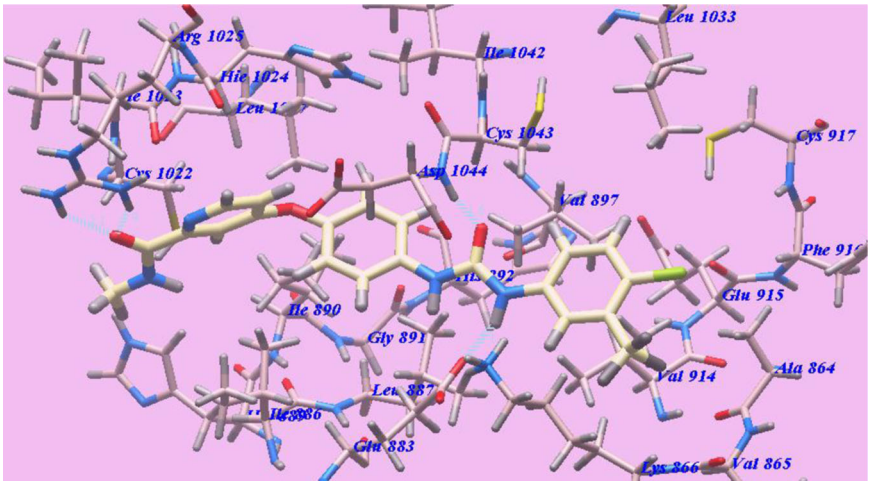


FIGURE 5 Predicted binding mode for sorafenib with 1WYN. H-bonded atoms are indicated by dotted lines

activity as sorafenib against HepG2 (IC_{50} = 9.18 ± 0.6 μM) and nearly the same activity against HCT-116 (IC_{50} = 5.47 ± 0.3 μM) and MCF-7 cell lines (IC_{50} = 7.26 ± 0.3 μM). With respect to the HepG2 hepatocellular carcinoma cell line, compounds **5_c**, **5_f**, **6_b**, **5_d**, and **6_c** displayed the highest anticancer activities (with IC_{50} = 5.93 ± 0.2, 6.58 ± 0.4, 8.10 ± 0.7, 8.75 ± 0.7, and 9.95 ± 0.9 μM, respectively). Compounds **4_b**, **4_c**, **4_g**, **5_a**, **5_b**, **6_a**, and **11_b** (with IC_{50} ranging from 11.48 ± 1.0 to 24.84 ± 1.8 μM) displayed good cytotoxicity. Compounds **4_d**, **4_f**, **11_a**, **12_a**, **12_c**, **15**, and **16** (with IC_{50} ranging from 35.94 ± 3.2 to 51.47 ± 3.6 μM) exhibited moderate cytotoxicity. Other compounds with (IC_{50} ranging from 67.42 ± 4.1 to 88.97 ± 4.8 μM) exhibited low cytotoxicity.

Cytotoxicity evaluation against colorectal carcinoma (HCT-116) cell line discovered that compounds **5_c** and **6_b** showed the highest anticancer activities with IC_{50} = 7.14 ± 0.4 and 7.91 ± 0.6 μM, respectively, and compounds **4_b**, 4_c, **5_a**, **5_d**, **5_f**, **6_c**, and **11_b** (with IC_{50} ranging from 9.10 ± 0.8 to 18.33 ± 1.7 μM) displayed good cytotoxicity. Compounds **4_d**, **4_f**, **4_g**, **5_b**, **6_a**, **12_a**, and **12_c** (with IC_{50} ranging from 20.76 ± 1.9 to 35.08 ± 2.8 μM) exhibited moderate cytotoxicity. Other compounds (with IC_{50} ranging from 44.3 ± 0.49 to 78.24 ± 4.7 μM) exhibited low cytotoxicity.

Cytotoxicity evaluation against MCF-7 cell line, revealed that compounds **5_c**, **5_d**, **and** **5_f** with (IC_{50} = 8.93 ± 0.6, 9.95 ± 0.8, and 10.11 ± 0.9 μM, respectively) exhibited the highest anticancer activities. Compounds **4_b**, 4_c, **5_a**, **6_b**, **6_c**, and **11_b** (with IC_{50} ranging from 12.31 ± 1.0 to 19.45 ± 1.6 μM) displayed good cytotoxicity. Compounds **4_d**, **4_g**, **5_b**, **6_a**, **12_a**, **12_c**, **15**, and **16** (with IC_{50} ranging from 26.86 ± 1.9 to 45.62 ± 2.7 μM) showed moderate cytotoxicity. On the contrary, other compounds displayed low cytotoxicity.

2.4.2 | In vitro VEGFR-2 kinase assay

The most active antiproliferative derivatives **5_{c-f}** and **$6_{b,c}$** were selected to evaluate their inhibitory activities against VEGFR-2 by using an antiphosphotyrosine antibody with the Alpha Screen system (PerkinElmer). The results were reported as IC_{50} value calculated from the concentration–inhibition response curve and summarized in Table 2. Sorafenib was used as positive control in this assay. The tested compounds displayed high to good inhibitory activity with IC_{50} values ranging from 0.07 to 0.36 μM. Among them, compounds **5_e** and **5_c** potently inhibited VEGFR-2 at lower IC_{50} values of 0.07 ± 0.01 and 0.08 ± 0.01 μM, respectively, compared with sorafenib IC_{50} value

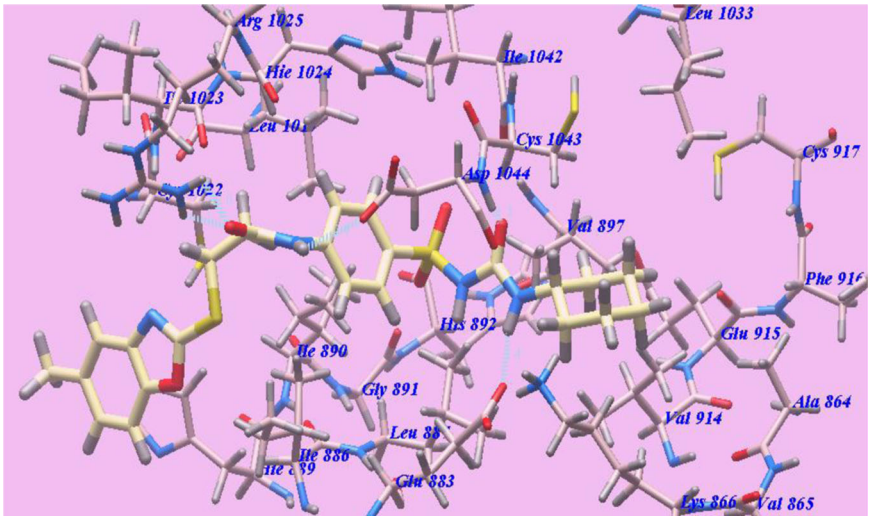


FIGURE 6 Predicted binding mode for **5_e** with 1WYN

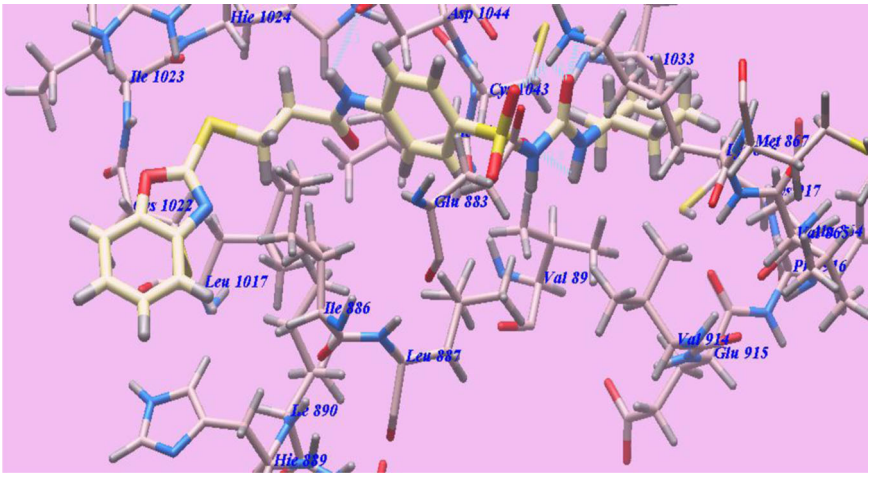


FIGURE 7 Predicted binding mode for 5_c with 1WYN

TABLE 2 In vitro cytotoxic activities of the newly synthesized compounds against HepG2, MCF-7, and HCT-116 cell lines and VEGFR-2 kinase assay

	IC_{50} (μM)[a]			
Compound	HepG2	HCT-116	MCF-7	VEGFR-2
4_a	88.97 ± 4.8	48.39 ± 3.4	59.19 ± 3.3	NT
4_b	24.84 ± 1.8	17.18 ± 1.5	16.42 ± 1.4	NT
4_c	15.34 ± 1.3	16.38 ± 1.4	18.30 ± 1.5	NT
4_d	44.71 ± 3.1	25.38 ± 2.2	26.86 ± 1.9	NT
4_e	67.42 ± 4.1	62.82 ± 3.9	73.25 ± 3.8	NT
4_f	18.02 ± 1.4	26.41 ± 2.3	34.72 ± 2.3	NT
5_a	13.15 ± 2.1	18.33 ± 1.7	19.45 ± 1.6	NT
5_b	14.12 ± 1.1	20.76 ± 1.9	28.13 ± 2.0	NT
5_c	5.93 ± 0.2	7.14 ± 0.4	8.93 ± 0.6	0.08 ± 0.01
5_d	8.75 ± 0.7	9.52 ± 0.5	9.95 ± 0.8	0.19 ± 0.02
5_e	4.13 ± 0.2	6.93 ± 0.3	8.67 ± 0.5	0.07 ± 0.01
5_f	6.58 ± 0.4	9.10 ± 0.8	10.11 ± 0.9	0.10 ± 0.02
6_a	21.37 ± 1.6	29.38 ± 2.5	36.80 ± 2.4	NT
6_b	8.10 ± 0.7	7.91 ± 0.6	12.31 ± 1.0	0.27 ± 0.02
6_c	9.95 ± 0.9	12.48 ± 1.1	15.70 ± 1.4	0.36 ± 0.03
11_a	35.94 ± 3.2	45.10 ± 3.3	54.85 ± 3.2	NT
11_b	11.48 ± 1.0	10.35 ± 0.9	13.79 ± 1.2	NT
12_a	42.96 ± 2.8	31.49 ± 2.6	45.62 ± 2.7	NT
12_b	NA	75.33 ± 4.5	78.25 ± 4.0	NT
12_c	41.93 ± 2.6	30.49 ± 2.6	38.48 ± 2.5	NT
12_d	NA	78.24 ± 4.7	81.32 ± 4.2	NT
13_a	68.22 ± 5.1	64.86 ± 4.8	75.23 ± 5.3	NT
13_b	73.94 ± 4.2	46.10 ± 3.4	55.85 ± 3.1	NT
14	NA	NA	NA	NT
15	48.5 ± 3.26	45.4 ± 3.44	42.21 ± 3.12	NT
16	36.25 ± 2.45	44.3 ± 0.49	40.91 ± 3.06	NT
Sorafenib	9.18 ± 0.6	5.47 ± 0.3	7.26 ± 0.3	0.10 ± 0.02

Abbreviations: NA, compounds having IC_{50} value > 100 μM; NT, compounds not tested for their VEGFR-2 inhibitory activity; VEGFR-2, vascular endothelial growth factor receptor-2.

[a]IC_{50} values are the mean ± standard deviation (SD) of three separate experiments.

(0.1 ± 0.02 μM). Compound **5$_f$** potently inhibited VEGFR-2 at low IC_{50} value (0.10 ± 0.02 μM) equipotent as sorafenib. Also, compounds **5$_d$**, **6$_c$**, and **6$_b$** possessed good VEGFR-2 inhibition with IC_{50} values of 0.19 ± 0.02, 0.27 ± 0.02, and 0.36 ± 0.03 μM, respectively.

The preliminary SAR study has focused on the effect of replacement of the urea linkers of sorafenib with different linkers, which interacted as H-bond donor through its NH with the side chain carboxylate of the essential amino acid residue Glutamate883 and with Aspartate1044 through its carbonyl group. Also, hydrophobic interactions through the attached (un)substituted hydrophobic moieties. The effect of replacement of pyridine moiety of sorafenib by the benzoxazole scaffold of the synthesized compounds on the antitumor activities also was noticed. The benzoxazole scaffold occupied the same hydrophobic pocket, which was occupied by the pyridine moiety of the standard ligand. On the contrary, different hydrophobic groups were introduced instead of the phenyl moiety of the reference ligand. Moreover, different substitutions were introduced to the phenyl group with different lipophilicity and electronic nature to study their effect in the anticancer activity. The data obtained revealed that the tested compounds displayed different levels of anticancer activity and possessed a distinctive pattern of selectivity against the HCT-116 and HepG2 cell lines, respectively. Generally, the spacers, linkers (HBA–HBD), lipophilicity, and electronic nature exhibited an important role in the anticancer activity. The sulfonylurea linkers of compounds **5$_{a–f}$** and sulfonylthiourea linkers as in compounds **6$_{a,b}$** were found to be essential for the higher anticancer activity than that of sulfonamide linker (SO_2NH) as in compounds **4$_{a–f}$**. The thiosemicarbazone (═N–NH–CS–NH_2) linker in compounds **11$_{a,b}$** exhibited higher activities than the hydrazone (═N–NH–CO) linker in compounds **12$_{a–d}$** and **13$_{a,b}$**, respectively. Also acetylpyrazoline linker in compound **16** showed higher activity than its parent chalcone (COCH═CH–) and oxime (═N–OH), as in compounds **15** and **14,** respectively. The free rotating open-chain HBA–HBD linkers exhibited higher activity than that of the cyclic one. The SCH_2CH_2CONH spacers resulted in higher activities than the SCH_2CONH one. The 5-methylbenzoxazole derivatives, for example, compound **5$_e$**, showed higher activities than the unsubstituted ones, for example, compound **5$_c$**. Alternatively, the distal phenyl group is not essential for the activity where the more lipophilic cyclohexyl moiety as in compounds **5$_e$** and **5$_c$** displayed the highest anticancer potency against all cell lines. Also, the more lipophilic electron-releasing cyclohexyl moiety as in compound **5$_e$** and **5$_c$** exhibited higher anticancer activity than the propyl moiety as in **6$_{b,c}$**.

From the structure of the synthesized derivatives and the data shown in Table 2 we can divide these tested compounds into six groups. The first group is compounds **4$_{a–f}$**, where the 5-methylbenzoxazole derivatives for example, compound **4$_d$** and **4$_f$** showed higher activities than the unsubstituted ones for example, compound **4$_a$** and **4$_e$**, respectively. The distal pyridine moiety as in compound **4$_c$** exhibited higher activity than the thiazole one as in compound **4$_b$**. In the second group **5$_{a–f}$**, the 5-methylbenzoxazole derivatives for example compound **5$_e$** showed higher activities than the unsubstituted ones for example compound **5$_c$**. Also the long-chain spacer as in compounds **5$_e$** and **5$_c$** exhibited higher activities than the shorter spacer as in compound **5$_a$**. Furthermore, the more lipophilic cyclohexyl moiety as in compounds **5$_e$** and **5$_c$** displayed higher anticancer potency against all cell lines than the distal phenyl group as in compounds **5$_f$** and **5$_d$**, respectively. In the third group, the unsubstituted benzoxazole derivative **6$_b$** displayed higher activity than the 5-methylbenzoxazole derivative **6$_c$**. The long-chain spacer as in compounds **6$_b$** and **6$_c$** exhibited higher activities than the shorter spacer as in compound **6$_a$**. The fourth group **11$_{a,b}$** revealed that the 5-methylbenzoxazole derivative **11$_b$** exhibited higher activity than the unsubstituted one **11$_a$**. In the fifth group, **12$_{a–d}$**, the 4-chloro substituted distal phenyl moiety as in compound **12$_c$** exhibited higher activity than the unsubstituted one **12$_a$**. Contrarily, the electron withdrawing Cl group at position 2 as compound **12$_b$** showed lower activity than the unsubstituted one **12$_a$**. On the other hand, the electron withdrawing group Cl at position 2 as in compound **12$_b$** exhibited more activity than the less electron withdrawing Br group as in compound **12$_d$**, which enabled us to deduce that the lipophilic and electron deficient groups at position 4 increased the activity while at position 2 decreased the activity. In the other group, the 5-methylbenzoxazole derivative **13$_b$** exhibited higher activity than the unsubstituted one **13$_a$**. The cyclic pyrazoline structure **16** showed higher activity than its parent chalcone **15** and the oxime **14**, respectively.

3 | CONCLUSION

The molecular design was performed to investigate the binding mode of the proposed compounds with VEGFR-2 receptor. The data obtained from the docking studies were fitted with that obtained from the biological screening. This higher effect may be due to the change of the linkers, which resemble that of sorafenib. All the tested compounds showed variable anticancer activities. Novel series of benzoxazole derivatives **4$_{a–f}$**–**16** were designed, synthesized, and evaluated for their anticancer activity against three human tumor cell lines hepatocellular carcinoma (HepG2), colorectal carcinoma (HCT-116), and breast cancer (MCF-7) targeting VEGFR-2 enzyme. HCT-116 was the most sensitive cell line to the influence of the new derivatives. In particular, compound **5$_e$** was found to be the most potent derivative overall in the tested compounds against HepG2, HCT-116, and MCF-7 cancer cell lines with IC_{50} = 4.13 ± 0.2, 6.93 ± 0.3, and 8.67 ± 0.5 μM, respectively. Compounds **5$_c$**, **5$_f$**, **6$_b$**, **5$_d$**, and **6$_c$** showed the highest anticancer activities against all HepG2 with IC_{50} of 5.93 ± 0.2, 6.58 ± 0.4, 8.10 ± 0.7, 8.75 ± 0.7, and 9.95 ± 0.9 μM, respectively; HCT-116 with IC_{50} of 7.14 ± 0.4, 9.10 ± 0.8, 7.91 ± 0.6, 9.52 ± 0.5 and 12.48 ± 1.1 μM, respectively; and MCF-7 with IC_{50} of 8.93 ± 0.6, 10.11 ± 0.9, 12.31 ± 1.0, 9.95 ± 0.8, and 15.70 ± 1.4 μM, respectively, in comparison with sorafenib as a reference drug with IC_{50} of 9.18 ± 0.6, 5.47 ± 0.3, and 7.26 ± 0.3 μM, respectively. The most active six compounds in this series **5$_{c–f}$** and **6$_{b,c}$** were further evaluated for their inhibitory activity against VEGFR-2. Compounds **5$_e$** and **5$_c$** potently inhibited VEGFR-2 at lower IC_{50} values of 0.07 ± 0.01 and 0.08 ± 0.01 μM,

respectively, compared with sorafenib IC_{50} value (0.11 ± 0.02 μM). Compound $\mathbf{5_f}$ potently inhibited VEGFR-2 at low IC_{50} value (0.10 ± 0.02 μM) equipotent as sorafenib. Also, compounds $\mathbf{5_d}$, $\mathbf{6_c}$, and $\mathbf{6_b}$ possessed good VEGFR-2 inhibition with IC_{50} values of 0.19 ± 0.02, 0.27 ± 0.02, and 0.36 ± 0.03 μM, respectively. The obtained results showed that the most active compounds could be useful as a template for future design, optimization, adaptation, and investigation to produce more potent and selective VEGFR-2 inhibitors with higher anticancer analogs.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All melting points (m.p.) were carried out by open capillary method on a Gallenkamp melting-point apparatus at the Faculty of Pharmacy, Al-Azhar University, and were uncorrected. The infrared spectra were recorded on Pye Unicam SP 1000 IR spectrophotometer at Pharmaceutical Analytical Unit, Faculty of Pharmacy, Al-Azhar University, using potassium bromide disc technique. Proton magnetic resonance ^{1}H NMR spectra were recorded on a Bruker 400 Mhz-NMR spectrometer at Faculty of Sciences, Cairo University, Cairo, Egypt. ^{13}C NMR spectra were recorded on an Mercury 400 Mhz-NMR spectrometer at Chemical Laboratory, Ministry of Defense, Cairo. TMS was used as internal standard and chemical shifts were measured in δ scale (ppm). The mass spectra were carried out on Direct Probe Controller Inlet part to Single Quadropole mass analyzer in Thermo Scientific GCMS model ISQ LT using Thermo X-Calibur software at the Regional Center for Mycology and Biotechnology, Al-Azhar University. Elemental analyses (C, H, N) were performed on a CHN analyzer at Regional Center for Mycology and Biotechnology, Al-Azhar University. All compounds were within ±0.4 of the theoretical values. The reactions were monitored by thin-layer chromatography (TLC) using TLC sheets precoated with UV fluorescent silica gel Merck 60 F254 plates and were visualized using UV lamp and different solvents as mobile phases.

The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

4.1.2 | Synthesis of compounds $\mathbf{1_{a,b}}$, $\mathbf{2_{a,b}}$, $\mathbf{3_{a–d}}$, 6, $\mathbf{8_{a–d}}$, and $\mathbf{9_{a–d}}$

Benzoxazole-2-thiol ($\mathbf{1_a}$), 5-methylbenzoxazole-2-thiol ($\mathbf{1_b}$), and their corresponding potassium salts ($\mathbf{2_{a,b}}$), 2-chloro-*N*-(4-sulfamoylphenyl)-acetamide ($\mathbf{3_a}$), 2-chloro-*N*-(4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl)acetamide ($\mathbf{3_b}$), 2-chloro-*N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)acetamide ($\mathbf{3_c}$), 3-chloro-*N*-(4-sulfamoylphenyl)propanamide ($\mathbf{3_d}$), *N*-(4-acetylphenyl)-2-chloroacetamide (**6**), ethyl substituted benzoate ($\mathbf{8_{a–d}}$), and substituted benzohydrazide ($\mathbf{9_{a–d}}$) were obtained according to the reported procedures.[19]

4.1.3 | General method for the synthesis of 2-[(5-(un)substituted benzoxazol-2-yl)thio]-*N*-{4-[*N*-(un)substituted sulfamoyl]phenyl}acetamide ($\mathbf{4_{a–d}}$) and 2-[(5-(un)substituted benzoxazol-2-yl)thio]-*N*-(4-sulfamoylphenyl)propanamide ($\mathbf{4_{e–f}}$)

To a mixture of compound $\mathbf{2_a}$ and/or $\mathbf{2_b}$ (0.002 mol) in dry *N*,*N*-dimethylformamide anhydrous (DMF; 50 ml), the appropriate chloroamide derivatives $\mathbf{3_{a–d}}$ (0.002 mol) were added. The reaction mixture was heated using a water bath for 12 hr. After cooling to room temperature, the reaction mixture was poured onto crushed ice. The precipitated solids were filtered, dried, and crystallized from ethanol to give the corresponding compounds $\mathbf{4_{a–g}}$.

2-(Benzoxazol-2-ylthio)-N-(4-sulfamoylphenyl)acetamide ($\mathbf{4_a}$)

Yield, 79%; m.p. 150–152°C; $IR_{\nu max}$ (cm^{-1}): 3,339, 3,240 (NH_2), 3,119 (NH), 3,060 (CH aromatic), 2,972 (CH aliphatic), 1,689 (C═O), 1,601 (C═N), 1,309, 1,150 (SO_2); ^{1}H NMR (400 MHz, dimethyl sulfoxide [DMSO]-d_6): 4.42 (s, 2H, $-SCH_2$), 7.25 (s, 2H, NH_2; D_2O exchangeable), 7.31 (dd, 1H, *J* = 5.7, 5.4 Hz, Ar-H, H-6 of benzoxazole), 7.33 (dd, 1H, *J* = 5.7, 4.8 Hz, Ar-H, H-5 of benzoxazole), 7.60 (d, 1H, *J* = 4.8 Hz, Ar-H, H-4 of benzoxazole), 7.62 (d, 1H, *J* = 5.4 Hz, Ar-H, H-7 of benzoxazole), 7.72 (d, 2H, *J* = 9 Hz, Ar-H, H-2, H-6 of phenyl), 7.76 (d, 2H, *J* = 9 Hz, Ar-H, H-3, H-5 of phenyl), 10.75 (s, 1H, NH; D_2O exchangeable); ^{13}C NMR (400 MHz, DMSO-d_6): δ = 20.03, 79.95, 114.30 (2C), 116.04 (2C), 117.46, 118.87, 126.99, 127.41, 128.50, 129.21 (2C), 160.95, and 162.98; MS (*m/z*): 363 (M^+, 100%, base peak), 225 (22.99%), 151 (22.16%), 132 (22.77%), 57 (21.79%); Anal. calcd. for $C_{15}H_{13}N_3O_4S_2$ (m.w. 363.41): C, 49.58; H, 3.61; N, 11.56; S, 17.64. Found: C, 49.96; H, 3.28; N, 11.40; S, 17.45.

3-(Benzoxazol-2-ylthio)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-acetamide ($\mathbf{4_b}$)

Yield, 75%; m.p. 217–219°C; $IR_{\nu max}$ (cm^{-1}): 3,365, 3,150 (2 NH), 3,097 (CH aromatic), 2,888 (CH aliphatic), 1,664 (C═O), 1,593 (C═N), 1,398, 1,182 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): 4.39 (s, 2H, $-SCH_2$), 6.78 (d, 1H, *J* = 4 Hz, Ar-H, H-5 of thiazole), 7.21 (d, 1H, *J* = 4 Hz, Ar-H, H-4 of thiazole), 7.28 (dd, 1H, *J* = *8.4*, 9 Hz, Ar-H, H-6 of benzoxazole), 7.45 (dd, 1H, *J* = *8.4*, 8 Hz, Ar-H, H-5 of benzoxazole), 7.58 (d, 1H, *J* = 8 Hz, Ar-H, H-4 of benzoxazole), 7.60 (d, 1H, *J* = 9 Hz, Ar-H, H-7 of benzoxazole), 7.70 (d, 2H, *J* = 8 Hz, Ar-H, H-2, H-6 of phenyl), 7.74 (d, 2H, *J* = 8 Hz, Ar-H, H-3, H-5 of phenyl), 10.75 (s, 1H, –CONH; D_2O exchangeable), 12.67 (s, 1H, $-SO_2NH$; D_2O exchangeable); MS (*m/z*): 446 (M^+, 11.20%), 372 (69.39%), 359 (100%, base peak), 151 (12.65%), and 63 (25.83%); Anal. calcd. for $C_{18}H_{14}N_4O_4S_3$ (m.w. 446.51): C, 48.42; H, 3.16; N, 12.55; S, 21.54. Found: C, 48.01; H, 3.12; N, 12.89; S, 21.85.

2-(Benzoxazol-2-ylthio)-N-(4-(N-(pyridin-2-yl)sulfamoyl)phenyl)-acetamide ($\mathbf{4_c}$)

Yield, 78%; m.p. 200–202°C; $IR_{\nu max}$ (cm^{-1}): 3,350, 3,114 (2 NH), 3,050 (CH aromatic), 2,936 (CH aliphatic), 1,675 (C═O), 1,600 (C═N), 1,379, 1,141 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): 4.36

(s, 2H, –SCH_2), 6.94 (dd, 1H, *J* = 7.6, 8.4 Hz, Ar-H, H-4 of pyridine), 7.00 (dd, 1H, *J* = 7.6, 8.4 Hz, Ar-H, H-5 of pyridine), 7.09 (dd, 1H, *J* = 8, 8.4 Hz, Ar-H, H-6 of benzoxazole), 7.34 (dd, 1H, *J* = 7.6, 8.4 Hz, Ar-H, H-5 of benzoxazole), 7.47 (d, 1H, *J* = 7.6 Hz, Ar-H, H-4, of benzoxazole), 7.66 (d, 1H, *J* = 8.4 Hz, Ar-H, H-6 of pyridine), 7.76 (d, 1H, *J* = 8 Hz, Ar-H, H-7 of benzoxazole), 7.80 (d, 2H, *J* = 8 Hz, Ar-H, H-2, H-6 of phenyl), 7.84 (d, 2H, *J* = 8 Hz, Ar-H, H-3, H-5 of phenyl), 7.96 (d, 1H, *J* = 8.4 Hz, Ar-H, H-3 of pyridine), 10.74 (s, 1H, –CONH; D_2O exchangeable), 11.13 (s, 1H, –SO_2NH; D_2O exchangeable); Anal. calcd. for $C_{20}H_{16}N_4O_4S_2$ (m.w. 440.49): C, 54.53; H, 3.66; N, 12.72; S, 14.56. Found: C, 54.75; H, 3.39; N, 12.94; S, 14.81.

*2-((5-Methylbenzoxazol-2-yl)thio)-N-(4-sulfamoylphenyl)acetamide (**4$_d$**)*

Yield, 75%; m.p. 183–185°C; $IR_{\nu max}$ (cm^{-1}): 3,326, 3,237 (NH_2), 3,124 (NH), 3,070 (CH aromatic), 2,930 (CH aliphatic), 1,673 (C=O), 1,597 (C=N), 1,329, 1,155 (SO_2); ^1H NMR (400 MHz, DMSO-d_6): 2.38 (s, 3H, CH_3), 4.40 (s, 2 H, –SCH_2), 7.11 (d, 1H, *J* = 8.4 Hz, Ar-H, H-6 of benzoxazole), 7.27 (s, 2H, NH_2; D_2O exchangeable), 7.41 (s, 1H, Ar-H, H-4 of benzoxazole), 7.49 (d, 1H, *J* = 8.4 Hz, Ar-H, H-7 of benzoxazole), 7.73 (d, 2H, *J* = 9 Hz, Ar-H, H-2, H-6 of phenyl), 7.77 (d, 2H, *J* = 9 Hz, Ar-H, H-3, H-5 of phenyl), 10.77 (s, 1H, NH; D_2O exchangeable); MS (*m/z*): 377 (M^+, 100%), 303 (37.05%), 297 (3.17%), 136 (3.63%), and 78 (10.91%); Anal. calcd. for $C_{16}H_{15}N_3O_4S_2$ (m.w. 377.43): C, 50.92; H, 4.01; N, 11.13; S, 16.99. Found: C, 50.71; H, 3.94; N, 11.33; S, 17.19.

*3-(Benzoxazol-2-ylthio)-N-(4-sulfamoylphenyl)propanamide (**4$_e$**)*

Yield, 70%; m.p. 190–192°C; $IR_{\nu max}$ (cm^{-1}): 3,304, 3,240 (NH_2), 3,190 (NH), 3,304 (CH aromatic), 2,927 (CH aliphatic), 1,661 (C=O), 1,602 (C=N), 1,338, 1,154 (SO_2); ^1H NMR (400 MHz, DMSO-d_6): 2.96 (t, 2H, *J* = 6.8 Hz, CH_2CO–), 4.49 (t, 2H, *J* = 6.8 Hz, –SCH_2), 7.23 (s, 2H, NH_2; D_2O exchangeable), 7.28 (dd, 1H, *J* = 8, 8.4 Hz, Ar-H, H-6 of benzoxazole), 7.35 (dd, 1H, *J* = 8, 7.6 Hz, Ar-H, H-5 of benzoxazole), 7.54 (d, 1H, *J* = 7.6 Hz, Ar-H, H-4 of benzoxazole), 7.57 (d, 1H, *J* = 8.4 Hz, Ar-H, H-7 of benzoxazole), 7.65 (d, 2H, *J* = 8.8 Hz, Ar-H, H-2, H-6 of phenyl), 7.72 (d, 2H, *J* = 8.8 Hz, Ar-H, H-3, H-5 of phenyl), 10.40 (s, 1H, NH; D_2O exchangeable); MS (*m/z*): 377 (M^+, 100%, base peak), 226 (6.82%), 151 (26.71%), 64 (31.66%), and 55 (83.65%); Anal. calcd. for $C_{16}H_{15}N_3O_4S_2$ (m.w. 377.43): C, 50.92; H, 4.01; N, 11.13; S, 16.99. Found: C, 51.26; H, 3.90; N, 11.56; S, 16.72.

*3-((5-Methylbenzoxazol-2-yl)thio)-N-(4-sulfamoylphenyl)-propanamide (**4$_f$**)*

Yield, 77%; m.p. 237–239°C; $IR_{\nu max}$ (cm^{-1}): 3,373, 3,291 (NH_2), 3,134 (NH), 3,050 (CH aromatic), 2,970 (CH aliphatic), 1,661 (C=O), 1,599 (C=N), 1,386, 1,158 (SO_2); ^1H NMR (400 MHz, DMSO-d_6): 2.34 (s, 3H, CH_3), 2.92 (t, 2H, *J* = 6.8, CH_2CO–), 4.47 (t, 2H, *J* = 6.8, –SCH_2), 7.09 (d, 1H, *J* = 8.4 Hz, Ar-H, H-6 of benzoxazole), 7.24 (s, 2H, NH_2; D_2O exchangeable), 7.35 (s, 1H, Ar-H, H-4 of benzoxazole), 7.41 (d, 1H, *J* = 8.4 Hz, Ar-H, H-7 of benzoxazole), 7.65 (d, 2H, *J* = 8.8 Hz, Ar-H, H-2 and H-6 of phenyl), 7.72 (d, 2H, *J* = 8.8 Hz, Ar-H, H-3 and H-5 of phenyl), 10.40 (s, 1H, NH; D_2O exchangeable); ^{13}C NMR (400 MHz, DMSO-d_6): δ = 20.38, 33.01, 41.12, 109.07, 110.53, 118.14 (2C), 124.29, 126.09 (2C), 130.97, 134.17, 137.86, 141.13, 144.10, 168.44, and 178.64; MS (*m/z*): 391 (M^+, 100%, base peak), 358 (12.47%), 226 (1.29%), 107 (11.57%), and 55 (47.19%); Anal. Calcd. for $C_{17}H_{17}N_3O_4S_2$ (m.w. 391.46): C, 52.16; H, 4.38; N, 10.73; S, 16.38. Found: C, 52.47; H, 4.20; N, 10.64; S, 16.33.

4.1.4 | General method for the synthesis of 2-(benzoxazol-2-ylthio)-*N*-(4-(*N*-(substitutedcarbamoyl)sulfamoyl)phenyl)acetamide (**5$_{a–b}$**) and 3-((5-(un)substituted benzoxazol-2-yl)-thio)-*N*-(4-(*N*-(substitutedcarbamoyl)sulfamoyl)-phenyl)propanamide (**5$_{b–c}$**)

A mixture of the appropriate sulfanilamide derivative **4$_a$** and/or **4$_{d–f}$** (0.01 mol) and anhydrous potassium carbonate (2.76 g, 0.02 mol) in 100 ml of dry acetone was refluxed while stirring for about 1.5 hr. The appropriate isocyanates, namely, cyclohexyl isocyanate and/or phenyl isocyanate (0.01 mol) were added drop-wise to the reaction mixture. Refluxing and stirring were continued during the course of the addition and for an additional 24 hr. The acetone was removed by evaporation under reduced pressure, and about 50 ml of water was added to dissolve the resulting residue. The solution was filtered. Acidification of the filtrate with diluted hydrochloric acid caused the precipitation of the product, which was filtered. Crystallization of the filter cake from 90% aqueous ethanol yielded the corresponding sulfonylurea derivatives (**5$_{a–f}$**).

*2-(Benzoxazol-2-ylthio)-N-(4-(N-(cyclohexylcarbamoyl)sulfamoyl)-phenyl)acetamide (**5$_a$**)*

Yield, 79%; m.p. 163–165°C; $IR_{\nu max}$ (cm^{-1}): 3,333, 3,300, 3,240 (3 NH), 3,099 (CH aromatic), 2,930 (CH aliphatic), 1,687 (C=O), 1,335, 1,156 (SO_2); ^1H NMR (400 MHz, DMSO-d_6): 1.06–1.13 (m, 4H, H-3 and H-5 of cyclohexyl), 1.16–1.29 (m, 2H, H-4 of cyclohexyl), 1.49–1.72 (m, 4H, H-2 and H-6 of cyclohexyl), 3.41 (m, 1H, H-1 of cyclohexyl), 5.56 (s, 2H, –SCH_2), 6.32 (s, 1H, N**H**-cyclohexyl; D_2O exchangeable), 7.24 (dd, 1H, *J* = 8, 8.4 Hz, Ar**-H**, H-6 of benzoxazole), 7.27 (dd, 1H, *J* = 8, 7.6 Hz, Ar**-H**, H-5 of benzoxazole), 7.54 (d, 1H, *J* = 7.6 Hz, Ar**-H**, H-4 of benzoxazole), 7.75 (d, 1H, *J* = 8.4 Hz, Ar**-H**, H-7 of benzoxazole), 7.80 (d, 2H, *J* = 8 Hz, Ar**-H**, H-2 and H-6 of phenyl), 7.90 (d, 2H, *J* = 8 Hz, Ar**-H**, H-3 and H-5 of phenyl), 10.24 (s, 1H, –N**H**ph; D_2O exchangeable), 10.56 (s, 1H, –SO_2N**H**; D_2O exchangeable); MS (*m/z*): 488 (M^+, 8.73%), 368 (67.97%), 359 (100%, base peak), 106 (20.25%), and 55 (80.53%); Anal. calcd. for $C_{22}H_{24}N_4O_5S_2$ (m.w. 488.58): C, 54.08; H, 4.95; N, 11.47; S, 13.12. Found: C, 54.54; H, 5.08; N, 11.72; S, 12.82.

*2-(Benzoxazol-2-ylthio)-N-(4-(N-(phenylcarbamoyl)sulfamoyl)phenyl)-acetamide (**5$_b$**)*

Yield, 70%; m.p. 146–148°C; $IR_{\nu max}$ (cm^{-1}): 3,350, 3,340, 3,275 (3 NH), 3,090 (CH aromatic), 2,980 (CH aliphatic), 1,637 (C=O), 1,321, 1,030 (SO_2); ^1H NMR (400 MHz, DMSO-d_6): 3.52 (s, 2H, –SCH_2), 6.90 (s, 1H, –N**H**ph′; D_2O exchangeable), 6.98 (dd, 1H, *J* = 8, 8 Hz, Ar-H,

H-4′ of phenyl), 7.21 (dd, 2H, *J* = 7.6, 8 Hz, Ar-H, H-3′ and H-5′ of phenyl), 7.25 (dd, 1H, *J* = 8, 8 Hz, Ar-H, H-6 of benzoxazole), 7.31 (dd, 1H, *J* = 8, 8.2 Hz, Ar-H, H-5 of benzoxazole), 7.35 (d, 2H, *J* = 7.6 Hz, Ar-H, H-2′ and H-6′ of phenyl), 7.53 (d, 1H, *J* = 8.2 Hz, Ar-H, H-4 of benzoxazole), 7.55 (d, 1H, *J* = 8 Hz, Ar-H, H-7 of benzoxazole), 7.69 (d, 2H, *J* = 8.4 Hz, Ar-H, H-2 and H-6 of phenyl), 7.85 (d, 2H, *J* = 8.4 Hz, Ar-H, H-3, and H-5 of phenyl), 8.76 (s, 1H, –NHph–; D_2O exchangeable), 10.55 (s, 1H, $-SO_2NH$; D_2O exchangeable); Anal. calcd. for $C_{22}H_{18}N_4O_5S_2$ (m.w. 482.53): C, 54.76; H, 3.76; N, 11.61; S, 13.29. Found: C, 54.30; H, 3.90; N, 11.72; S, 13.30.

*3-(Benzoxazol-2-ylthio)-N-(4-(N-(cyclohexylcarbamoyl)sulfamoyl)-phenyl)propanamide (**5c**)*

Yield, 75%; m.p. 204–206°C; $IR_{\nu max}$ (cm^{-1}): 3,329, 3,265, 3,150 (3 NH), 3,070 (CH aromatic), 2,929 (CH aliphatic), 1,678 (C═O), 1,330, 1,166 (SO_2); ^1H NMR (400 MHz, DMSO-d_6): 1.05–1.16 (m, 4H, H-3 and H-5 of cyclohexyl), 1.19–1.26 (m, 2H, H-4 of cyclohexyl), 1.48–1.72 (m, 4H, H-2 and H-6 of cyclohexyl), 2.99 (t, 2H, *J* = 7.6, CH_2CO–), 3.31 (m, 1H, H-1 of cyclohexyl), 4.48 (t, 2H, *J* = 7.6, $-SCH_2$), 6.30 (s, 1H, –NH-cyclohexyl; D_2O exchangeable), 7.30 (dd, 1H, *J* = 7.6, 8 Hz, Ar-H, H-6 of benzoxazole), 7.34 (dd, 1H, *J* = 7.6, 7.2 Hz, Ar-H, H-5 of benzoxazole), 7.54 (d, 1H, *J* = 7.2 Hz, Ar-H, H-4 of benzoxazole), 7.57 (d, 1H, *J* = 8 Hz, Ar-H, H-7 of benzoxazole), 7.68 (d, 2H, *J* = 8.4 Hz, Ar-H, H-2 and H-6 of phenyl), 7.79 (d, 2H, *J* = 8.4 Hz, Ar-H, H-3 and H-5 of phenyl), 10.26 (s, 1H, –CONH; D_2O exchangeable), and 10.48 (s, 1H, $-SO_2NH$; D_2O exchangeable); Anal. calcd. for $C_{23}H_{26}N_4O_5S_2$ (m.w. 502.60): C, 54.96; H, 5.21; N, 11.15; S, 12.76. Found: C, 54.99; H, 5.28; N, 10.98; S, 12.95.

*3-(Benzoxazol-2-ylthio)-N-(4-(N-(phenylcarbamoyl)sulfamoyl)phenyl)-propanamide (**5d**)*

Yield, 77%; m.p. 187–189°C; $IR_{\nu max}$ (cm^{-1}): 3,324, 3,300, 3,150 (3 NH), 3,050 (CH aromatic), 2,922 (CH aliphatic), 1,637 (C═O), 1,326, 1,152 (SO_2); ^1H NMR (400 MHz, DMSO-d_6): 2.96 (t, 2H, *J* = 6.8 Hz, $-CH_2CO$), 4.49 (t, 2H, *J* = 6.8 Hz, $-SCH_2$), 6.98 (s, 1H, –NHph′; D_2O exchangeable), 7.21 (dd, 1H, *J* = 8, 8 Hz, Ar-H, H-4′ of phenyl), 7.27 (dd, 1H, *J* = 7.2, 8.4 Hz, Ar-H, H-6 of benzoxazole), 7.31 (dd, 1H, *J* = 7.2, 6.4 Hz, Ar-H, H-5 of benzoxazole), 7.35 (dd, 2H, *J* = 8, 8 Hz, Ar-H, H-3′ and H-5′ of phenyl), 7.37 (d, 2H, *J* = 8 Hz, Ar-H, H-2′ and H-6′ of phenyl), 7.53 (d, 1H, *J* = 6.4 Hz, Ar-H, H-4 of benzoxazole), 7.55 (d, 1H, *J* = 8.4 Hz, Ar-H, H-7 of benzoxazole), 7.69 (d, 2H, *J* = 8.4 Hz, Ar-H, H-2 and H-6 of phenyl), 7.85 (d, 2H, *J* = 8.4 Hz, Ar-H, H-3 and H-5 of phenyl), 8.73 (s, 1H, –NHph–; D_2O exchangeable), 10.47 (s, 1H, $-SO_2NH$; D_2O exchangeable); MS (*m/z*): 495 (M^+–1, 0.93%), 339 (10.50%), 298 (10.90%), 64 (40.88%), and 55 (100%, base peak); Anal. calcd. for $C_{23}H_{20}N_4O_5S_2$ (m.w. 496.56): C, 55.63; H, 4.06; N, 11.28; S, 12.91. Found: C, 55.71; H, 4.38; N, 11.57; S, 13.01.

*N-(4-(N-(Cyclohexylcarbamoyl)sulfamoyl)phenyl)-3-((5-methylbenzoxazol-2-yl)thio)propanamide (**5e**)*

Yield, 80%; m.p. 232–234°C; $IR_{\nu max}$ (cm^{-1}): 3,342, 3,300, 3,102 (3 NH), 3,060 (CH aromatic), 2,930 (CH aliphatic), 1,686 (C═O), 1,335, 1,156 (SO_2); ^1H NMR (400 MHz, DMSO-d_6): 1.04–1.18 (m, 4H, H-3 and H-5 of cyclohexyl), 1.20–1.25 (m, 2H, H-4 of cyclohexyl), 1.45–1.64 (m, 4H, H-2 and H-6 of cyclohexyl), 2.31 (s, 3H, CH_3 of benzoxazole), 2.93 (t, 2H, *J* = 6.8 Hz, CH_2CO–), 3.17 (m, 1H, H-1 of cyclohexyl), 4.47 (t, 2H, *J* = 6.8 Hz, $-SCH_2$), 6.29 (s, 1H, –NH-cyclohexyl; D_2O exchangeable), 7.08 (d, 1H, *J* = 8.4 Hz, Ar-H, H-6 of benzoxazole), 7.33 (s, 1H, Ar-H, H-4 of benzoxazole), 7.40 (d, 1H, *J* = 8.4 Hz, Ar-H, H-7 of benzoxazole), 7.67 (d, 2H, *J* = 8.8 Hz, Ar-H, H-2 and H-6 of phenyl), 7.79 (d, 2H, *J* = 8.8 Hz, Ar-H, H-3 and H-5 of phenyl), 10.22 (s, 1H, –NHph; D_2O exchangeable), 10.47 (s, 1H, $-SO_2NH$; D_2O exchangeable); MS (*m/z*): 516 (M^+, 3.02%), 422 (62.83%), 265 (35.22%), 102 (99.01%), and 54 (100%, base peak); Anal. calcd. for $C_{24}H_{28}N_4O_5S_2$ (m.w. 516.63): C, 55.80; H, 5.46; N, 10.84; S, 12.41. Found: C, 55.64; H, 5.29; N, 10.68; S, 12.59.

*3-((5-Methylbenzoxazol-2-yl)thio)-N-(4-(N-(phenylcarbamoyl)sulfamoyl)phenyl)propanamide (**5f**)*

Yield, 77%; m.p. 217–219°C; $IR_{\nu max}$ (cm^{-1}): 3,380, 3,317, 3,118 (3 NH), 3,090 (CH aromatic), 2,980 (CH aliphatic), 1,670 (C═O), 1,392, 1,155 (SO_2); ^1H NMR (400 MHz, DMSO-d_6): 2.29 (s, 3H, CH_3), 2.92 (t, 2H, *J* = 6.8 Hz, $-CH_2CO$), 4.47 (t, 2H, *J* = 6.8 Hz, $-SCH_2$), 6.99 (dd, 1H, *J* = 7.6, 7.6 Hz, Ar-H, H-4′ of phenyl), 7.05 (d, 1H, *J* = 8.4 Hz, Ar-H, H-6 of benzoxazole), 7.23 (dd, 2H, *J* = 7.6, 8 Hz, Ar-H, H-3′ and H-5′ of phenyl), 7.31 (d, 2H, *J* = 8 Hz, Ar-H, H-2′ and H-6′ of phenyl), 7.35 (s, 1H, Ar-H, H-4 of benzoxazole), 7.39 (d, 1H, *J* = 8.4 Hz, Ar-H, H-7 of benzoxazole), 7.70 (d, 2H, *J* = 8.8 Hz, Ar-H, H-2 and H-6 of phenyl), 7.87 (d, 2H, *J* = 8.8 Hz, Ar-H, H-3 and H-5 of phenyl), 8.77 (s, 1H, –NHph′; D_2O exchangeable), 10.49 (s, H, –NHph–; D_2O exchangeable), and 10.63 (s, 1H, $-SO_2NH$; D_2O exchangeable); Anal. calcd. for $C_{24}H_{22}N_4O_5S_2$ (m.w. 510.58): C, 56.46; H, 4.34; N, 10.97; S, 12.56. Found: C, 56.67; H, 4.18; N, 10.58; S, 12.37.

4.1.5 | General method for the synthesis of *N*-(4-(*N*-(ethylcarbamothioyl)sulfamoyl)phenyl)-2-((5-methylbenzoxazol-2-yl)thio)acetamide (6_a) and *N*-(4-(*N*-(ethylcarbamothioyl)sulfamoyl)phenyl)-3-((5-(un)-substituted benzoxazol-2-yl)thio)propanamide (6_{b-c})

Ethyl isothiocyanate (0.87 g, 0.01 mol) was added drop-wise to a mixture of the appropriate sulfanilamide derivative **4d–f** (0.01 mol) and anhydrous potassium carbonate (2.76 g, 0.02 mol) in 100 ml of dry acetone. The reaction mixture was refluxed while stirring for 24 hr. The acetone was removed by evaporation under reduced pressure, and about 50 ml of water was added to dissolve the resulting residue. The solution was filtered. Acidification of the filtrate with diluted hydrochloric acid caused the precipitation of the product, which was filtered. Crystallization of the filter cake from 90% aqueous ethanol yielded the corresponding sulfonylthiourea derivatives (6_{a-c}).

*N-(4-(N-(Ethylcarbamothioyl)sulfamoyl)phenyl)-2-((5-methylbenzoxazol-2-yl)thio)acetamide (**6a**)*

Yield, 78%; m.p. 220–222°C; $IR_{\nu max}$ (cm^{-1}): 3,450, 3,363, 3,260 (3 NH), 3,080 (CH aromatic), 2,979 (CH aliphatic), 1,628 (C═O),

1,332, 1,152 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): 1.04 (t, 3H, J = 7.4 Hz, –$CH_2\underline{CH_3}$), 2.16 (s, 3H, CH_3 of benzoxazole), 3.35 (s, 2H, –SCH_2), 3.97 (q, 2H, J = 7.4 Hz, –$\underline{CH_2}CH_3$), 6.98 (d, 1H, J = 8 Hz, Ar-H, H-6 of benzoxazole), 7.32 (s, 1H, Ar-H, H-4 of benzoxazole), 7.39 (d, 1H, J = 8 Hz, Ar-H, H-7 of benzoxazole), 7.82 (d, 2H, J = 8.4 Hz, Ar-H, H-2 and H-6 of phenyl), 7.92 (d, 2H, J = 8.4 Hz, Ar-H, H-3 and H-5 of phenyl), 8.47 (s, 1H, –N<u>H</u>-ethyl; D_2O exchangeable), 11.16 (s, 1H, –CONH; D_2O exchangeable), 11.39 (s, 1H, –SO_2NH; D_2O exchangeable); MS (*m/z*): 464 (M^+, 5.74%), 379 (66.79%), 373 (100%, base peak), 111 (43.35%, base peak), and 73 (49.19%); Anal. calcd. for $C_{19}H_{20}N_4O_4S_3$ (m.w. 464.57): C, 49.12; H, 4.34; N, 12.06; S, 20.70. Found: C, 49.45; H, 4.57; N, 12.37; S, 20.98.

*3-(Benzoxazol-2-ylthio)-N-(4-(N-(ethylcarbamothioyl)sulfamoyl)-phenyl)propanamide (**6$_b$**)*

Yield, 75%; m.p. 177–179°C; $IR_{\nu max}$ (cm^{-1}): 3,360, 3,296, 3,114 (3 NH), 3,058 (CH aromatic), 2,982 (CH aliphatic), 1,670 (C=O), 1,393, 1,151 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): 1.16 (t, 3H, J = 8.4 Hz, CH_3), 2.79 (t, 2H, J = 8 Hz, CH_2CO), 3.12 (q, 2H, J = 8.4 Hz, –$C\underline{H_2}CH_3$), 4.48 (t, 2H, J = 8 Hz, –SCH_2), 7.02 (s, 1H, –N<u>H</u>-ethyl; D_2O exchangeable), 7.33 (dd, 1H, J = 7.8, 8 Hz, Ar-H, H-6 of benzoxazole), 7.38 (dd, 1H, J = 7.8, 7.6 Hz, Ar-H, H-5 of benzoxazole), 7.43 (d, 1H, J = 7.6 Hz, Ar-H, H-4 of benzoxazole), 7.53 (d, 1H, J = 8 Hz, Ar-H, H-7 of benzoxazole), 7.68 (d, 2H, J = 7.4 Hz, Ar-H, H-2 and H-6 of phenyl), 7.80 (d, 2H, J = 7.4 Hz, Ar-H, H-3 and H-5 of phenyl), 10.12 (s, 1H, –CONH; D_2O exchangeable), and 10.46 (s, 1H, –SO_2NH; D_2O exchangeable); Anal. calcd. for $C_{19}H_{20}N_4O_4S_3$ (m.w. 464.57): C, 49.12; H, 4.34; N, 12.06; S, 20.70. Found: C, 49.39; H, 4.17; N, 12.52; S, 20.16.

*N-(4-(N-(Ethylcarbamothioyl)sulfamoyl)phenyl)-3-((5-methylbenzoxazol-2-yl)thio)propanamide (**6$_c$**)*

Yield, 72%; m.p. 203–205°C; $IR_{\nu max}$ (cm^{-1}): 3,322, 3,222, 3,190 (3 NH), 3,070 (CH aromatic), 2,975 (CH aliphatic), 1,690 (C=O), 1,340, 1,165 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): 1.01 (t, 3H, J = 7.2 Hz, –$CH_2\underline{CH_3}$), 2.32 (s, 3, CH_3 of benzoxazole), 2.94 (t, 2H, J = 6.8 Hz, CH_2CO–), 3.32 (q, 2H, J = 7.2 Hz, –$\underline{CH_2}CH_3$), 4.48 (t, 2H, J = 6.8 Hz, –SCH_2), 7.08 (d, 1H, J = 8 Hz, Ar-H, H-6 of benzoxazole), 7.36 (s, 1H, Ar-H, H-4 of benzoxazole), 7.40 (d, 1H, J = 8 Hz, Ar-H, H-7 of benzoxazole), 7.73 (d, 2H, J = 8.8 Hz, Ar-H, H-2 and H-6 of phenyl), 7.81 (d, 2H, J = 8.8 Hz, Ar-H, H-3 and H-5 of phenyl), 8.67 (s, 1H, –N<u>H</u>-ethyl; D_2O exchangeable), 10.74 (s, 1H, CONH; D_2O exchangeable), and 11.47 (s, 1H, SO_2NH; D_2O exchangeable); ^{13}C NMR (400 MHz, DMSO-d_6): δ = 13.68, 21.38 (2C), 34.13, 42.16, 110.07, 111.56, 119.02, 119.12, 125.29, 127.05, 129.13, 131.96, 133.38, 135.19, 143.76, 145.09, 169.74, 178.28, and 179.63; Anal. calcd. for $C_{20}H_{22}N_4O_4S_3$ (m.w. 478.60): C, 50.19; H, 4.63; N, 11.71; S, 20.10. Found: C, 49.17; H, 4.59; N, 11.38; S, 19.85.

4.1.6 | General method for the synthesis of *N*-(4-acetylphenyl)-2-((5-(un)substituted benzoxazol-2-yl)-thio)acetamide ($8_{a,b}$)

A mixture of the appropriate potassium salt of benzoxazole-2-thiol **2$_{a,b}$** (0.01 mol) and *N*-(4-acetylphenyl)-2-chloroacetamide **7** (2.12 g, 0.01 mol) in DMF (50 ml) was heated using a water bath for 8 hr. After cooling to room temperature, the reaction mixture was poured onto crushed ice. The beige and brown precipitates, respectively, were collected by filtration, dried and crystallized from absolute ethanol to give the target compounds **8$_{a,b}$**, respectively.

*N-(4-Acetylphenyl)-2-(benzoxazol-2-ylthio)acetamide (**8$_a$**)*

Yield, 79%; m.p. 155–157°C; $IR_{\nu max}$ (cm^{-1}): 3,324 (NH), 3,060 (CH aromatic), 2,913 (CH aliphatic), 1,671 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): 2.42 (s, 3H, CH_3), 4.44 (s, 2H, CH_2), 7.31 (dd, 1H, J = 7.6, 9.6 Hz, Ar-H, H-6 of benzoxazole), 7.60 (dd, 1H, J = 7, 7.6 Hz, Ar-H, H-5 of benzoxazole), 7.63 (d, 1H, J = 7 Hz, Ar-H, H-4 of benzoxazole), 7.65 (d, 1H, J = 9.6 Hz, Ar-H, H-7 of benzoxazole), 7.72 (d, 2H, J = 8.4 Hz, Ar-H, H-2 and H-6 of phenyl), 7.93 (d, 2H, J = 8.4 Hz, Ar-H, H-3 and H-5 of phenyl), 10.78 (s, 1H, NH; D_2O exchangeable); MS (*m/z*): 326 (M^+, 13.87%), 313 (96.03%), 183 (37.60%), 77 (100, base peak %), and 42 (65.35%); Anal. calcd. for $C_{17}H_{14}N_2O_3S$ (326.37): C, 62.56; H, 4.32; N, 8.58; S, 9.82. Found: C, 62.25; H, 4.16; N, 8.99; S, 10.02.

*N-(4-Acetylphenyl)-2-(5-methylbenzoxazol-2-ylthio)acetamide (**8$_b$**)*

Yield, 70%; m.p. 183–185°C; $IR_{\nu max}$ (cm^{-1}): 3,330 (NH), 3,103 (CH aromatic), 2,996 (CH aliphatic), 1,686 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): 2.36 (s, 3H, CH_3 of benzoxazole), 2.50 (s, 3H, $COCH_3$), 4.39 (s, 2H, –SCH_2), 7.09 (d, 1H, J = 8.4 Hz, Ar-H, H-6 of benzoxazole), 7.39 (s, 1H, Ar-H, H-4 of benzoxazole), 7.48 (d, 1H, J = 8.4 Hz, Ar-H, H-7 of benzoxazole), 7.68 (d, 2H, J = 8.8 Hz, Ar-H, H-2 and H-6 of phenyl), 7.91 (d, 2H, J = 8.8 Hz, Ar-H, H-3 and H-5 of phenyl), 10.74 (s, 1H, NH; D_2O exchangeable); ^{13}C NMR (400 MHz, DMSO-d_6): δ = 21.26, 26.88, 37.25, 110.00, 110.99, 118.88 (2 C), 125.61, 130.02 (2 C), 132.48, 134.54, 141.83, 143.41, 150.05, 164.08, 166.10, 196.96; MS (*m/z*): 340 (M^+, 78%), 232 (21.84%), 191 (28.55%), 62 (42.95%), and 43 (100%, base peak); Anal. calcd. for $C_{18}H_{16}N_2O_3S$ (340.40): C, 63.51; H, 4.74; N, 8.23; S, 9.42. Found: C, 63.56; H, 4.97; N, 7.94; S, 9.61.

4.1.7 | General method for the synthesis of *N*-(4-(1-(2-carbamothioylhydrazono)ethyl)-phenyl)-2-((5-(un)substituted benzoxazol-2-yl)thio)-acetamide ($11_{a,b}$)

The appropriate acetyl derivative **8$_{a,b}$** (0.001 mol) was treated with thiosemicarbazide (0.001 mol) in ethanol (50 ml) in the presence of catalytic amount of glacial acetic acid and refluxed for 8 hr. Resulting solids were filtered, washed with water, dried, and recrystallized from ethanol to afford the corresponding derivatives **11$_{a,b}$**, respectively.

*2-(Benzoxazol-2-ylthio)-N-(4-(1-(2-carbamothioylhydrazono)ethyl)-phenyl)acetamide (**11$_a$**)*

Yield, 79%; m.p. 191–193°C; IR$_{\nu max}$ (cm^{-1}): 3,449, 3,370 (NH$_2$), 3,263, 3,310 (2 NH), 3,050 (CH aromatic), 2,927 (CH aliphatic), 1,689 (C═O); ^{1}H NMR (400 MHz, DMSO-d$_6$): 2.27 (s, 3H, CH$_3$), 4.41 (s, 2H, –SCH$_2$), 7.31 (dd, 1H, Ar-H, *J* = 8, 8 Hz, H-6 of benzoxazole), 7.34 (dd, 1H, Ar-H, *J* = 8.8, 8 Hz, H-5 of benzoxazole), 7.57 (d, 1, *J* = 8.8 Hz, Ar-H, H-4 of benzoxazole), 7.63 (d, 1H, *J* = 8 Hz, Ar-H, H-7 of benzoxazole), 7.66 (d, 2H, *J* = 8.4 Hz, Ar-H, H-2 and H-6 of phenyl), 7.91 (d, 2H, *J* = 8.4 Hz, Ar-H, H-3 and H-5 of phenyl), 8.25 (s, 2H, NH$_2$; D$_2$O exchangeable), 10.17 (s, 1H, CONH; D$_2$O exchangeable), 10.57 (s, 1H, NHCS; D$_2$O exchangeable); ^{13}C NMR (400 MHz, DMSO-d$_6$): *δ* = 24.90, 33.79, 110.54, 111.54 (2 C), 118.98 (2 C), 124.77, 125.46, 128.89 (2 C), 132.05, 146.91, 157.06 (2 C), 169.56 (2 C), and 179.55; Anal. calcd. for C$_{18}$H$_{17}$N$_5$O$_2$S$_2$ (m.w. 399.49): C, 54.12; H, 4.29; N, 17.53; S, 16.05. Found: C, 54.57; H, 4.49; N, 17.48; S, 16.26.

*N-(4-(1-(2-Carbamothioylhydrazono)ethyl)phenyl)-2-((5-methylbenzoxazol-2-yl)thio)acetamide (**11$_b$**)*

Yield, 70%; m.p. 211–213°C; IR$_{\nu max}$ (cm^{-1}): 3,369, 3,307 (NH$_2$), 3,245, 3,172 (2 NH), 3,091 (CH aromatic), 2,961 (CH aliphatic), 1,687 (C═O); ^{1}H NMR (400 MHz, DMSO-d$_6$): 2.27 (s, 3H, CH$_3$), 2.39 (s, 3H, CH$_3$ of benzoxazole), 4.39 (s, 2H, –SCH$_2$), 7.10 (d, 1H, Ar-H, *J* = 8 Hz, H-6 of benzoxazole), 7.37 (s, 1H, Ar-H, H-4 of benzoxazole), 7.45 (d, 1H, *J* = 8 Hz, Ar-H, H-7 of benzoxazole), 7.57 (d, 2H, *J* = 8.6 Hz, Ar-H, H-2 and H-6 of phenyl), 7.85 (d, 2H, *J* = 8.6 Hz, Ar-H, H-3 and H-5 of phenyl), 8.25 (s, 2H, NH$_2$; D$_2$O exchangeable), 10.17 (s, 1H, CONH; D$_2$O exchangeable), and 10.56 (s, 1H, NHCS; D$_2$O exchangeable); Anal. calcd. for C$_{19}$H$_{19}$N$_5$O$_2$S$_2$ (m.w. 413.51): C, 55.19; H, 4.63; N, 16.94; S, 15.51. Found: C, 55.56; H, 4.43; N, 16.97; S, 15.64.

4.1.8 | General method for the synthesis of 2-(benzoxazol-2-ylthio)-*N*-(4-(1-(2-((un)substituted benzoyl)hydrazono)ethyl)phenyl)acetamide (12$_{a-d}$)

N-(4-Acetylphenyl)-2-(benzoxazol-2-ylthio)acetamide (**8$_a$**; 0,33 gm, 0.001 mol) was treated with the appropriate benzohydrazide derivative, namely, benzohydrazide, 2-chlorobenzohydrazide, 4-chlorobenzohydrazide, and/or 2-bromobenzohydrazide **10$_{a-d}$** (0.001 mol) in ethanol (20 ml) in the presence of catalytic amount of glacial acetic acid and refluxed for 8 hr. The resulting solids were filtered, washed with water, dried, and recrystallized from ethanol to afford the corresponding compounds **12$_{a-d}$**, respectively.

*2-(Benzoxazol-2-ylthio)-N-(4-(1-(2-benzoylhydrazono)ethyl)phenyl)-acetamide (**12$_a$**)*

Yield, 79%; m.p. 260–262°C; IR$_{\nu max}$ (cm^{-1}): 3,320, 3,235 (2 NH), 3,070 (CH aromatic), 2,936 (CH aliphatic), 1,664 (C═O); ^{1}H NMR (400 MHz, DMSO-d$_6$): 2.31 (s, 3H, CH$_3$), 4.39 (s, 2H, –SCH$_2$), 7.30 (dd, 1H, Ar-H, *J* = 5.6, 7.2 Hz, H-6 of benzoxazole), 7.47 (dd, 1H, Ar-H, *J* = 9.2, 5.6 Hz, H-5 of benzoxazole), 7.54 (dd, 2H, *J* = 6.8, 7.2 Hz, Ar-H, H-3′ and H-5′ of phenyl), 7.62 (dd, 1H, *J* = 6.8, 6.8 Hz, Ar-H, H-4′ of phenyl), 7.64 (d, 1H, *J* = 9.2 Hz, Ar-H, H-4 of benzoxazole), 7.67 (d, 1H, *J* = 7.2 Hz, Ar-H, H-7 of benzoxazole), 7.82 (d, 2H, *J* = 9.2 Hz, Ar-H, H-2 and H-6 of phenyl), 7.85 (d, 2H, *J* = 9.2 Hz, Ar-H, H-3 and H-5 of phenyl), 7.88 (d, 2H, *J* = 7.2 Hz, Ar-H, H-2′ and H-6′ of phenyl), 10.58 (s, 1H, CONH; D$_2$O exchangeable), 10.70 (s, 1H, NHCO; D$_2$O exchangeable); MS (*m/z*): 444.99 (M$^+$, 25.22%), 443.98 (74.46%), 370.02 (100%, base peak), 104.66 (12.51%), and 76.99 (11.17%); Anal. calcd. for C$_{24}$H$_{20}$N$_4$O$_3$S (m.w. 444.51): C, 64.85; H, 4.54; N, 12.60; S, 7.21. Found: C, 64.98; H, 4.71; N, 12.89; S, 7.32.

*2-(Benzoxazol-2-ylthio)-N-(4-(1-(2-(2-chlorobenzoyl)hydrazono)-ethyl)phenyl)acetamide (**12$_b$**)*

Yield, 77%; m.p. 190–192°C; IR$_{\nu max}$ (cm^{-1}): 3,299, 3,187 (2 NH), 3,053 (CH aromatic), 2,929 (CH aliphatic), 1,660 (C═O); ^{1}H NMR (400 MHz, DMSO-d$_6$): 2.26 (s, 3H, CH$_3$), 4.35 (s, 2H, –SCH$_2$), 7.30 (dd, 1H, Ar-H, *J* = 7.6, 8 Hz, H-6 of benzoxazole), 7.32 (dd, 1H, Ar-H, *J* = 7.4, 8 Hz, H-5 of benzoxazole), 7.34–7.62 (m, 4H, Ar-H, H-3′, H-4′, H-5′ and H-6′ of phenyl), 7.63 (d, 1H, *J* = 7.4 Hz, Ar-H, H-4 of benzoxazole), 7.65 (d, 1H, *J* = 7.6 Hz, Ar-H, H-7 of benzoxazole), 7.80 (d, 2H, *J* = 8.4 Hz, Ar-H, H-2 and H-6 of phenyl), 7.87 (d, 2H, *J* = 8.4 Hz, Ar-H, H-3 and H-5 of phenyl), 10.47, 10.61 (s, s, 1H, CONH; D$_2$O exchangeable), 10.91, 11.16 (s, s, 1H, NHCO; D$_2$O exchangeable); ^{13}C NMR (400 MHz, DMSO-d$_6$): *δ* = 14.86, 37.21, 110.69, 118.70, 119.18, 124.81 (2 C), 125.14 (2 C), 126.95, 127.55, 128.98, 129.25, 130.00, 130.91, 133.50, 137.17, 140.35, 148.45, 151.75, 154.64, 163.35, 164.29, and 169.96; MS (*m/z*): 481 (M$^+$+2, 11.30%), 479 (M$^+$, 44.90%), 404 (100%, base peak), 314 (14.21%), 139 (82.24%), 77 (5.50%); Anal. calcd. for C$_{24}$H$_{19}$ClN$_4$O$_3$S (m.w. 478.95): C, 60.19; H, 4.00; N, 11.70; S, 6.69. Found: C, 60.42; H, 4.02; N, 11.56; S, 6.57.

*2-(Benzoxazol-2-ylthio)-N-(4-(1-(2-(4-chlorobenzoyl)hydrazono)-ethyl)phenyl)acetamide (**12$_c$**)*

Yield, 75%; m.p. 247–249°C; IR$_{\nu max}$ (cm^{-1}): 3,327, 3,255 (2 NH), 3,056 (CH aromatic), 2,924 (CH aliphatic), 1,651 (C═O); ^{1}H NMR (400 MHz, DMSO-d$_6$): 2.31 (s, 3H, CH$_3$), 4.39 (s, 2H, –SCH$_2$), 7.28 (dd, 1H, Ar-H, *J* = 7, 8 Hz, H-6 of benzoxazole), 7.30 (dd, 1H, Ar-H, *J* = 7, 8.4 Hz, H-5 of benzoxazole), 7.32 (d, 2H, *J* = 7.2 Hz, Ar-H, H-3′ and H-5′ of phenyl), 7.55 (d, 2H, *J* = 8.4 Hz, Ar-H, H-4 of benzoxazole), 7.57 (d, 2H, *J* = 8 Hz, Ar-H, H-7 of benzoxazole), 7.60 (d, 2H, *J* = 8 Hz, Ar-H, H-2 and H-6 of phenyl), 7.63 (d, 2H, *J* = 8 Hz, Ar-H, H-3 and H-5 of phenyl), 7.82 (d, 2H, *J* = 7.2 Hz, Ar-H, H-2′ and H-6′ of phenyl), 10.59 (s, 1H, CONH; D$_2$O exchangeable), 10.77 (s, 1H, NHCO; D$_2$O exchangeable); ^{13}C NMR (400 MHz, DMSO-d$_6$): *δ* = 21.37, 37.21, 110.06 (2 C), 118.64 (2 C), 119.15 (2 C), 125.61 (2 C), 127.70, 128.82, 130.24, 133.52, 134.54 (2 C), 141.86 (2 C), 150.05 (2 C), 164.15, and 165.65 (2 C); Anal. calcd. for C$_{24}$H$_{19}$ClN$_4$O$_3$S (m.w. 478.95): C, 60.19; H, 4.00; N, 11.70; S, 6.69. Found: C, 60.07; H, 3.84; N, 11.50; S, 6.68.

*2-(Benzoxazol-2-ylthio)-N-(4-(1-(2-(2-bromobenzoyl)hydrazono)-ethyl)phenyl)acetamide (**12$_d$**)*

Yield, 75%; m.p. 186–188°C; IR$_{\nu max}$ (cm^{-1}): 3,311, 3,210 (2 NH), 3,070 (CH aromatic), 2,930 (CH aliphatic), 1,660 (C═O); ^{1}H NMR (400 MHz, DMSO-d$_6$): 2.27 (s, 3H, CH$_3$), 4.36 (s, 1H, –SCH$_2$), 7.29 (dd,

1H, Ar-H, J = 8.4, 8 Hz, H-6 of benzoxazole), 7.32 (dd, 1H, Ar-H, J = 7.4, 8 Hz, H-5 of benzoxazole), 7.34 (dd, 1H, Ar-H, J = 8.8, 8.8 Hz, H-5′ of phenyl), 7.41 (d, 1H, J = 7.4 Hz, Ar-H, H-4 of benzoxazole), 7.43 (d, 1H, J = 8.4 Hz, Ar-H, H-7 of benzoxazole), 7.45 (d, 2H, J = 8.4 Hz, Ar-H, H-2 and H-6 of phenyl), 7.51 (d, 1H, J = 7.2 Hz, Ar-H, H-3′ of phenyl), 7.57 (dd, 1H, J = 7.2, 8.8 Hz, Ar-H, H-4′ of phenyl), 7.64 (d, 1H, J = 8.8 Hz, Ar-H, H-6′ of phenyl), 7.81 (d, 2H, J = 8.4 Hz, Ar-H, H-3 and H-5 of phenyl), 10.48, 10.60 (s, s, 1H, CONH; D_2O exchangeable), 10.91, 11.16 (s, s, 1H, NHCO; D_2O exchangeable); MS (*m/z*): 525 (M^++2, 13.38%), 523 (M^+, 33.27%), 358 (100%, base peak), 326 (76.72%), 184 (32.96%), and 63 (75.27%); Anal. calcd. for $C_{24}H_{19}BrN_4O_3S$ (m.w. 523.41): C, 55.07; H, 3.66; N, 10.70; S, 6.13. Found: C, 54.81; H, 3.95; N, 10.36; S, 5.99.

4.1.9 | General method for the synthesis of *N*-(4-(1-(2-(2-cyanoacetyl)hydrazono)ethyl)phenyl)-2-(((un)-substituted benzoxazol-2-yl)thio)acetamide ($13_{a,b}$)

A mixture of the appropriate acetyl derivative $\mathbf{8_{a,b}}$ (0.001 mol) and 2-cyanoacetohydrazide (0.01 g, 0.001 mol) in ethanol (25 ml) was refluxed for 3 hr, then left to cool. The solid product formed upon pouring onto ice/water was filtered and recrystallized from ethanol to give the corresponding derivatives $\mathbf{13_{a,b}}$, respectively.

*2-(Benzoxazol-2-ylthio)-N-(4-(1-(2-(2-cyanoacetyl)hydrazono)ethyl)-phenyl)acetamide (**13$_a$**)*

Yield, 79%; m.p. 207–209°C; $IR_{\nu max}$ (cm^{-1}): 3,350, 3,179 (2 NH), 3,057 (CH aromatic), 2,925 (CH aliphatic), 2,260 (CN), 1,673 (C═O); ^1H NMR (400 MHz, DMSO-d_6): 2.20 (s, 3H, CH_3), 4.20 (s, 2H, –SCH_2), 4.39 (s, 2H, J = 6.8 Hz, –CH_2CN), 7.30 (dd, 1H, Ar-H, J = 8.8, 9 Hz, H-6 of benzoxazole), 7.33 (dd, 1H, Ar-H, J = 8.8, 9.2 Hz, H-5 of benzoxazole), 7.60 (d, 1H, J = 9.2 Hz, Ar-H, H-4 of benzoxazole), 7.64 (d, 1H, J = 9 Hz, H-7 of benzoxazole), 7.76 (d, 2H, J = 8.4 Hz, Ar-H, H-2 and H-6 of phenyl), 7.87 (d, 2H, J = 8.4 Hz, Ar-H, H-3 and H-5 of phenyl), 10.60 (s, 1H, CONHph; D_2O exchangeable), and 10.96 (s, 1H, NHCO; D_2O exchangeable); Anal. calcd. for $C_{20}H_{17}N_5O_3S$ (m.w. 407.45): C, 58.96; H, 4.21; N, 17.19; S, 7.87. Found: C, 59.25; H, 4.35; N, 16.92; S, 7.62.

*N-(4-(1-(2-(2-Cyanoacetyl)hydrazono)ethyl)phenyl)-2-((5-methylbenzo[d]oxazol-2-yl)thio)-acetamide (**13$_b$**)*

Yield, 70%; m.p. 241–243°C; $IR_{\nu max}$ (cm^{-1}): 3,320, 3,183 (2 NH), 3,056 (CH aromatic), 2,925 (CH aliphatic), 2,258 (CN), 1,685 (C═O); ^1H NMR (400 MHz, DMSO-d_6): 2.20 (s, 3H, CH_3), 2.37 (s, 3H, CH_3 of benzoxazole), 4.20 (s, 2H, –SCH_2), 4.37 (s, 2H, J = 6.8 Hz, –CH_2CN), 7.10 (d, 1H, Ar-H, J = 8.4 Hz, H-6 of benzoxazole), 7.40 (s, 1H, Ar-H, H-4 of benzoxazole), 7.48 (d,1H, J = 8.4 Hz, Ar-H, H-7 of benzoxazole), 7.60 (d, 2H, J = 8 Hz, Ar-H, H-2 and H-6 of phenyl), 7.76 (d, 2H, J = 8 Hz, Ar-H, H-3 and H-5 of phenyl), 10.57 (s, 1H, CONH; D_2O exchangeable), and 10.96 (s, 1H, CONHph; D_2O exchangeable); ^{13}C NMR (400 MHz, DMSO-d_6): δ = 14.11, 21.36, 25.33, 37.20, 110.06, 116.72, 118.62, 119.08, 119.17, 125.61, 127.46, 127.73, 133.26, 134.54, 140.16, 141.84, 149.10, 150.04, 164.15, 165.63, and 166.17; Anal. calcd. for $C_{21}H_{19}N_5O_3S$ (m.w. 421.48): C, 59.84; H, 4.54; N, 16.62; S, 7.61. Found: C, 59.91; H, 4.59; N, 16.40; S, 7.55.

4.1.10 | Synthesis of 2-(benzoxazol-2-ylthio)-*N*-(4-(1-(hydroxyimino)ethyl)phenyl)acetamide (14)

A mixture of equimolar amounts of the acetyl derivative $\mathbf{8_a}$ (0.33 g, 0.001 mol) and hydroxylamine hydrochloride (0.07 g, 0.001 mol) in absolute ethanol (50 ml) was heated under reflux for 20 hr and then left to cool. The separated solid was filtered, washed with water, dried, and recrystallized from ethanol.

Yield, 65%; m.p. 205–207°C; $IR_{\nu max}$ (cm^{-1}): 3,310 (NH), 3,065 (CH aromatic), 2,915 (CH aliphatic), 1,672 (C═O); ^1H NMR (400 MHz, DMSO-d_6): 2.12 (s, 3H, CH_3), 3.75 (s, 2H, –SCH_2), 7.53 (dd, 1H, Ar-H, J = 8, 8.4 Hz, H-6 of benzoxazole), 7.56 (dd, 1H, Ar-H, J = 8, 8.8 Hz, H-5 of benzoxazole), 7.65 (d, 1H, J = 8.8 Hz, Ar-H, H-4 of benzoxazole), 7.70 (d, 2H, J = 8.4 Hz, Ar-H, H-7 of benzoxazole), 7.87 (d, 2H, J = 8.4 Hz, Ar-H, H-2 and H-6 of phenyl), 7.91 (d, 2H, J = 8.4 Hz, Ar-H, H-3 and H-5 of phenyl), 10.26 (s, 1H, CONH; D_2O exchangeable), and 11.07 (s, 1H, NOH; D_2O exchangeable); Anal. calcd. for $C_{17}H_{15}N_3O_3S$ (m.w. 341.39): C, 59.81; H, 4.43; N, 12.31; S, 9.39. Found: C, 59.81; H, 4.43; N, 12.31; S, 9.39.

4.1.11 | Synthesis of 2-(benzoxazol-2-ylthio)-*N*-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)acetamide (15)

To a mixture of the acetyl derivative $\mathbf{8_a}$ (0.65 g, 0.002 mol) in ethyl alcohol (50 ml) and 5% NaOH in ethyl alcohol (10 ml), 4-methoxybenzaldehyde (0.27 g, 0.002 mol) was added drop-wise within 15 min. The reaction mixture was refluxed for 3 hr. The formed precipitate was cooled, filtered, air dried, and then recrystallized from ethanol to give the corresponding chalcone **15**.

Yield, 83%; m.p. 146–148°C; $IR_{\nu max}$ (cm^{-1}): 3,114 (NH), 3,080 (CH aromatic), 2,905 (CH aliphatic), 1,665 (C═O); ^1H NMR (400 MHz, DMSO-d_6): 3.02 (s, 3H, $COCH_3$), 4.65 (s, 2H, –SCH_2), 6.70 (d, 1H, J = 9.2 Hz, COCH═), 6.81 (d, 2H, J = 8.8 Hz, Ar-H, H-3′ and H-5′ of phenyl′), 7.28 (dd, 1H, Ar-H, J = 8.4, 8.8 Hz, H-6 of benzoxazole), 7.35 (dd, 1H, Ar-H, J = 8.4, 8 Hz, H-5 of benzoxazole), 7.41 (d, 1H, J = 8 Hz, Ar-H, H-4 of benzoxazole), 7.43 (d, 1H, J = 8.8 Hz, Ar-H, H-7 of benzoxazole), 7.47 (d, 2H, J = 8.8 Hz, Ar-H, H-2′ and H-6′ of phenyl′), 7.74 (d, 2H, J = 8 Hz, Ar-H, H-2 and H-6 of phenyl), 7.96 (d, 2H, J = 8 Hz, Ar-H, H-3 and H-5 of phenyl), 8.08 (d, 1H, J = 9.2 Hz, = CH-ph′), and 12.81 (s, 1H, NH; D_2O exchangeable); Anal. calcd. for $C_{25}H_{20}N_2O_4S$ (m.w. 444.51): C, 67.55; H, 4.54; N, 6.30; S, 7.21. Found: C, 67.33; H, 4.45; N, 6.50; S, 7.33.

4.1.12 | Synthesis of *N*-(4-(1-acetyl-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenyl)-2-(benzoxazol-2-ylthio)acetamide (16)

A mixture of the chalcone **15** (0.89 g, 0.002 mol) and hydrazine hydrate (1 ml, 98%) in ethanol (50 ml) in the presence of glacial acetic acid (5 ml) was refluxed for 6 hr. After cooling, the separated

precipitate was filtered, air dried, and recrystallized from ethanol to give the corresponding *N*-acetylpyrazoline derivative **16**.

Yield, 65%; m.p. 281–283°C; $IR_{\nu max}$ (cm^{-1}): 3,323 (NH), 3,090 (CH aromatic), 2,978 (CH aliphatic), 1,675 (C=O); ^1H NMR (400 MHz, DMSO-d_6): 1.66 (s, 3H, $COCH_3$), 3.70 (d, 2H, J = 12 Hz, CH_2 of pyrazol), 3.75 (s, 2H, OCH_3), 3.76 (s, 3H, SCH_2), 6.35 (t, 1H, CH of pyrazol), 6.77 (dd, 1H, J = 8.4, 7 Hz, H-6 of benzoxazole), 6.89 (d, 2H, J = 8.4 Hz, Ar-H, H-2 and H-6 of phenyl), 7.05 (dd, 1H, Ar-H, J = 8.4, 8.8 Hz, H-5 of benzoxazole), 7.16 (d, 1H, J = 8.8 Hz, Ar-H, H-4 of benzoxazole), 7.41 (d, 2H, J = 8.4 Hz, Ar-H, H-3 and H-5 of phenyl), 7.81 (d, 1H, J = 7 Hz, H-7 of benzoxazole), and 9.70 (s, 1H, NH; D_2O exchangeable); ^{13}C NMR (400 MHz, DMSO-d_6): δ = 14.11, 21.36, 25.33, 37.20, 110.06, 116.72, 118.62, 119.08, 119.17, 125.61, 127.46, 127.73, 133.26, 134.54, 140.16, 141.84, 149.10, 150.04, 164.15, 165.63, 166.17; 26.80, 41.09, 55.79 (2 C), 108.29, 114.82 (2 C), 114.97 (2 C), 115.46, 119.44, 120.46, 123.38 (2 C), 128.21 (2 C), 130.29 (2 C), 130.81 (2 C), 132.26, 134, 142.21, 147.65, 158.6, 161.39, and 186.73; Anal. calcd. for $C_{27}H_{24}N_4O_4S$ (m.w. 500.57): C, 64.79; H, 4.83; N, 11.19; S, 6.40. Found: C, 64.71; H, 4.89; N, 10.99; S, 6.51.

4.2 | Docking studies

In the present work, all the target compounds were subjected to docking study to explore their binding mode toward VEGFR-2 enzyme. All modeling experiments were performed using molsoft program, which provides a unique set of tools for the modeling of protein/ligand interactions. It predicts how small flexible molecule such as substrates or drug candidates bind to a protein of known three-dimensional structure represented by grid interaction potentials (http://www.molsoft.com/icm_pro.html). Each experiment used the biological target VEGFR-2 downloaded from the Brookhaven Protein Databank (http://www.rcsb.org/pdb/explore/explore.do?structureId=1YWN). To qualify the docking results in terms of accuracy of the predicted binding conformations in comparison with the experimental procedure, the reported VEGFR-2 inhibitor drugs vatalanib and sorafenib were used as reference ligands.

4.3 | Biological assays

4.3.1 | In vitro cytotoxic activity

The cytotoxicity assays were performed at Pharmacology & Toxicology Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt. Cancer cells from different cancer cell lines hepatocellular carcinoma (HepG2), breast cancer (MCF-7), and colorectal carcinoma (HCT-116), were purchased from American Type Cell Culture Collection (ATCC; Manassas) and grown on the appropriate growth medium Roswell Park Memorial Institute medium (RPMI 1640) supplemented with 100 mg/ml of streptomycin, 100 U/ml of penicillin and 10% of heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO_2 atmosphere at 37°C. Cytotoxicity assay by MTT.

Exponentially growing cells from different cancer cell lines were trypsinized, counted, and seeded at the appropriate densities (2,000–1,000 cells/0.33 cm^2 per well) into 96-well microtiter plates. Cells then were incubated in a humidified atmosphere at 37°C for 24 hr. Then, cells were exposed to different concentrations of compounds (0.1, 10, 100, and 1,000 μM) for 72 hr. Then the viability of treated cells was determined using MTT technique as follows. Media were removed, cells were incubated with 200 μl of 5% MTT solution/well (Sigma-Aldrich, MO) and were allowed to metabolize the dye into colored-insoluble formazan crystals for 2 hr. The remaining MTT solution were discarded from the wells and the formazan crystals were dissolved in 200 μl/well acidified isopropanol for 30 min, covered with aluminum foil and with continuous shaking using a MaxQ 2000 plate shaker (Thermo Fisher Scientific Inc, MI) at room temperature. Absorbance was measured at 570 nm using a Stat Fax 4200 plate reader (Awareness Technology, Inc., FL). The cell viability was expressed as percentage of control and the concentration that induces 50% of maximum inhibition of cell proliferation (IC_{50}) were determined using GraphPad Prism version 5 software (GraphPad software Inc, CA).[41–43]

4.3.2 | In vitro VEGFR-2 kinase assay

The kinase activity of VEGFR-2 was carried out in Pharmacology and Toxicology Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt, and measured by use of an antiphosphotyrosine antibody with the Alpha Screen system (PerkinElmer) according to manufacturer's instructions.[6] Enzyme reactions were performed in 50 mM Tris-HCl, pH 7.5, 5 mM $MnCl_2$, 5 mM $MgCl_2$, 0.01% Tween-20, and 2 mM DTT, containing 10 μM adenosine triphosphate (ATP), 0.1 μg/ml biotinylated poly-GluTyr (4:1) and 0.1 nM of VEGFR-2 (Millipore, UK). Before catalytic initiation with ATP, the tested compounds at final concentrations ranging from 0 to 300 μg/ml and enzyme were incubated for 5 min at room temperature. The reactions were quenched by the addition of 25 μl of 100 mM EDTA (ethylenediaminetetraacetic acid), 10 μg/ml AlphaScreen streptavidine cytotoxicity evaluation donor beads and 10 μg/ml acceptor beads in 62.5 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) pH 7.4, 250 mM NaCl, and 0.1% bovine serum albumin. Plate was incubated in the dark overnight and then read by ELISA reader (PerkinElmer). Wells containing the substrate and the enzyme without compounds were used as reaction control. Wells containing biotinylated poly-GluTyr (4:1) and enzyme without ATP were used as basal control. Percent inhibition was calculated by the comparison of compounds treated to control incubations. The concentration of the test compound causing 50% inhibition (IC_{50}) was calculated from the concentration–inhibition response curve (triplicate determinations) and the data were compared with sorafenib (Sigma-Aldrich) as standard VEGFR-2 inhibitor.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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