Synthesis, Molecular Docking, And Antitumor Evaluation of Some New Pyrazole, Pyridine, And Thiazole Derivatives Incorporating Sulfonamide Residue

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Abstract

Cancer is the second leading cause of death worldwide. There is always a huge demand for novel anticancer. New series of pyrazole, pyridine, thiazole derivatives containing sulfonamide moiety were prepared and screened for their antitumor activity against breast cancer cell line (MCF-7). The results of this investigation revealed that compounds **3** and **8** had significant anticancer activity against the MCF-7 cancer cell line with IC₅₀ values 19.2 and 14.2 μ M, respectively, in relation to the standard drug, doxorubicin.

Introduction

Cancer is one of the most dreadful diseases in the world and despite immense advances in the field of basic and clinical research, which have resulted in higher cure rates for a number of malignancies^{1,2}. Tumor growth and metastasis depend on various factors, including the physiological process of angiogenesis. For example, elevated expression of vascular endothelial growth factor, epidermal growth factor, fibroblast growth factor, and platelet-derived growth factor are associated with tumor angiogenesis, metastases, survival, and resistance to apoptosis. Therefore, these growth factors have represented potential molecular targets for inhibition of tumor growth and progression³⁻⁶. Presently, a large number of anti-cancer drugs in clinical practice have shown pervasive side effects, low specificity, and multidrug resistance, so, there is always an urgent demand to develop novel anticancer drugs, and diverse new natural or synthetic compounds are developed continuously by scientists⁷. Sulfa drugs were the first broadly effective antibacterial to be used systemically and paved the way for the antibiotic revolution in medicine (see Fig. 1). In addition, Sulfonyl or sulfonamide hybrids were broadly explored for their anticancer activities via different mechanisms⁸⁻¹⁷ and it was found that they possess minimum side effects along with multi-drug resistance activity.

Therefore, and in continuation of our interest to develop a series of novel small molecules as potential antitumor agents that can block biologically relevant molecular targets¹⁸⁻²², we prepared certain pyrazoles, pyridines, thiazoles derivatives containing sulfonamide pharmacophores and studied their activity against breast cancer cell line (MCF-7).

Results And Discussion

2.1. Chemistry Section

Compounds **1a** and **1b** were prepared according to literature procedure²³. Condensation of **1a** and **1b** and phenylhydrazine provided the hydrazones derivatives **2a** and **2b**. The Vilsmeier- Haack reaction using 2.5 equiv of phosphoryl chloride performed a double addition of reagent to afford, ultimately after hydrolysis, the desired pyrazole 4-carbaldehyde derivative **3** (Scheme 1). The structure of **3** was established using both spectral and analytical analyses, in addition to X-ray analysis (Fig. 2). The IR spectrum of **3** showed

absorption bands at $\nu = 1672$ (CHO), 1633 (N = CH-), and $1342, 1153$ (SO₂); while ¹H NMR showed signals at 10.52 (s, 1H, CHO) and 9.28 (s, 1H, pyrazole H-5) besides the rest of protons in its expected locations.

Treatment of the pyrazole carbaldehyde derivative **3** with some selected nitrogenous active reagents, namely (thiosemicarbazide, carbohydrazide, and cyanoacetohydrazide), afforded the condensed adduct **4**, **5**, and **6** respectively without any observation of cyclized products. The versatile α, β -unsaturated nitrile **7** is obtained upon treatment of **3** with ethyl cyanoacetate in the presence of few drops of triethylamine which was directly subjected to react with acetophenone in boiling ethanol containing a catalytic amount of ammonium acetate to afford 1,2 dihydropyridine derivatives **8**. The structure of **8** was established by both spectral and analytical analysis, the infra-red spectrum of compound **8** showed absorption peaks $\nu = 3360$ (NH), 2218 (CN), 1655 (CO), in addition, the ¹H NMR exhibited signals at $\delta = 12.54$ (NH), 9.28 (s, 1H, pyrazole H-5), and 7.74 (pyridine H-5), supported the suggested structure. Treatment of **3** with different aldehydes, namely acetophenone and 2-acetylthiophene, gave the corresponding chalcone derivatives **9a** and **9b**. The ¹H-NMR of **9a** (as a represented example) shows two doublet protons with J coupling 15.30 Hz, indicating that the chalcone derivatives exist in E-configuration (Scheme 2).

The pyrazole derivative **10** was achieved in good yield, via the treatment of **9a** with hydrazine hydrate in boiling ethanol.

Bromination of compounds **2a** and **2b** using bromine in acetic acid afforded the bis-dibromide derivatives **11a** and **11b** as sole products in very high yields. The structures of **12a** and **12b** were established by both spectral and analytical analyses. The appearance of the singlet signal at $\delta = 7.57$ ppm (CH) is supported the suggested structures.

The monobromo derivative **11a** could be isolated as a side product, indicated that the mechanism of the reaction involving the formation of the monobromo as intermediate. Treatment of compounds with urea or N-phenylurea afforded the thiazoles derivatives **13 a-d** in good yields (see, scheme 3 and experimental section)

2.2. Anticancer Activity Screening

2.2.1. Cytotoxicity Assay

The selectivity and sensitivity of malignancy and normal cells were checked by cytotoxicity assay of the experimental compounds. Ten of the newly synthesized compounds were examined for their anticancer action counter to MCF-7 cancer cell line through a high-throughput screening technique, and the results are furnished in Table 1. The screening of the IC₅₀ results in Table 1 demonstrated that compound **8** revealed high anticancer activity against the MCF-7 cancer cell line with IC₅₀ values $14.2 \mu\text{M}$. Meanwhile, this compound has the topmost IC₅₀ value, it can be estimated as a primary hit. Compounds **3**, **13a**, and **13b** exhibited high proliferative activity against the chosen cell line with IC₅₀ values $19.2, 28.1,$ and $35.1 \mu\text{M}$, respectively. On the other hand, two Compounds (**2a** and **10**) demonstrated trivial anticancer

activity against the MCF-7 cell line with IC₅₀ values 210.8 and 149.3 μ M, respectively. From the previously stated study, we could realize that compounds 3 and 8 presented a good anticancer activity through all the established compounds expressed by their IC₅₀ values associate with doxorubicin IC₅₀ = 1.19 μ M. The biological profile of these compounds could be optimized by additional studies and could be used as lead compounds.

Table 1
Docking, binding energy calculations, and antitumor activity assay against MCF-7 of the newly synthesized compounds:

Comp. No.	MCF-7 IC ₅₀ (μ M)	Binding Energy (kcal/mol)	C-Docker Energy (-)	C-Docker Interaction Energy (-)
2a	149.2	11.2958	31.8572	53.8868
2b	88.2	9.5428	23.3008	57.2528
3	19.2	-10.32	10.2987	53.3724
8	14.2	-26.3	29.77	50.25
9a	89.6	10.7781	26.3799	73.4747
10	210.8	15.6467	0.17243	64.7635
13a	28.1	-5.8561	23.7322	43.0261
13b	35.1	-3.3595	14.2812	44.1445
13c	78.3	3.6573	22.5583	44.5716
13d	62.3	1.3936	19.0755	57.9311

2.2.2. Molecular Modeling

Molecular docking for synthesized compounds was performed to explore their biological profile due to their binding with CDK2. Subsequently, their bound conformations and binding affinities were estimated. The structure preparation of the receptor was done by the removal of co-crystallized ligands and water molecules before energy minimization using the CHARMM force field. Hydrogens were added to the protein followed by adding the charges. The CDK2 coordinates were gained from the protein data bank (PDB 6GUH) and the structure was adjusted by Accelrys Discovery Studio 2.5. The missing residues were added as well as the structure was relaxed to correct the protein errors. Finally, the active site of CDK2 was distinct and all the synthesized compounds were docked and their binding energies were calculated. The synthesized derivatives were docked utilizing the default parameters of the C-Docker protocol (see Figures. 3 and 4), (Table 1).

Materials And Methods

A digital Gallen-KampMFB-595 instrument was used to measure melting points using open capillary tubes. The results were not corrected. KBr pellets were used to measure IR spectra using a Shimadzu FTIR-440 spectrometer. The MS-50 Kratos (A.E.I.) spectrometer, equipped with a data system, was used to obtain mass spectra at 70 eV. Chemical shifts in ppm units for ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) were measured using a Bruker model Ultra Shield NMR spectrometer in DMSO- d_6 using tetramethyl silane (TMS) as an internal reference. Cairo University's Microanalytical Center conducted the elemental studies (percent C, H, and N). The appropriate precautions in handling moisture-sensitive compounds were taken. Solvents were dried by standard techniques. The monitoring of the progress of all reactions and homogeneity of the synthesized compounds were carried out and were run using thin-layer chromatography (TLC) aluminum sheets silica gel 60 F254 (Merck).

General procedure for synthesizing of hydrazones derivatives 2a and 2b: To **1a** and **1b** (10 mmol) in acetic acid (100 mL) and water (10 mL) phenylhydrazine (11 mmol) was added. The reaction mixture was stirred at room temperature until completion (TLC, 5 h). The solid which was formed was filtered off, washed with water, dried and crystallized from acetic acid to give:

4-[5-methyl-4-(1-(2-phenylhydrazineylidene) ethyl)-1H-1,2,3-triazol-1-yl]benzene-sulfonamide (2a). Colorless crystals, yield 90%, m.p. = 256–258 °C. IR (KBr, cm^{-1}) ν = 3323, 3165 (NH_2), 3136 (NH), 1371, 1141 (SO_2); ^1H NMR (500 MHz, δ , ppm, DMSO- d_6): δ = 9.35 (s, 1H, NH), 8.06 (d, 2H, J = 8.5 Hz, Ar-H), 7.88 (d, 2H, J = 8.5 Hz, Ar-H), 7.60 (s, 2H, NH_2), 6.73–7.17 (m, 5H, Ph-), 2.62 (s, 3H, CH_3 -), 2.43 (s, 3H, CH_3 -). Anal. Calcd. For. $\text{C}_{17}\text{H}_{18}\text{N}_6\text{O}_2\text{S}$ (370.43): C, 55.12; H, 4.90; N, 22.69. Found: C, 55.20; H, 4.80; N, 22.80.

N,N-dimethyl-N'-[(4-(5-methyl-4-(1-(2-phenylhydrazineylidene)ethyl)-1H-1,2,3-triazol-1-yl)sulfonyl]formimidamide (2b). Colorless crystals, yield 88%, m.p. = 179–180 °C. IR (KBr, cm^{-1}) ν = 3321, (NH), 1629 (N = CH-), 1373, 1149 (SO_2); ^1H NMR (500 MHz, δ , ppm, DMSO- d_6): δ = 9.33 (s, 1H, NH), 8.29 (s, 1H, N = CH), 8.02 (d, 2H, J = 8.5 Hz, Ar-H), 7.82 (d, 2H, J = 8.5 Hz, Ar-H), 6.73–7.17 (m, 5H, Ph-), 3.16 (s, 3H, N- CH_3), 2.94 (s, 3H, N- CH_3), 2.63 (s, 3H, CH_3 -), 2.46 (s, 3H, CH_3 -). MS, m/z (%): 425 [M^+] (100). Anal. Calcd. For. $\text{C}_{20}\text{H}_{23}\text{N}_7\text{O}_2\text{S}$ (425.51): C, 56.45; H, 5.45; N, 23.04. Found: C, 56.30; H, 5.60; N, 23.10.

N'-[(4-(4-(4-formyl-1-phenyl-1H-pyrazol-3-yl)-5-methyl-1H-1,2,3-triazol-1-yl)phenyl)sulfonyl]-N,N-dimethylformimidamide (3). Phosphorous oxychloride (25 mmol) was added to DMF (100 mL) at 0 °C and stirred for 30 min. **2a** or **2b** (10 mmol) were added slowly to this mixture and stirred for 5 h. The crude reaction was then quenched into water (1 L) and stirred for an additional 1 h the solid formed was separated, washed with water, dried, and crystallized from methanol to afford **3**.

Colorless crystals, yield 88%, m.p. = 250–251 °C. IR (KBr, cm^{-1}) ν = 1672, (CO), 1633 (N = CH-), 1342, 1153 (SO_2); ^1H NMR (500 MHz, δ , ppm, DMSO- d_6): δ = 10.52 (s, 1H, CHO), 9.28 (s, 1H, pyrazole H-5), 8.02 (s, 1H, N = CH), 8.00 (d, 2H, J = 8.6 Hz, Ar-H), 7.82 (d, 2H, J = 8.6 Hz, Ar-H), 7.86–7.54 (m, 5H, Ph-), 3.14 (s, 3H, N- CH_3), 2.92 (s, 3H, N- CH_3), 2.66 (s, 3H, CH_3 -). MS, m/z (%): 463 [M^+] (100). Anal. Calcd. For. $\text{C}_{22}\text{H}_{21}\text{N}_7\text{O}_3\text{S}$ (463.52): C, 57.01; H, 4.57; N, 21.15. Found: C, 57.20; H, 4.60; N, 21.10.

General procedure for the synthesis of compounds 4, 5, and 6. A solution of **3** (4.63 g, 10 mmol) and thiosemicarbazide or carbothidihydrazide or cyanacetic acid hydrazide (11 mmol) in a mixture of acetic acid/ ethanol (1:1) was refluxed for 6 h. The solid formed on boiling was filtered off, dried and crystallized from acetic acid to afford:

2-[(3-(1-(4-(N-(dimethylamino) methylene) sulfamoyl) phenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-1-phenyl-1H-pyrazol-4-yl)methylene]hydrazine-1-carbothioamide (4). Pale yellow crystals in 86% yield; m.p. 234–236 °C. IR (KBr, cm^{-1}) ν = 3427 (NH), 3329, 3253 (NH_2), 1637 (C = N), 1346, 1143 (SO_2); ^1H NMR (500 MHz, δ , ppm, DMSO- d_6): δ = 11.55 (s, 1H, NH), 9.29 (s, 1H, pyrazole H-5), 8.74 (s, 1H, CH = N), 8.30 (s, 2H, NH_2), 8.04 (d, 2H, J = 8.5 Hz, Ar-H), 7.91 (d, 2H, J = 8.5 Hz, Ar-H), 7.89 (s, 1H, N = CH), 7.59–7.37 (m, 5H, Ph-H), 3.17 (s, 3H, NCH_3), 2.94 (s, 3H, NCH_3), 2.68 (s, 3H, CH_3). Anal. Calcd. For. $\text{C}_{23}\text{H}_{24}\text{N}_{10}\text{O}_2\text{S}_2$ (536.63): C, 51.58; H, 4.51; N, 26.10. Found: C, 51.60; H, 4.70; N, 26.20.

N'-[(4-(4-(4-(2-(hydrazinecarbothioyl)hydrazineylidene)methyl)-1-phenyl-1H-pyrazol-3-yl)-1H-5-methyl-1H-1,2,3-triazol-1-yl) phenyl)sulfonyl]-N,N-dimethylformimidamine (5). Pale yellow crystals in 86% yield; m.p. 202–204 °C. IR (KBr, cm^{-1}) ν = 3232 – 3138 (2NH, NH_2), 1633 (C = N), 1338, 1147 (SO_2); ^1H NMR (500 MHz, δ , ppm, DMSO- d_6): δ = 11.92 (s, 1H, NH), 11.77 (s, 2H, NH_2), 9.92 (s, 1H, NH), 8.79 (s, 1H, pyrazole H-5), 8.29 (s, 1H, CH = N), 8.04 (d, 2H, J = 8.5 Hz, Ar-H), 7.90 (d, 2H, J = 8.5 Hz, Ar-H), 7.87 (s, 1H, N = CH), 7.58–7.40 (m, 5H, Ph-H), 3.17 (s, 3H, NCH_3), 2.94 (s, 3H, NCH_3), 2.69 (s, 3H, CH_3). Anal. Calcd. For. $\text{C}_{23}\text{H}_{25}\text{N}_{11}\text{O}_2\text{S}_2$ (551.65): C, 50.08; H, 4.57; N, 27.93. Found: C, 50.20; H, 4.70; N, 27.80.

N'-[(4-(4-(4-(2-(2-cyanoacetyl) hydrazineylidene)methyl)-1-phenyl-1H-pyrazol-3-yl)-1H-5-methyl-1H-1,2,3-triazol-1-yl) phenyl)sulfonyl]-N,N-dimethylformimidamine (6). Colorless crystals in 79% yield; m.p. 152–154 °C. IR (KBr, cm^{-1}) ν = 3217 (NH), 2250 (CN), 1697 (CO), 1627 (C = N) (CO), 1344, 1149 (SO_2); ^1H NMR (500 MHz, δ , ppm, DMSO- d_6): δ = 11.81 (s, 1H, NH), 9.10 (s, 1H, pyrazole H-5), 8.74 (s, 1H, CH = N), 8.29 (s, 1H, N = CH), 8.02 (d, 2H, J = 8.6 Hz, Ar-H), 7.89 (d, 2H, J = 8.6 Hz, Ar-H), 7.58–7.37 (m, 5H, Ph-H), 4.25 (s, 2H, CH_2), 3.17 (s, 3H, NCH_3), 2.94 (s, 3H, NCH_3), 2.69 (s, 3H, CH_3). Anal. Calcd. For. $\text{C}_{25}\text{H}_{24}\text{N}_9\text{O}_3\text{S}$ (530.17): C, 56.59; H, 4.56; N, 23.76. Found: C, 56.30; H, 4.50; N, 23.60.

Ethyl-2-cyano-3-[3-(1-(4-(N-(dimethylamino) methylene) sulfamoyl) phenyl)-5-methyl-1H-1,2,3-triazole-4-yl]acrylate (7). A solution of **3** (10 mmol), ethyl cyanoacetate (1.13 mL, 10 mmol) in the presence of 3 drops of triethylamine in absolute ethanol (20 mL) was refluxed for 5 h. The solution was cooled and poured onto crushed ice. The resulting solid was filtered, dried and recrystallized from ethanol to yield **7**. Yellow crystals in 90% yield; m.p. 230–231 °C; IR (KBr, cm^{-1}) ν = 2220 (CN), 1703 (COOEt), 1624 (C = C), 1336, 1151 (SO_2); ^1H NMR (500 MHz, δ , ppm, DMSO- d_6): δ = 9.21 (s, 1H, pyrazole H-5), 8.17 (s, 1H, N = CH), 8.00 (s, 1H, CH = C), 7.89 (d, 2H, J = 8.6 Hz, Ar-H), 7.86 (d, 2H, J = 8.6 Hz, Ar-H), 7.56–7.45 (m, 5H, Ph-H), 4.29 (q, 2H, J = 7.6 Hz, CH_2 , ester), 3.13 (s, 3H, NCH_3), 2.92 (s, 3H, NCH_3), 2.66 (s, 3H, CH_3), 1.27 (t, 3H, J = 7.65 Hz, CH_3), MS, m/z (%): 558 [M^+] (100). Anal. Calcd. For. $\text{C}_{27}\text{H}_{26}\text{N}_8\text{O}_4\text{S}$ (558.62): C, 58.05; H, 4.69; N, 20.06. Found: C, 58.20; H, 4.80; N, 20.10.

N'-[4-(4-(4-(3-cyano-2-oxo-6-phenyl-1,2-dihydropyridin-4-yl)-1-phenyl-1*H*-pyrazol-3-yl)-5-methyl-1*H*-1,2,3-triazol-1-yl)phenyl)sulfonyl]-*N,N*-dimethylformimidamide (**8**). A mixture of **4** (2.79 g, 5 mmol), ammonium acetate (2.73 g, 10 mmol) and acetophenone (5 mmol) in absolute ethanol (50 mL) was refluxed for 12 h. The solid which was precipitated during heating, was filtered, washed with water, dried, and crystallized from acetic acid to afford **8** as pale yellow crystals in 80% yield; m.p. 264–266 °C. IR (KBr, cm⁻¹) ν = 3360 (NH), 2218 (CN), 1655 (CO), 1337, 1154 (SO₂); ¹H NMR (500 MHz, δ , ppm, DMSO-*d*₆): δ = 12.54 (brs., 1H, NH), 9.28 (s, 1H, pyrazole H-5), 8.12 (s, 1H, N = CH), 8.00 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.90 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.74 (s, 1H, pyridine H-5), 7.56–7.30 (m, 10H, 2 Ph-H), 3.15 (s, 3H, NCH₃), 2.94 (s, 3H, NCH₃), 2.66 (s, 3H, CH₃-). Anal. Calcd. For. C₃₃H₂₇N₉O₃S (629.70): C, 62.94; H, 4.32; N, 20.02. Found: C, 62.80; H, 4.50; N, 20.10.

General procedure for the synthesis of chalcones 9a-c: To a well-stirred solution of acetophenone or 2-acetylthiophene or 2-acetylpyridine **3** (10 mmol) in alcoholic NaOH (5 %, 25 ml) at 0–5 °C a solution of **3** (2.32 g, 5 mmol) was added gradually. Stirring was continued for 10 h at r.t. the resulting precipitate was filtrated, washed with water, dried, and crystallized from acetic acid to give.

N,N-dimethyl-*N'*-[4-(5-methyl-4-(4-(3-oxo-3-phenylprop-1-en-1-yl)-1-phenyl-1*H*-pyrazol-3-yl)-1*H*-1,2,3-triazol-1-yl)phenyl)sulfonyl]formimidamide (**9a**). Yellow crystals in 86% yield; m.p. 190–191 °C. IR (KBr, cm⁻¹) ν = 1678 (CO), 1631 (C = N), 1597 (C = C), 1340, 1163 (SO₂); ¹H NMR (DMSO-*d*₆): δ = 9.49 (s, 1H, pyrazole H-5), 8.60 (s, 1H, CH = N), 8.10 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.92 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.80 (d, 1H, *J* = 15.30 Hz, CH = CH), 7.70 (d, 1H, *J* = 15.30 Hz, CH = CH), 7.59–7.45 (m, 10H, 2 Ph-H), 3.13 (s, 3H, NCH₃), 2.94 (s, 3H, NCH₃), 2.67 (s, 3H, CH₃-). Anal. Calcd. For. C₃₀H₂₇N₇O₃S (565.65): C, 63.70; H, 4.81; N, 17.33. Found: C, 63.80; H, 4.70; N, 17.50.

N,N-dimethyl-*N'*-[4-(5-methyl-4-(4-(3-oxo-3-(thiophen-2-yl)prop-1-en-1-yl)-1-phenyl-1*H*-pyrazol-3-yl)-1*H*-1,2,3-triazol-1-yl)phenyl)sulfonyl]formimidamide (**9b**). Yellow crystals in 80% yield; m.p. 166–168 °C. IR (KBr, cm⁻¹) ν = 1643 (CO), 1631 (C = N), 1597 (C = C), 1342, 1159 (SO₂); ¹H NMR (DMSO-*d*₆): δ = 9.49 (s, 1H, pyrazole H-5), 8.60 (s, 1H, CH = N), 8.48 (d, 1H, *J* = 16.70 Hz, CH = CH), 7.94 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.92 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.59–7.45 (m, 9H, CH = CH, Ph-H, 3 thiophene-H), 3.16 (s, 3H, NCH₃), 2.90 (s, 3H, NCH₃), 2.66 (s, 3H, CH₃-), MS, *m/z* (%): 571 [M⁺] (43). Anal. Calcd. For. C₂₈H₂₅N₇O₃S₂ (571.67): C, 58.83; H, 4.41; N, 17.15. Found: C, 58.70; H, 4.60; N, 17.20.

4-(4-(1',5-diphenyl-1*H*,2*H*-[3,4'-bipyrazol]-3'-yl)-5-methyl-1*H*-1,2,3-triazol-1-yl)benzenesulfonamide (**10**). To a solution of appropriate chalcones **9a** (2.78g, 5 mmol) in ethanol (30 ml), hydrazine hydrate 80% (12 mmol) was added. The reaction mixture was refluxed for 6 h. Left to cool to room temperature. The formed solid product was filtered, dried, and recrystallized from EtOH to afford **10** as colorless crystals in 82% yield; m.p. 180–182 °C. IR (KBr, cm⁻¹) ν = 3218 (NH), 1631 (C = N), 1597 (C = C), 1340, 1163 (SO₂); ¹H NMR (DMSO-*d*₆): δ = 11.21 (s, 1H, NH), 9.49 (s, 1H, pyrazole H-5), 8.60 (s, 1H, CH = N), 8.20 (s, 1H, pyrazole-H), 7.94 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.92 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.59–7.45 (m, 10H, 2 Ph-H), 3.19 (s, 3H,

NCH₃), 2.92 (s, 3H, NCH₃) 2.65 (s, 3H, CH₃-). Anal. Calcd. For. C₂₇H₂₂N₈O₂S (522.16): C, 62.06; H, 4.24; N, 21.44. Found: C, 62.20; H, 4.80; N, 21.70.

General procedure for the synthesis of compounds 11a, 12a, and 12b. To a stirred cold solution of compound **1a** or **1b** (10 mmol) in acetic acid, bromine (15 mmol) is add-drop wisely within 1h and the stirring is continued at room temperature for another 4 hrs. The reaction mixture was heated at 80°C for another 6 hrs. The solid which was formed on heating filtered off, washed with ethanol (10 ml), dried, and crystallized from acetic acid to give :

4-[4-(2-bromoacetyl)-5-methyl-1H-1,2,3-triazol-1-yl] benzenesulfonamide (11 a). Colorless crystals, m.p. 230-232°C. IR (KBr, cm⁻¹) ν = 3340, 3194 (NH₂), 1681 (C = O), 1330, 1161 (SO₂). ¹H-NMR (500 MHz, δ , ppm, DMSO-d₆): δ = 8.01 (d, 2H, J = 8.6 Hz, Ar-H), 7.90 (d, 2H, J = 8.6 Hz, Ar-H), 7.57 (s, 2H, NH₂), 4.66 (s, 2H, CH₂), 2.62 (s, 3H, CH₃). Anal. Calcd for C₁₁H₁₁BrN₄O₃S (359.20): C, 36.78; H, 3.09; N, 15.60. Found C, 36.90; H, 3.20; N, 15.70.

4-[4-(2,2-dibromoacetyl)-5-methyl-1H-1,2,3-triazol-1-yl] benzene sulfonamide (12a). Colorless crystals in 90% yield; m.p. 222–224 °C. IR (KBr, cm⁻¹) ν = 3226, 3103 (NH₂), 1705 (C = O), 1338, 1163 (SO₂). ¹H-NMR (500 MHz, δ , ppm, DMSO-d₆): δ = 8.0 (d, 2H, J = 8.5 Hz, Ar-H), 7.92 (d, 2H, J = 8.5 Hz, Ar-H), 7.62 (s, 2H, NH₂), 7.57 (s, 1H, CH), 2.62 (s, 3H, CH₃). ¹³C NMR (125 MHz, δ , ppm, DMSO-d₆) δ c 10.09 (CH₃), 41.86 (CH), 126.32, 127.26, 137.02 (= CH triazole), 137.42 (= CH triazole), 141.58, 145.56, 181.32 (C = O). Anal. Calcd for C₁₁H₁₀Br₂N₄O₃S (438.09): C, 30.16; H, 2.30; N, 12.79. Found C, 30.10; H, 2.40; N, 12.90.

N-[[4-[4-(2,2-dibromoacetyl)-5-methyl-1H-1,2,3-triazol-1-yl] phenyl] sulfonyl]-N, N-dimethylformimidamide (12b). Colorless crystals in 92% yield; m.p. 214–216 °C. IR (KBr, cm⁻¹) ν = 2991 (CH), 1699 (C = O), 1338, 1151 (SO₂). ¹H-NMR (500 MHz, δ , ppm, DMSO-d₆): δ = 8.28 (s, 1H, CH = N), 8.02 (d, 2H, J = 8.6 Hz, Ar-H), 7.86 (d, 2H, J = 8.6 Hz, Ar-H), 7.57 (s, 1H, CH), 3.16 (s, 3H, NCH₃), 2.93 (s, 3H, NCH₃), 2.62 (s, 3H, CH₃). ¹³C NMR (125 MHz, δ , ppm, DMSO-d₆) δ c 10.05 (CH₃), 35.20 (NCH₃), 41.04 (CH), 126.06, 127.50, 136.92 (= CH triazole), 137.35 (= CH triazole), 141.59, 144.60, 160.16 (N = C), 181.31 (C = O). MS, m/z (%): 493 [M⁺] (56), 445 [M⁺-N(CH₃)₂] (4). Anal. Calcd for C₁₄H₁₅Br₂N₅O₃S (493.17): C, 34.10; H, 3.07; N, 14.20. Found C, 34.20; H, 3.20; N, 14.30.

General procedure for the synthesis of compounds 13a-d: A solution of **12a** or **12b** (10 mmol), and thiourea or *N*-phenylurea in a mixture of acetic acid/ ethanol (50 ml, 1:1) was refluxed for 10h. The solid formed on boiling was filtered of, dried and crystallized from acetone to afford:

4-[4-(2-aminothiazol-5-yl)-5-methyl-1H-1,2,3-triazol-1-yl] benzene sulfonamide (13a). Pale yellow crystals, 83% yield m.p. 268–270 °C. IR (KBr, cm⁻¹) ν = 3329, 3228 (2NH₂), 2889 (CH), 1624 (C = N), 1334, 1155 (SO₂). ¹H-NMR (500 MHz, δ , ppm, DMSO-d₆): δ = 8.66 (brs., 2H, NH₂), 8.07 (d, 2H, J = 8.5 Hz, Ar-H), 7.90 (d, 2H, J = 8.5 Hz, Ar-H), 7.88 (s, 2H, NH₂), 7.16 (s, 1H, thiazole-H4), 2.52 (s, 3H, CH₃). ¹³C NMR (125 MHz, δ , ppm, DMSO-d₆) δ c = 9.99, 103.89, 125.82, 127.31, 130.11, 133.07, 135.47, 137.62, 145.20, 169.99. MS,

m/z (%): 336 [M⁺] (22). Anal. Calcd for C₁₂H₁₂N₆O₂S (336.39): C, 42.85; H, 3.60; N, 24.98. Found C, 42.70; H, 3.50; N, 24.90.

N'-((4-(4-(2-aminothiazol-5-yl)-5-methyl-1H-1,2,3-triazol-1-yl)phenyl)sulfonyl)-N,N-dimethylformimidamide (13 b). Yellow crystals, 82% yield, m.p. 234–236 ° C. IR (KBr, cm⁻¹) ν = 3354, 3263 (NH₂), 2999 (CH), 1635(C = N), 1340, 1143 (SO₂). ¹H-NMR (500 MHz, δ , ppm, DMSO-d₆): δ = 8.66 (brs, 2H, NH₂), 8.28 (s, 1H, CH = N), 8.01 (d, 2H, J = 8.5 Hz, Ar–H), 7.82 (d, 2H, J = 8.5 Hz, Ar–H), 7.14 (s, 1H, thiazole- H4), 3.16 (s, 3H, NCH₃), 2.93 (s, 3H, NCH₃), 2.52 (s, 3H, CH₃). ¹³C NMR (125 MHz, δ , ppm, DMSO-d₆) δ_c = 9.9, 103.9, 125.5 (2C), 125.8, 127.2, 127.3 (2C), 130.1, 132.9, 135.4, 137.6, 144.8, 145.2, 169.90. Anal. Calcd for C₁₅H₁₇N₇O₂S₂ (391.47): C, 46.02; H, 4.38; N, 25.05. Found C, 46.10; H, 4.20; N, 25.20.

4-[5-methyl-4-(2-(phenylamino)thiazol-5-yl)-1H-1,2,3-triazol-1-yl]benzene sulfonamide (13c). Yellow crystals 85% yield, m.p. 254 – 25 ° C. IR (KBr, cm⁻¹) ν = 3329, 3242(NH₂), 3147 (NH), 1618(C = N), 1338, 1166 (SO₂). ¹H-NMR (500 MHz, δ , ppm, DMSO-d₆): δ 10.51 (s, 1H, NH), 8.07 (d, 2H, J = 8.5 Hz, Ar–H), 7.93 (d, 2H, J = 8.5 Hz, Ar–H), 7.65 (m, 3H, Ph-H), 7.29 (s, 1H, thiazol –H4), 7.20 (s, 2H, NH₂), 6.96 (m, 2H, Ph-H), 2.58 (s, 3H, CH₃). ¹³C NMR (125 MHz, DMSO-d₆) δ_c = 10.1, 48.6, 92.4, 104.2, 117.3, 121.9, 125.6 (2C), 127.2 (2C), 129.0, 133.0, 138.00, 138.2, 139.1, 140.5, 144.2, 162.6. Anal. Calcd for C₁₈H₁₆N₆O₂S₂ (412.49): C, 52.41; H, 3.91; N, 20.37. Found C, 52.60; H, 3.60; N, 20.50.

N,N-dimethyl amino-N'-[(4-(5-methyl-4-(2-(phenylamino)thiazol-5-yl)-1H-1,2,3-triazol-1-yl)phenyl)sulfonyl]formimidamide (13 d). Pale yellow crystals, 78% yield, m.p. 265–267 ° C. IR (KBr, cm⁻¹) ν = 3263 (NH), 1635(C = N), 1340, 1143 (SO₂). ¹H-NMR (500 MHz, δ , ppm, DMSO-d₆): δ = 10.36 (s, 1H, NH), 8.28 (s, 1H, CH = N), 8.01 (d, 2H, J = 8.5 Hz, Ar–H), 7.84 (d, 2H, J = 8.5 Hz, Ar–H), 7.67 (s, 1H, thiazole- H4), 7.28–6.93 (m, 5H, Ph-H), 3.16 (s, 3H, NCH₃), 2.93 (s, 3H, NCH₃), 2.69 (s, 3H, CH₃). ¹³C NMR (125 MHz, DMSO-d₆) δ_c = 10.1, 35.2, 41.2, 92.37, 104.2, 117.0, 125.6 (2C), 125.7 (2C), 127.5 (2C), 129.0, 129.1, 139.82, 140.5, 141.1, 142.2, 160.2, 162.6, 163.8. Anal. Calcd for C₂₁H₂₁N₇O₂S (467.57): C, 53.95; H, 4.53; N, 20.97. Found C, 53.80; H, 4.70; N, 20.80.

X-ray crystallography of compound 3.

A single crystal of compound **3** was obtained by slow evaporation at room temperature, from dimethylformamide (DMF). The crystal structure was solved and refined using MaXus (Bruker Nonius, Delft and Mac Science, Japan)²⁴. Mo K α radiation (λ = 0.71073 Å) and a graphite monochromator were used for data collection. The chemical formula and ring labeling system are shown in Fig. 2. Crystal data for compound **3**: C₂₂H₂₁N₇O₃S, M_r = 463.520; system, Monoclinic; space group, P2₁/c; a = 11.3957 (3) Å; b = 13.1843 (5) Å; c = 5.1206 (5) Å; α = 90.00°; β = 106.413 (3)°; γ = 90.00°; V = 2179.21 (12) Å³; Z = 4; D_x = 1.413 Mg m⁻³; range for data θ = 2.910–27.485 °; (Mo-K α) μ = 0.190 mm⁻¹; T = 298 K; 16702 measured reflections; 4921 independent reflections; 2336 observed reflections; $R(\text{all})$ = 0.1595; $R(\text{gt})$ = 0.0623; $wR(\text{ref})$ = 0.2021; $wR(\text{gt})$ = 0.1604; $S(\text{ref})$ = 0.995; 4921 reflections; $\delta/\sigma_{\text{max}}$ = 0.004; $\delta\rho_{\text{max}}$ = 0.278 e Å⁻³; $\delta\rho_{\text{min}}$

= -0.346eÅ³. Crystallographic data for the structure 5 have been deposited with the Cambridge Crystallographic Data Center (CCDC) under the number 1574927. Copies of the data can be obtained, free of charge, on application to CCDC 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44-1223- 336033; e-mail: deposit@ccdc.cam.ac.uk or at www.ccdc.cam.ac.uk].

Molecular docking studies

Materials and methods

Three-dimensional structure of human cyclin-dependent kinase CDK2, PDB:2FVD, was obtained from protein data bank. Energy minimization of newly designed compounds was done by employing discovery studio 2.5 for structure refinement. The geometry of all designed analogues is typed with CHARMM²⁵ force field; then partial charges are calculated by Momany Rone method²⁶. Further, they are optimized through a smart minimizer algorithm, which performs 1000 steps of steepest descent with a root mean-square (RMS) gradient tolerance of 0.1. Same as the preparation of ligands for the target, its active site was also passed with the energy minimization process and it was done using charm force field which is defined by the equation given below:

where E = total energy, E_b = bond potential energy, E_q and E_f = bond angle potential energy, E_w = torsion energy, E_{vdw} = van der Waals interaction energy, E_{el} = electrostatic potential energy, E_{hb} = hydrogen bond energy, E_{cr} = energy constraints, and E_{cj} = energy function²⁵.

CDOCKER (CHARMM-based DOCKER), a docking program provided by discovery studio 2.5, uses a CHARMM based molecular dynamics (MD) scheme to dock ligands into a receptor binding site, and then random conformations will be produced using high-temperature molecular dynamics. When these conformations are translated to the active site, candidate poses are then generated using random rigid body rotations followed by simulated annealing. CDOCKER offers all the advantages of full ligand flexibility (including bonds, angles, and dihedrals) and reasonable computation times. CDOCKER uses soft core potentials, which are found to be effective in exploring the conformational space of macromolecules used in various docking studies. The nonbonded interactions which involve van der Waals (vdW) and electrostatics are softened at different levels, except during the final minimization step²⁷. Initially, ten conformations for each inhibitor are generated in the active site of the target enzyme, which is created as a spherical region with a diameter of 10 Å. Simulated annealing is performed using a flexible ligand and a rigid protein. Receptor–ligand interactions are calculated from grid extension 8.0, random conformations are generated using specific molecular dynamics steps, and the system is heated to 700 K in 2000 steps, cooling steps to 5000, and cooling temperature to 300 K. The final refinement step of minimization is performed using full potential. Minimized docking poses are then clustered, based on a heavy atom RMSD approach. The ranking is based on the total docking energy, which is composed of the ligand's intramolecular energy and the ligand-receptor interaction.

Declarations

Author contributions

Authors participated in the idea of this research and they carried out the synthesis, purification and characterization of all compounds by the different spectroscopic techniques. Both authors have read and approved the final version submitted

Competing interests

The authors declare no competing interests.

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Figures

Figure 1

Chemical structure of sulfonamides (SAs).

Figure 2

X-ray structure of 3.

Figure 3

Hydrogen bond interactions of compound 8 with the active site residues. It forms 3 HB with Lys33, Thr14, and Lys 89.

Figure 4

Hydrogen bond interactions of compound 3 with the active site residues of CDK2. It forms 1 HB with Lys33.

Supplementary Files

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