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# Anticancer and radio-sensitizing evaluation of some new sulfonamide derivatives bearing pyridone, thiophene, and hydrazone moieties

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**Abstract** A series of new pyridone **5**, **6**, **8a–j**, hydrazone **7a–j**, and thiophene **9–12** derivatives bearing a sulfonamide moiety were synthesized from the starting material 4-chloro-*N*-(4-(1-(2-(2-cyanoacetyl)hydrazono)ethyl)phenyl) benzenesulfonamide **4**. The target compounds were in vitro evaluated for their cytotoxic activity against a human liver cancer cell line (HepG2). Compounds **4** and **8d–j** showed higher cytotoxic activity compared to doxorubicin, as a positive control. The radio-sensitizing ability of the promising compounds **4**, **8d**, and **8h** was studied which showed an enhanced cytotoxic activity after combination with γ-radiation. Molecular modeling was performed in CA II/IX mimic active site to predict the binding possibility of the target compounds. All the synthesized compounds showed appropriate fitting with the amino acids in the binding pocket on the basis of their *S* score data and binding interactions. This binding possibility might contribute at least in part, to their anticancer activity.

**Electronic supplementary material** The online version of this article (doi:10.1007/s11164-017-2903-x) contains supplementary material, which is available to authorized users.

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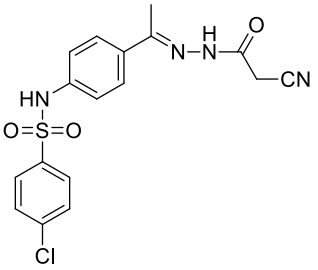
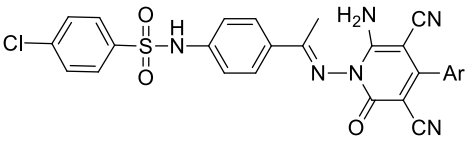
## Graphical Abstract

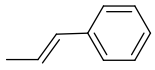
A novel series of sulfonamide derivatives bearing a biologically active pyridone, thiophene, and hydrazone moieties was synthesized and screened for their cytotoxic activity against HepG2 cell line. The most potent compounds in this study **4**, **8d**, and **8h** were evaluated for their radio-sensitizing activity.



**4**; $IC_{50}$ (before irradiation)= 8 μM
$IC_{50}$ (after irradiation)= 6.5 μM

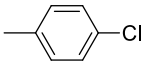


**8d**; Ar =



$IC_{50}$ (before irradiation)= 16.4 μM
$IC_{50}$ (after irradiation)= 10.7 μM

**8h**; Ar =



IC50 (before irradiation)= 13.3 μM
IC50 (after irradiation)= 9.1 μM

**Keywords** Sulfonamide · Pyridone · Thiophene · Hydrazone · Anticancer · Radiosensitizers

## Introduction

Radiotherapy is the second most successful treatment for cancer next to surgery [1]. Radiation delivers energy to tissues, causing ionization and excitation of atoms. The effect of radiation is exerted through the generation of single and double strand breaks, apoptosis of cells as they progress through the cell cycle and through the generation of short-lived free radicals (ROS), which damage proteins and membranes [2]. It is now well known that in the presence of chemotherapy, an increased response occurs within the irradiated tissue.

Pyridones constitute an important class of anticancer agents [3] as they act through many mechanisms including inhibition of tyrosine kinases such as FGFR and VEGFR, Met and TAM family kinases [4, 5]. They were also identified as inhibitors of the serine/threonine kinase PIM-1, which plays an important role in cell cycle progression, signal transduction pathways, and apoptosis [6, 7]. Phosphodiesterase inhibitors act through PDE3, PDE4, and PDE5 inhibition [8],

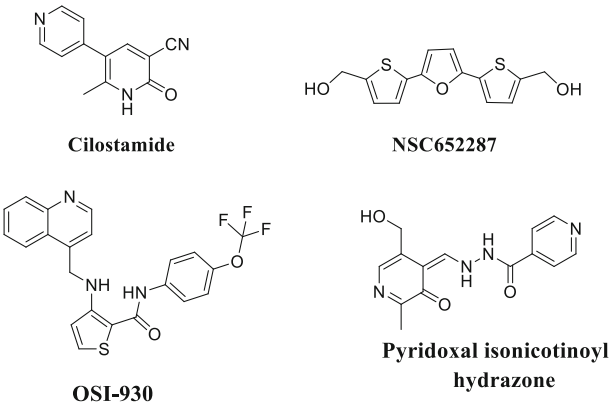



**Fig. 1** Anticancer agents containing pyridone, thiophene, and hydrazone moieties

e.g. cilostamide (Fig. 1), a selective PDE3 inhibitor, that shows synergism with the anti-apoptotic action of PDE4 inhibitors in leukemia cells [9]. Survivin inhibitor is a member of the inhibitor of apoptosis family (IAP) [9]. Farnesyl transferase inhibitors (FTIs) act by preventing the proper functioning of the Ras protein [10]. Thiophene derivatives also show interesting antitumor activity [11], e.g. NSC652287, that act through the induction of protein cross-linking [12] and OSI-930, a selective inhibitor of Kit and tyrosine kinases through the inactivation of cytochrome P450 (Fig. 1) [13]. In addition, hydrazones form the backbone of many anticancer agents that act by inhibiting the dihydrofolate reductase enzyme [14], inducing apoptosis through the inhibition of tubulin polymerization [15], arresting the cell cycle at the G2/M phase [16, 17] c-Met tyrosine kinase inhibition [18], and cytotoxic activity through the chelation of Fe(III), e.g. pyridoxal isonicotinoyl hydrazone (Fig. 1) [19].

The considerable anticancer activity of sulfonamides makes them candidates of choice to be combined with other pharmacologically active moieties for achieving better cytotoxic activity. Sulfonamides' anticancer activity is exerted through many mechanisms, the most prominent of which is the inhibition of carbonic anhydrase isozymes [20, 21]. Sulfonamides possess a zinc binding group that coordinates with the Zn(II) ion of the CA active site and establish additional interactions promoted by the aryl substituent in the region nearby the catalytic site [22, 23], and this is consistent with the "tail approach" [24]. This moiety known as tail extends the inhibitor allowing it to make multiple interactions with the amino acids towards the outside of the active site. The addition of these tails can also alter the properties of the CA inhibitor [22].

Based on this, and as a continuation of our work [25–27], we planned to synthesize a novel series of sulfonamides bearing a biologically active pyridone, hydrazone, thiophene, and fused thiophene derivatives, as these moieties may act as a tail to improve the binding in the active site of CA, and they also possess anticancer activity [3–5, 11, 28–30], in order to be evaluated against human liver cancer cell line (HepG2) alone and in combination with γ-radiation.

## Experimental

### Chemistry

Melting points were uncorrected and measured using a Stuart melting point apparatus (Stuart Scientific, Redhill, UK). The IR spectra were recorded on ABB Bomem FT-IR spectrometer MB 104 (ABB Bomem Inc., Québec, Canada) using KBr pellets. $^{1}$H-NMR spectra were recorded using a Bruker 300 and 400 NMR spectrometers (Bruker, Flawil, Switzerland) operating at 300 and 400 MHz, respectively. $^{13}$C-NMR spectra were recorded using a Bruker 100 NMR spectrometers operating at 100 MHz. Mass spectra were run on Shimadzu GCMS/QP 5050 mass spectrometer (Shimadzu, Tokyo, Japan). Microanalyses were measured with an elemental analyses system GmbH VarioEL V300 element analyzer (Elemental Analysensysteme GmbH, Chennai, India) and were found within the limit of 0.4% of theoretical values for all the synthesized compounds. The compound's purity was checked by thin layer chromatography (TLC) with precoated aluminum sheets silica gel Merck 60 F254 and was visualized by UV lamp (Merck, Darmstadt, Germany). The developing solvent system was benzene/methanol (7:3) and the spots were visualized in UV chamber. The spectroscopic data and elemental analyses were consistent with the assigned structures. IR spectra were performed at the National Center for Radiation Research and Technology, Atomic Energy Authority. While $^{1}$H-NMR and $^{13}$C-NMR spectra were performed at the Chemical Warfare department, Ministry of Defense. Mass spectra were performed at the Microanalytical Laboratories of Al-Azhar University. Elemental analyses were performed at the Microanalytical Laboratories of the Faculty of Science, Cairo University. 4-Chlorobenzene sulfonyl chloride and 4-aminoacetophenon were purchased from Sigma-Aldrich (St. Louis, MO).

*4-Chloro-N-(4-(1-((4,6-diamino-3-cyano-2-oxopyridin-1(2H)-yl)imino)ethyl)phenyl) benzenesulfonamide (**5**)*

A mixture of compound **4** (3.9 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mol) was refluxed in dioxane (15 mL) containing triethylamine 5 drops for 6 h. The reaction mixture was poured into ice-water and filtered. The obtained solid was crystallized from ethanol to give **5**. Yield, 50.1%; m.p. 168–170 °C. IR (KBr, $cm^{-1}$): 3383, 3334, 3283 (NH, $2NH_2$), 3010 (CH arom.), 2926, 2872 (CH aliph.), 2191 (CN), 1682 (CO), 1606 (C=N), 1337, 1162 ($SO_2$), 748 (C–Cl). $^{1}$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.16 (s, 3H, $CH_3$), 4.16 (s, 1H, CH-pyridone), 7.08–7.83 (m, 8H, Ar–H), 7.60 (s, 1H, $SO_2NH$), 10.60, 10.94 (2 s, 4H, 2 $NH_2$). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.5 ($CH_3$), 66.8 (C–CN), 88.5 (CH-pyridone), 116.7 (CN), 119.8 (2), 127.7, 127.9 (2), 129.0 (2), 129.9 (2), 130.1, 130.5, 133.9 (C-phenyl), 138.3 (N–C–$NH_2$), 148.8 (CO), 157.5 (C=N), 166.2 (C–$NH_2$). MS m/z (%): 457 ($M^+$) (19.4), 459.5 (8.4), 438.7 (100). Anal. Calcd. For $C_{20}H_{17}ClN_6O_3S$ (456.91): C, 52.57; H, 3.75; N, 18.39; Found: C, 52.88; H, 3.96; N, 18.59.

*N-(4-(1-((4-Amino-3-cyano-6-hydroxy-2-oxopyridin-1(2H)-yl)imino)ethyl)phenyl)-4-chlorobenzenesulfonamide (**6**)*

A mixture of compound **4** (3.9 g, 0.01 mol) and ethyl cyanoacetate (1.13 g, 0.01 mol) was refluxed in dioxane (15 mL) containing triethylamine 5 drops for 6 h. The reaction mixture was poured into ice-water and filtered. The solid obtained was crystallized from ethanol to give **6**. Yield, 70.6%; m.p. 118–120 °C. IR (KBr, $cm^{-1}$): 3420 (OH), 3384, 3237, 3210 (NH, $NH_2$), 3100 (CH arom.), 2927, 2853 (CH aliph.), 2261 (CN), 1713 (CO), 1602 (C=N), 1330, 1184 ($SO_2$), 743 (C–Cl). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 2.19 (s, 3H, $CH_3$), 4.18 (s, 1H, CH-pyridone), 7.10–7.86 (m, 8H, Ar–H), 7.67 (s, 1H, $SO_2NH$), 10.62 (s, 2H, $NH_2$), 10.96 (s, 1H, OH). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 66.8 (C–CN), 75.5 (CH-pyridone), 116.7 (CN), 119.8 (2), 127.7, 127.9 (2), 129.0 (2), 129.9 (2), 130.1, 130.3, 133.9 (C-phenyl), 148.8 (CO), 157.5 (C=N), 165.3 (C–OH), 166.2 (C–$NH_2$). MS m/z (%): 458 ($M^+$) (2.6), 460 (0.9), 60.8 (100). Anal. Calcd. For $C_{20}H_{16}ClN_5O_4S$ (457.89): C, 52.45; H, 3.52; N, 15.29; Found: C, 52.71; H, 3.82; N, 15.46.

**General procedure for the synthesis of compounds 7a–j**

A mixture of **4** (3.9 g, 0.01 mol) and aromatic aldehydes (0.01 mol) namely, furfural, benzaldehyde, 4-methyl benzaldehyde, cinnamaldehyde, 2-methoxybenzaldehyde, 4-methoxybenzaldehyde, 2-chlorobenzaldehyde, 4-chlorobenzaldehyde, 4-dimethyl amino benzaldehyde, piperonal, in ethanol (15 mL) containing piperidine (0.5 mL) was refluxed for 3 h. The reaction mixture was poured into ice-water containing few drops of dil. HCl and the obtained solid was crystallized from dioxane to give **7a–j**, respectively.

*Chloro-N-(4-(1-(2-(2-cyano-3-(furan-2-yl)acryloyl)hydrazono)ethyl)phenyl) benzenesulfonamide (**7a**)*

Yield, 71.1%; m.p. 253–255 °C. IR (KBr, $cm^{-1}$): 3368, 3300 (2NH), 3033 (CH arom.), 2934, 2865 (CH aliph.), 2203 (CN), 1700 (CO), 1610 (C=N), 1341, 1162 ($SO_2$), 771 (C–Cl). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 2.25 (s, 3H, $CH_3$), 6.84–8.01 (m, 11H, Ar–H), 7.43 (s, 1H, $SO_2NH$), 8.14 (s, 1H, CH), 10.53 (s, 1H, NHCO). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.3 ($CH_3$), 100.1, 113.8 (C-furan), 113.9 (C–CN), 116.2 (CN), 119.1 (2) (C-phenyl), 119.4, 121.9 (2), 127.5 (2), 127.7 (2), 128.6, 128.7 129.3 (C-phenyl), 129.5 (C-furan), 133.2 (C=N), 137.8 (C-furan), 138.3 (CH), 148.7 (CO). Anal. Calcd. For $C_{22}H_{17}ClN_4O_4S$ (468.91): C, 56.35; H, 3.65; N, 11.95; Found: C, 56.43; H, 3.70; N, 12.13.

*4-Chloro-N-(4-(1-(2-(2-cyano-3-phenylacryloyl)hydrazono)ethyl)phenyl) benzenesulfonamide (**7b**)*

Yield, 90.2%; m.p. 242–244 °C. IR (KBr, $cm^{-1}$): 3369, 3348 (2NH), 3040 (CH arom.), 2929, 2864 (CH aliph.), 2203 (CN), 1688 (CO), 1595 (C=N), 1353, 1163 ($SO_2$), 753 (C–Cl). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 2.17 (s, 3H, $CH_3$), 7.17–7.99

(m, 13H, Ar–H), 7.43 (s, 1H, $SO_2NH$), 7.98 (s, 1H, CH), 10.11 (s, 1H, NHCO). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 100.2 (C–CN), 113.7 (CN), 113.9 (2), 116.1, 119.0, 119.4 (2), 121.9 (2), 127.4 (2), 127.5 (2), 127.7 (2), 128.6, 129.3, 129.7, 133.2 (C-phenyl), 137.8 (C=N), 138.3 (CH), 148.7 (CO). MS m/z (%): 479 ($M^+$) (9.4), 481 (4.4), 194 (100). Anal. Calcd. For $C_{24}H_{19}ClN_4O_3S$ (478.95): C, 60.19; H, 4.00; N, 11.70; Found: C, 60.33; H, 4.29; N, 12.10.

*4-Chloro-N-(4-(1-(2-(2-cyano-3-(p-tolyl)acryloyl)hydrazono)ethyl)phenyl) benzenesulfonamide (**7c**)*

Yield, 73.2%; m.p. 144–146 °C. IR (KBr, $cm^{-1}$): 3375, 3215 (2NH), 3067 (CH arom.), 2940, 2853 (CH aliph.), 2208 (CN), 1675 (CO), 1615 (C=N), 1342, 1158 ($SO_2$), 752 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.26 (s, 3H, $CH_3$), 2.41 (s, 3H, $CH_3$ tolyl), 7.18–8.09 (m, 12H, Ar–H), 7.47 (s, 1H, $SO_2NH$), 8.05 (s, 1H, CH), 9.95 (s, 1H, NHCO). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 26.6 ($CH_3$), 100.3 (C–CN), 113.7 (CN), 113.9 (2), 116.1, 119.4 (2), 121.9 (2), 127.4 (2), 127.5, 127.7 (2), 127.9 (2), 128.6, 129.4, 129.6, 133.2 (C-phenyl), 137.8 (C=N), 138.3 (CH), 148.7 (CO). Anal. Calcd. For $C_{25}H_{21}ClN_4O_3S$ (492.98): C, 60.91; H, 4.29; N, 11.36 Found: C, 61.21; H, 4.43; N, 11.71.

*4-Chloro-N-(4-(1-(2-(2-cyano-5-phenylpenta-2,4-dienoyl)hydrazono)ethyl)phenyl) benzenesulfonamide (**7d**)*

Yield, 95.1%; m.p. 190–192 °C. IR (KBr, $cm^{-1}$): 3377, 3339 (2NH), 3021 (CH arom.), 2934, 2860 (CH aliph.), 2210 (CN), 1671 (CO), 1618 (C=N), 1347, 1160 ($SO_2$), 745 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.17 (s, 3H, $CH_3$), 7.19–7.85 (m, 13H, Ar–H), 7.40 (s, 1H, $SO_2NH$), 8.04, 8.07 (2d, 2H, CH=CH, $J = 8.0$ Hz), 8.66 (s, 1H, CH), 10.53 (s, 1H, NHCO). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 100.2 (C–CN), 113.7 (CN), 113.9 (2) (C-phenyl), 114.3 (CH=CH), 116.1, 117.3, 119.1 (2), 121.9 (2), 127.4 (2), 127.5, 127.7 (C-phenyl), 128.3 (CH–CH), 128.6, 129.3, 129.6, 133.2 (C-phenyl), 134.0 (CH–$C_6H_5$), 137.8 (C=N), 138.3 (CH), 148.7 (CO). Anal. Calcd. For $C_{26}H_{21}ClN_4O_3S$ (504.99): C, 61.84; H, 4.19; N, 11.09; Found: C, 62.14; H, 4.33; N, 11.32.

*4-Chloro-N-(4-(1-(2-(2-cyano-3-(2-methoxyphenyl)acryloyl)hydrazono)ethyl)phenyl) benzenesulfonamide (**7e**)*

Yield, 60.9%; m.p. 128–130 °C. IR (KBr, $cm^{-1}$): 3379, 3329 (2NH), 3073 (CH arom.), 2938, 2842 (CH aliph.), 2243 (CN), 1655(CO), 1608 (C=N), 1303, 1188 ($SO_2$), 747 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.17 (s, 3H, $CH_3$), 3.91 (s, 3H, $OCH_3$). 6.99–7.99 (m, 12H, Ar–H), 7.43 (s, 1H, $SO_2NH$), 8.03 (s, 1H, CH), 10.36 (s, 1H, NHCO). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 71.6 ($OCH_3$), 100.2 (C–CN), 105.2 (C-phenyl), 113.7 (CN), 113.9 (2), 114.3, 115.1, 116.1, 119.4, 120.2, 121.9 (2), 127.7 (2), 127.9 (2), 130.2, 130.5, 134.0 (C-phenyl), 138.3 (CH), 140.8 (C=N), 145.3 (C-phenyl), 148.7 (CO). Anal. Calcd. For

$C_{25}H_{21}ClN_4O_4S$ (508.98): C, 58.99; H, 4.16; N, 11.01; Found: C, 59.22; H, 4.32; N, 11.17.

*4-Chloro-N-(4-(1-(2-(2-cyano-3-(4-methoxyphenyl)acryloyl)hydrazono)ethyl)phenyl) benzenesulfonamide (**7f**)*

Yield, 67.8%; m.p. 126–128 °C. IR (KBr, $cm^{-1}$): 3392, 3319 (2NH), 3076 (CH arom.), 2935, 2834 (CH aliph.), 2180 (CN), 1661 (CO), 1620 (C=N), 1350, 1169 ($SO_2$), 743 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.17 (s, 3H, $CH_3$), 3.81 (s, 3H, $OCH_3$). 6.94–8.08 (m, 12H, Ar–H), 7.47 (s, 1H, $SO_2NH$), 8.62 (s, 1H, CH), 9.87 (s, 1H, NHCO). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 69.5 ($OCH_3$), 100.2 (C–CN), 105.2 (2) (C-phenyl), 115.3 (CN), 119.7 (2), 127.7, 127.9, 129.0 (4), 130.0 (2), 130.1 (2), 132.2, 137.4 (2) (C-phenyl), 137.8 (C=N), 138.6 (CH), 140.2 (C-phenyl), 148.7 (CO). Anal. Calcd. For $C_{25}H_{21}ClN_4O_4S$ (508.98): C, 58.99; H, 4.16; N, 11.01; Found: C, 59.15; H, 4.38; N, 11.13.

*4-Chloro-N-(4-(1-(2-(3-(2-chlorophenyl)-2-cyanoacryloyl)hydrazono)ethyl)phenyl) benzenesulfonamide (**7g**)*

Yield, 72.1%; m.p. 126–128 °C. IR (KBr, $cm^{-1}$): 3365, 3323 (2NH), 3021 (CH arom.), 2931, 2856 (CH aliph.), 2213 (CN), 1679 (CO), 1587 (C=N), 1342, 1167 ($SO_2$), 756 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.17 (s, 3H, $CH_3$), 7.00–8.15 (m, 12H, Ar–H), 7.45 (s, 1H, $SO_2NH$), 8.17 (s, 1H, CH), 10.33 (s, 1H, NHCO). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 100.2 (C–CN), 113.7 (CN), 113.9 (2), 115.3, 118.1, 118.4, 119.2 (2), 125.0 (2), 127.8, 127.9, 128.0 (2), 128.4, 128.5, 129.5, 129.8, 132.0 (C-phenyl), 137.8 (C=N), 138.2 (CH), 142.2 (CO). Anal. Calcd. For $C_{24}H_{18}Cl_2N_4O_3S$ (513.40): C, 56.15; H, 3.53; N, 10.91; Found: C, 56.31; H, 3.49; N, 10.85.

*4-Chloro-N-(4-(1-(2-(3-(4-chlorophenyl)-2-cyanoacryloyl)hydrazono)ethyl)phenyl) benzenesulfonamide (**7h**)*

Yield, 66.2%; m.p. 103–105 °C. IR (KBr, $cm^{-1}$): 3357, 3341 (2NH), 3059 (CH arom.), 2942, 2851 (CH aliph.), 2214 (CN), 1678 (CO), 1605 (C=N), 1341, 1156 ($SO_2$), 762 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.17 (s, 3H, $CH_3$), 7.12–8.10 (m, 12H, Ar–H), 7.47 (s, 1H, $SO_2NH$), 8.43 (s, 1H, CH), 10.68 (s, 1H, NHCO). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 100.2 (C–CN), 113.7 (CN), 113.9 (2), 116.2, 118.4 (2), 119.2 (2), 125.0 (2), 127.9 (2), 128.0, 128.2 (2), 128.5, 129.5, 129.8, 132.1 (C-phenyl), 137.8 (C=N), 138.2 (CH), 142.2 (CO). Anal. Calcd. For $C_{24}H_{18}Cl_2N_4O_3S$ (513.40): C, 56.15; H, 3.53; N, 10.91; Found: C, 56.02; H, 3.34; N, 10.75.

*4-Chloro-N-(4-(1-(2-(2-cyano-3-(4-(dimethylamino)phenyl)acryloyl)hydrazono)ethyl)phenyl) benzenesulfonamide (**7i**)*

Yield, 36.8%; m.p. 228–230 °C. IR (KBr, $cm^{-1}$): 3355, 3308 (2NH), 3095 (CH arom.), 2918, 2879 (CH aliph.), 2202 (CN), 1671 (CO), 1611 (C=N), 1338, 1160

($SO_2$), 757 (C–Cl). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 2.24 (s, 3H, $CH_3$), 3.07 (s, 6H, $2CH_3$), 6.82–7.94 (m, 12H, Ar–H), 7.76 (s, 1H, $SO_2NH$), 8.01 (s, 1H, CH), 10.55 (s, 1H, NHCO). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 50.3 (2) ($N(CH_3)_2$), 100.2 (C–CN), 102.3 (2) (C-phenyl), 113.7 (CN), 113.9 (2), 114.2, 116.1, 121.9 (2), 127.7 (2), 127.9 (2), 128.1 (2), 128.6, 129.6, 133.2 (C-phenyl), 137.8 (C=N), 138.0 (C-phenyl), 138.3 (CH), 148.7 (CO). Anal. Calcd. For $C_{26}H_{24}ClN_5O_3S$ (522.02): C, 59.82; H, 4.63; N, 13.42; Found: C, 60.13; H, 4.89; N, 13.60.

*N-(4-(1-(2-(3-(Benzo[d][1,3]dioxol-5-yl)-2-cyanoacryloyl)hydrazono)ethyl)phenyl)-4-chlorobenzenesulfonamide (**7j**)*

Yield, 50.3%; m.p. 251–253 °C. IR (KBr, $cm^{-1}$): 3387, 3370 (2NH), 3092 (CH arom.), 2915, 2867 (CH aliph.), 2216 (CN), 1667 (CO), 1598 (C=N), 1348, 1179 ($SO_2$), 753 (C–Cl). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 2.25 (s, 3H, $CH_3$), 6.17 (s, 2H, $CH_2$), 7.11–7.79 (m, 11H, Ar–H), 7.55 (s, 1H, $SO_2NH$), 8.09 (s, 1H, CH), 10.57 (s, 1H, NHCO). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 99.8 ($CH_2$), 100.2 (C–CN), 103.3, 105.6 (C-phenyl), 113.7 (CN), 113.9 (2), 114.2, 116.2, 117.5, 119.4 (2), 121.9 (2), 127.7 (2), 130.2, 130.5, 134.0 (C-phenyl), 137.8 (C=N), 137.9, 138.0 (C-phenyl), 138.3 (CH), 148.7 (CO). Anal. Calcd. For $C_{25}H_{19}ClN_4O_5S$ (522.96): C, 57.42; H, 3.66; N, 10.71; Found: C, 57.22; H, 3.38; N, 10.43.

## General procedure for the synthesis of compounds 8a–j

*Method (A)*: A mixture of **4** (3.9 g, 0.01 mol) and arylidene malononitrile (0.01 mol) in ethanol (15 mL) containing piperidine (0.5 mL) was refluxed for 4 h. The reaction mixture was poured into ice-water containing few drops of dil. HCl and filtered. The solid obtained was crystallized from dioxane to give **8a–j**, respectively.
*Method (B)*: A mixture of compounds **7a–j** (0.01 mol) and malononitrile (0.66 g, 0.01 mol) in dioxane (15 mL) containing piperidine (0.5 mL) was refluxed for 5 h. The reaction mixture was cooled and poured into ice-water containing few drops of dil. HCl and filtered. The obtained solid was crystallized from dioxane to give **8a–j**.

*N-(4-(1-((6-Amino-3,5-dicyano-4-(furan-2-yl)-2-oxopyridin-1(2H)-yl)imino)ethyl)phenyl)-4-chlorobenzenesulfonamide (**8a**)*

Yield, 35.2%; m.p. 133–135 °C. IR (KBr, $cm^{-1}$): 3330, 3245, 3200 ($NH_2$, NH), 3060 (CH arom.), 2918, 2847 (CH aliph.), 2211, 2200 (2 CN), 1676 (CO), 1601 (C=N), 1341, 1170 ($SO_2$), 761 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.16 (s, 3H, $CH_3$), 7.01–7.75 (m, 11H, Ar–H), 7.56 (s, 1H, $SO_2NH$), 10.73 (s, 2H, $NH_2$). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 76.5 (C–CN), 109.4, 112.8 (C-furan), 115.8 (2) (CN), 116.4 (2) (C-phenyl), 121.5 (C–CN), 127.5, 128.7 (2), 129.1 (2), 130.0 (2), 137.5, 138.0, 140.0 (C-phenyl), 140.0 (C–$NH_2$), 143.7, 149.8 (C-furan), 150.0 (CO), 159.5 (C=N), 169.4 (C-pyridone). Anal. Calcd. For $C_{25}H_{17}ClN_6O_4S$ (532.96): C, 56.34; H, 3.22; N, 15.77; Found: C, 56.45; H, 3.39; N, 15.89.

*N-(4-(1-((6-Amino-3,5-dicyano-2-oxo-4-phenylpyridin-1(2H)-yl)imino)ethyl)phenyl)-4-chlorobenzenesulfonamide (**8b**)*

Yield, 67.8%; m.p. 126–128 °C. IR (KBr, $cm^{-1}$): 3334, 3238, 3204 ($NH_2$, NH), 3048 (CH arom.), 2920, 2851 (CH aliph.), 2215, 2198 (2 CN), 1670 (CO), 1602 (C=N), 1349, 1161 ($SO_2$), 755 (C–Cl). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 2.14 (s, 3H, $CH_3$), 7.14–8.05 (m, 13H, Ar–H), 7.54 (s, 1H, $SO_2NH$), 10.61 (s, 2H, $NH_2$). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 76.5, 115.3 (C–CN), 115.8 (2) (CN), 116.4 (2), 127.5, 127.9, 128.1 (2), 128.7 (2), 128.9 (2), 129.1 (2), 130.0 (2), 132.5, 137.5, 138.1, 140.0 (C-phenyl), 140.0 (C–$NH_2$), 160.0 (CO), 163.4 (C=N), 169.0 (C-pyridone). Anal. Calcd. For $C_{27}H_{19}ClN_6O_3S$ (543): C, 59.72; H, 3.53; N, 15.48; Found: C, 59.99; H, 3.64; N, 15.54.

*N-(4-(1-((6-Amino-3,5-dicyano-2-oxo-4-(p-tolyl)pyridin-1(2H)-yl)imino)ethyl)phenyl)-4-chlorobenzenesulfonamide (**8c**)*

Yield, 78.7%; m.p. 132–134 °C. IR (KBr, $cm^{-1}$): 3328, 3247, 3213 ($NH_2$, NH), 3040 (CH arom.), 2940, 2922 (CH aliph.), 2212, 2195 (2 CN), 1667 (CO), 1606 (C=N), 1344, 1162 ($SO_2$), 756 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.27 (s, 3H, $CH_3$ tolyl), 2.38 (s, 3H, $CH_3$), 7.15–8.04 (m, 12H, Ar–H), 7.41 (s, 1H, $SO_2NH$), 10.61 (s, 2H, $NH_2$). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.5 ($CH_3$), 29.1 ($CH_3$ tolyl), 83.6, 120.8 (C–CN), 128.2 (2) (CN), 128.7 (2), 127.5, 129.2 (2), 129.5 (2), 130.0 (3), 130.1 (5), 131.8 (2), 134.6 (C-phenyl), 138.5 (C–$NH_2$), 147.8 (CO), 157.1 (C=N), 163.7 (C-pyridone). Anal. Calcd. For $C_{28}H_{21}ClN_6O_3S$ (557.02): C, 60.37; H, 3.80; N, 15.09; Found: C, 60.55; H, 4.12; N, 15.17.

*N-(4-(1-((6-Amino-3,5-dicyano-2-oxo-4-(styryl)pyridin-1(2H)-yl)imino)ethyl)phenyl)-4-chlorobenzenesulfonamide (**8d**)*

Yield, 27.3%; m.p. 176–178 °C. IR (KBr, $cm^{-1}$): 3353, 3247, 3213 ($NH_2$, NH), 3060 (CH arom.), 2934, 2856 (CH aliph.), 2203, 2186 (2 CN), 1687 (CO), 1600 (C=N), 1345, 1161 ($SO_2$), 757 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.21 (s, 3H, $CH_3$), 7.33–8.01 (m, 13H, Ar–H), 7.61 (d, 1H, CH=CH, $J$ = 8.4 Hz), 7.63 (d, 1H, CH–$C_6H_5$, $J$ = 8.4 Hz), 7.57 (s, 1H, $SO_2NH$), 10.61 (s, 2H, $NH_2$). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.2 ($CH_3$), 76.1, 110.3 (C–CN), 115.8 (2) (CN), 116.4 (2) (C-phenyl), 125.9 (C-styryl) 127.5, 127.9, 128.5 (2), 128.7 (2), 128.9 (2), 129.1 (2), 130.0 (2) (C-phenyl), 131.1 (C-styryl), 135.4, 137.5, 137.7, 140.0 (C-phenyl), 140.0 (C–$NH_2$), 160.5 (CO), 163.4 (C=N), 170.5 (C-pyridone). Anal. Calcd. For $C_{29}H_{21}ClN_6O_3S$ (569.03): C, 61.21; H, 3.72; N, 14.77; Found: C, 61.46; H, 3.83; N, 14.95.

*N-(4-(1-((6-Amino-3,5-dicyano-4-(2-methoxyphenyl)-2-oxopyridin-1(2H)-yl)imino)ethyl)phenyl)-4-chlorobenzenesulfonamide (**8e**)*

Yield, 48.1%; m.p. 143–145 °C. IR (KBr, $cm^{-1}$): 3320, 3227, 3217 ($NH_2$, NH), 3043 (CH arom.), 2920, 2846 (CH aliph.), 2215, 2190 (2 CN), 1664 (CO), 1601 (C=N), 1338, 1168 ($SO_2$), 743 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.17 (s,

3H, $CH_3$), 3.84 (s, 3H, $OCH_3$), 6.93–8.16 (m, 12H, Ar–H), 7.48 (s, 1H, $SO_2NH$), 10.59 (s, 2H, $NH_2$). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 40.3 ($OCH_3$), 66.4 (C–CN), 111.4 (C-phenyl), 115.3 (C–CN), 115.8 (2) (CN), 116.4 (2), 119.8, 120.9, 127.5, 128.7 (2), 128.9, 129.1 (2), 129.7, 130.0 (2), 137.5, 137.8, 140.0 (C-phenyl), 140.0 (C–$NH_2$), 157.5 (C-phenyl), 160.0 (CO), 163.5 (C=N), 169.1 (C-pyridone). Anal. Calcd. For $C_{28}H_{21}ClN_6O_4S$ (573.02): C, 58.69; H, 3.69; N, 14.67; Found: C, 58.81; H, 3.91; N, 14.86.

*N-(4-(1-((6-Amino-3,5-dicyano-4-(4-methoxyphenyl)-2-oxopyridin-1(2H)-yl)imino)ethyl)phenyl)-4-chlorobenzenesulfonamide (**8f**)*

Yield, 34.5%; m.p. 143–145 °C. IR (KBr, $cm^{-1}$): 3328, 3297, 3181 ($NH_2$, NH), 3037 (CH arom.), 2946, 2919 (CH aliph.), 2217, 2190 (2 CN), 1655 (CO), 1601 (C=N), 1336, 1162 ($SO_2$), 756 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.17 (s, 3H, $CH_3$), 3.8 (s, 3H, $OCH_3$), 7.12–8.03 (m, 12H, Ar–H), 7.63 (s, 1H, $SO_2NH$), 10.60 (s, 2H, $NH_2$). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 33.3 ($OCH_3$), 66.2 (C–CN), 114.2 (2) (C-phenyl), 115.3 (C–CN), 115.8 (2) (CN), 116.4 (2), 124.8, 127.5, 128.7 (2), 129.1 (2), 129.7 (2), 130.0 (2), 130.2, 130.5, 134.0 (C-phenyl), 137.8 (C–$NH_2$), 160.0 (CO), 163.4 (C=N), 169.4 (C-pyridone). Anal. Calcd. For $C_{28}H_{21}ClN_6O_4S$ (573.02): C, 58.69; H, 3.69; N, 14.67; Found: C, 58.87; H, 3.84; N, 14.85.

*N-(4-(1-((6-Amino-4-(2-chlorophenyl)-3,5-dicyano-2-oxopyridin-1(2H)-yl)imino)ethyl)phenyl)-4-chlorobenzenesulfonamide (**8g**)*

Yield, 39.1%; m.p. 128–130 °C. IR (KBr, $cm^{-1}$): 3328, 3247, 3213 ($NH_2$, NH), 3040 (CH arom.), 2965, 2930 (CH aliph.), 2217, 2195 (2 CN), 1668 (CO), 1601 (C=N), 1341, 1171 ($SO_2$), 748 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.17 (s, 3H, $CH_3$), 7.20–8.05 (m, 12H, Ar–H), 7.50 (s, 1H, $SO_2NH$), 10.62 (s, 2H, $NH_2$). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 66.6, 115.3 (C–CN), 115.8 (2) (CN), 116.4 (2), 126.7, 127.5, 127.9, 128.7 (2), 129.1 (2), 129.3, 129.9, 130.0 (2), 135.3, 135.8, 137.5, 137.8, 140.0 (C-phenyl), 140.0 (C–$NH_2$) 160.0 (CO), 163.1 (C=N), 169.4 (C-pyridone). Anal. Calcd. For $C_{27}H_{18}Cl_2N_6O_3S$ (577.44): C, 56.16; H, 3.14; N, 14.55; Found: C, 56.35; H, 3.27; N, 14.79.

*N-(4-(1-((6-Amino-4-(4-chlorophenyl)-3,5-dicyano-2-oxopyridin-1(2H)-yl)imino)ethyl)phenyl)-4-chlorobenzenesulfonamide (**8h**)*

Yield, 26.6%; m.p. 161–163 °C. IR (KBr, $cm^{-1}$): 3322, 3247, 3214 ($NH_2$, NH), 3068 (CH arom.), 2917, 2850 (CH aliph.), 2218, 2215 (2 CN), 1666 (CO), 1603 (C=N), 1356, 1172 ($SO_2$), 767 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.15 (s, 3H, $CH_3$), 7.08–8.03 (m, 12H, Ar–H), 7.47 (s, 1H, $SO_2NH$), 10.62 (s, 2H, $NH_2$). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 73.5, 115.3 (C–CN), 115.8 (2) (CN), 116.4 (2), 127.5, 128.7 (2), 128.9 (2), 129.1 (2), 130.0 (2), 130.5 (2), 131.0, 133.5, 137.5, 137.8, 140.0 (C-phenyl), 140.0 (C–$NH_2$), 160.0 (CO), 163.4 (C=N), 169.5 (C-pyridone). Anal. Calcd. For $C_{27}H_{18}Cl_2N_6O_3S$ (577.44): C, 56.16; H, 3.14; N, 14.55; Found: C, 56.32; H, 3.23; N, 14.68.

*N-(4-(1-((6-Amino-3,5-dicyano-4-(4-(dimethylamino)phenyl)-2-oxopyridin-1(2H)-yl)imino)ethyl)phenyl)-4-chlorobenzenesulfonamide (**8i**)*

Yield, 42.2%; m.p. 151–153 °C. IR (KBr, $cm^{-1}$): 3387, 3247, 3213 ($NH_2$, NH), 3036 (CH arom.), 2937, 2857 (CH aliph.), 2183, 2148 (2 CN), 1657 (CO), 1586 (C=N), 1366, 1170 ($SO_2$), 760 (C–Cl). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 2.22 (s, 3H, $CH_3$), 3.06 (s, 6H, $2CH_3$), 6.80–7.99 (m, 12H, Ar–H), 7.75 (s, 1H, $SO_2NH$), 10.62 (s, 2H, $NH_2$). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 40.1 (2), ($N(CH_3)_2$), 71.3 (C–CN), 111.7 (2) (C-phenyl), 115.3 (C–CN), 115.8 (2) (CN), 116.4 (2), 122.0, 127.5, 128.7 (2), 129.1 (2), 130.0 (2), 130.3 (2), 137.5, 137.8, 140.0 (C-phenyl), 140.0 (C–$NH_2$), 150.3 (C-phenyl), 160.0 (CO), 164.3 (C=N), 169.1 (C-pyridone). Anal. Calcd. For $C_{29}H_{24}ClN_7O_3S$ (586.06): C, 59.43; H, 4.13; N, 16.73; Found: C, 59.59; H, 4.19; N, 16.84.

*N-(4-(1-((6-Amino-4-(benzo[d][1,3]dioxol-5-yl)-3,5-dicyano-2-oxopyridin-1(2H)-yl)imino)ethyl)phenyl)-4-chlorobenzenesulfonamide (**8j**)*

Yield, 65.0%; m.p. 118–120 °C. IR (KBr, $cm^{-1}$): 3387, 3300, 3266 ($NH_2$, NH), 3060 (CH arom.), 2937, 2857 (CH aliph.), 2183, 2148 (2 CN), 1659 (CO), 1567 (C=N), 1356, 1152 ($SO_2$), 751 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.17 (s, 3H, $CH_3$), 6.07 (s, 2H, O–$CH_2$–O), 6.96–8.04 (m, 11H, Ar–H), 7.70 (s, 1H, $SO_2NH$), 10.64 (s, 2H, $NH_2$). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 72.5 (C–CN), 101.2 ($CH_2$), 108.0, 112.8 (C-phenyl), 115.3 (C–CN), 115.8 (2) (CN), 118.1 (2), 118.3, 125.8, 127.5, 128.5 (2), 129.7 (2), 129.9 (2), 137.5, 138.1, 140.0 (C-phenyl), 140.0 (C–$NH_2$), 148.0, 148.7 (C-phenyl), 160.0 (CO), 163.4 (C=N), 169.4 (C-pyridone). Anal. Calcd. For $C_{28}H_{19}ClN_6O_5S$ (587.01): C, 57.29; H, 3.26; N, 14.32; Found: C, 57.61; H, 3.43; N, 14.67.

*4-Chloro-N-(4-(1-(2-(2,4-diamino-5-cyanothiophene-3-carbonyl)hydrazono)ethyl) phenyl) benzenesulfonamide (**9**)*

A mixture of compound **4** (3.9 g, 0.01 mol), malononitrile (0.66 g, 0.01 mol), and elemental sulfur (0.32 g, 0.01 mol) were refluxed in ethanol (15 mL) containing triethylamine 5 drops for 6 h. The reaction mixture was poured into ice-water and filtered. The obtained solid was crystallized from dioxane to give **9**. Yield, 52.2%; m.p. > 300 °C. IR (KBr, $cm^{-1}$): 3392, 3324, 3198 (2NH, $2NH_2$), 3066 (CH arom.), 2931, 2865 (CH aliph.), 2208 (CN), 1695 (CO), 1598 (C=N), 1334, 1167 ($SO_2$), 752 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.16 (s, 3H, $CH_3$), 7.58–7.87 (m, 8H, Ar–H), 7.68 (s, 1H, $SO_2NH$), 7.79 (s, 2H, $NH_2$), 10.56 (s, 1H, NH), 10.95 (s, 2H, $NH_2$). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.4 ($CH_3$), 66.6 (C–CN), 115.3 (C-thiophene), 115.3 (CN), 119.7 (2), 127.7, 127.9 (2), 129.0 (2), 130.0 (2) (C-phenyl), 132.2 (C–$NH_2$), 137.5, 137.8, 138.6 (C-phenyl), 148.7 (C=N), 154.8 (CO), 166.2 (S–C–$NH_2$). Anal. Calcd. For $C_{20}H_{17}ClN_6O_3S_2$ (488.97): C, 49.13; H, 3.50; N, 17.19; Found: C, 49.01; H, 3.42; N, 16.91.

*Ethyl-3,5-diamino-4-(2-(1-(4-(4-chlorophenylsulfonamido)phenyl) ethylidene)hydrazinecarbonyl) thiophene-2-carboxylate (**10**)*

A mixture of compound **4** (3.9 g, 0.01 mol), ethyl cyanoacetate (1.13 g, 0.01 mol) and elemental sulfur (0.32 g, 0.01 mol) were refluxed in ethanol (15 mL) containing triethylamine 5 drops for 6 h. The reaction mixture was poured into ice-water and filtered. The obtained solid was crystallized from dioxane to give **10**. Yield, 50.6%; m.p. > 300 °C. IR (KBr, $cm^{-1}$): 3389, 3298, 3200 (NH, $2NH_2$), 3080 (CH arom.), 2925, 2855 (CH aliph.), 1742, 1679 (2 CO), 1601 (C=N), 1367, 1174 ($SO_2$), 748 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 1.03 (t, 3H, $\underline{CH_3}CH_2$, $J = 8.8$ Hz), 2.16 (s, 3H, $CH_3$), 4.17 (q, 2H, $\underline{CH_2}CH_3$, $J = 8.8$ Hz), 7.08–8.03 (m, 8H, Ar–H), 7.68 (s, 1H, $SO_2NH$), 7.79 (s, 2H, $\underline{NH_2}$), 10.54 (s, H, NH), 10.95 (s, 2H, $NH_2$). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 20.1 ($\underline{CH_3}CH_2$), 24.4 ($CH_3$), 60.3 ($\underline{CH_2}CH_3$), 119.7 (C-thiophene), 119.7 (2) (C-phenyl), 126.0 (C–CO ester), 127.7, 127.9 (2), 129.0 (2), 129.9 (2) (C-phenyl), 132.2 (C–$NH_2$), 137.5, 137.8, 138.6 (C-phenyl), 148.7 (C=N), 163.5 (CO ester), 166.2 (CO), 167.5 (S–C–$NH_2$). Anal. Calcd. For $C_{22}H_{22}ClN_5O_5S_2$ (536.02): C, 49.30; H, 4.14; N, 13.07; Found: C, 49.08; H, 3.82; N, 12.89.

*N-(4-(1-(2-(2-Amino-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carbonyl)hydrazono)ethyl)phenyl)-4-chlorobenzenesulfonamide (**11**)*

To a solution mixture of compound **4** (3.9 g, 0.01 mol), absolute ethanol (15 mL), triethylamine 5 drops and cyclopentanone (0.84 g, 0.01 mol), elemental sulfur (0.32 g, 0.01 mol) were added. The reaction mixture was refluxed for 2 h, then poured into ice-water and filtered. The solid obtained was crystallized from dioxane to give **11**. Yield, 34.0%; m.p. 166–168 °C. IR (KBr, $cm^{-1}$): 3382, 3315, 3257 (2NH, $NH_2$), 3086 (CH arom.), 2937, 2843 (CH aliph.), 1656 (CO), 1599 (C=N), 1370, 1183 ($SO_2$), 752 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.16 (s, 3H, $CH_3$), 2.24 (m, 2H, $CH_2$ cyclopentane), 3.04, 3.09 (2t, 4H, $2CH_2$ cyclopentane, $J = 8.0$ Hz), 7.13–7.77 (m, 8H, Ar–H), 7.64 (s, 1H, $SO_2NH$), 10.63 (s, 1H, NH), 10.97 (s, 2H, $NH_2$). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 24.4 ($CH_3$), 25.3, 26.9, 39.4 ($CH_2$ cyclopentane), 119.7 (C–CO), 119.7 (2) (C-phenyl), 127.7 (C-thiophene), 128.3, 129.0 (2), 130.1 (2), 130.3 (2), 132.1, 133.6 (C-phenyl), 138.3 (C-thiophene), 139.5 (C-phenyl), 148.7 (C=N), 154.8 (C–$NH_2$), 166.2 (CO). Anal. Calcd. For $C_{22}H_{21}ClN_4O_3S_2$ (489.01): C, 54.03; H, 4.33; N, 11.46; Found: C, 54.44; H, 4.49; N, 11.61.

*N-(4-(1-(2-(2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonyl)hydrazono)ethyl)phenyl)-4-chlorobenzenesulfonamide (**12**)*

To a solution mixture of compound **4** (3.9 g, 0.01 mol), absolute ethanol (15 mL), triethylamine 5 drops and cyclohexanone (0.98 g, 0.01 mol), elemental sulfur (0.32 g, 0.01 mol) were added. The reaction mixture was refluxed for 2 h, then poured into ice-water and filtered. The solid obtained was crystallized from dioxane to give **12**. Yield, 79.1%; m.p. 138–140 °C. IR (KBr, $cm^{-1}$): 3387, 3332, 3262 (2NH, $NH_2$), 3078 (CH arom.), 2930, 2857 (CH aliph.), 1680 (CO), 1603 (C=N), 1335, 1191 ($SO_2$), 739 (C–Cl). $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 1.63, 2.44 (m,

8H, 4 $CH_2$ cyclohexane), 2.14 (s, 3H, $CH_3$), 7.06–7.82 (m, 8H, Ar–H), 7.53 (s, 1H, $SO_2NH$), 10.62 (s, 1H, NH), 10.96 (s, 2H, $NH_2$). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 24.4 ($CH_3$), 25.3 (2), 26.9 (2) ($CH_2$ cyclohexane), 119.7 (C–CO), 127.7 (2) (C-phenyl), 127.7 (C-thiophene), 128.3, 129.0 (2), 130.1 (2), 130.3 (2), 132.1, 133.6 (C-phenyl), 138.3 (C-thiophene), 139.5 (C-phenyl), 148.7 (C=N), 154.8 (C–$NH_2$), 166.2 (CO). Anal. Calcd. For $C_{23}H_{23}ClN_4O_3S_2$ (503.04): C, 54.92; H, 4.61; N, 11.14; Found: C, 55.23; H, 4.93; N, 11.37.

## In vitro anticancer evaluation against human tumor liver cancer (HepG2)

The in vitro anticancer screening was performed on liver human carcinoma cell line (HepG2) using the sulforhodamine B stain (SRB) assay for 26 new compounds and one reported compound. The in vitro anticancer screening was done by the pharmacology unit at the National Cancer Institute, Cairo University.

The sulforhodamine B (SRB) assay [31], remains one of the most widely used methods for in vitro cytotoxic screening. It relies on the ability of SRB to bind to protein components of the cells that have been fixed to tissue-culture plates by trichloroacetic acid (TCA). The amount of dye extracted from stained cells is equivalent to the cell mass [32]. Cells were plated in the 96-multiwell plate (104 cells/well) for 24 h before adding the target compounds. Compounds were added to 1 mL DMSO and diluted with saline to make serial dilutions of the target compounds (12.5, 25, 50, and 100 μM). Triplicate wells were prepared for concentration to determine the standard error. Color intensity was measured using enzyme-linked immunosorbent assay (ELISA) reader. The relationship between surviving fraction and drug concentration was plotted to get the $IC_{50}$ of the compound after the specified time, and compared to the reference drug doxorubicin, the results are given in Table 1.

## Radiosensitizing evaluation

Irradiation of the promising compounds was performed at the National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, using Gamma cell-40 ($^{137}$Cs) source. The most active compounds **4**, **8d** and **8h** were further re-evaluated for their in vitro cytotoxic activity in combination with γ-irradiation (8 Gy). Cells were incubated in the 96-multiwell plate for 24 h before γ-irradiation with a single dose of 8 Gy. Cells were further incubated for 48 h at 37 °C in 5% $CO_2$ atmosphere. After that, cells were fixed, washed and stained with 0.4% (wt/vol) SRB dissolved in 1% acetic acid, for 30 min. Excess unbound dye was washed off four times using 1% acetic acid and the attached stain was recovered with Tris–EDTA buffer. Color intensity was measured at a wavelength of 570 nm. In another plate, cells were incubated with the most potent compounds: **4**, **8d**, and **8h** in molar concentrations of 12.5, 25, 50, and 100 μM. After 2 h, cells were subjected to a single dose of γ-radiation at a dose level of 8 Gy with a dose rate of 0.758 rad/s for 18 min, then cytotoxicity was measured 48 h after irradiation. The surviving fractions were measured and expressed as mean values ± SE. The results were analyzed using 1-way ANOVA test and the results are given in Table 2.

**Table 1** $IC_{50}$ values of the newly synthesized compounds against HepG2 cell line

| Cpd. no. | Compound concentration (μM) | | | | $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| | Surviving fraction (mean ± SE)[a] | | | | |
| | 12.5 (μM) | 25 (μM) | 50 (μM) | 100 (μM) | |
| **DOX.** | 0.551 ± 0.026 | 0.480 ± 0.003 | 0.139 ± 0.005 | 0.130 ± 0.016 | 32 |
| **4** | 0.180 ± 0.056 | 0.159 ± 0.025 | 0.078 ± 0.008 | 0.064 ± 0.044 | 8 |
| **5** | 0.731 ± 0.032 | 0.594 ± 0.049 | 0.444 ± 0.017 | 0.321 ± 0.022 | 41.2 |
| **6** | 0.688 ± 0.021 | 0.625 ± 0.029 | 0.500 ± 0.024 | 0.434 ± 0.024 | 50 |
| **7a** | 0.688 ± 0.029 | 0.625 ± 0.022 | 0.608 ± 0.028 | 0.563 ± 0.015 | – |
| **7b** | 0.844 ± 0.027 | 0.719 ± 0.032 | 0.438 ± 0.025 | 0.393 ± 0.007 | 44.4 |
| **7c** | 0.881 ± 0.009 | 0.563 ± 0.006 | 0.438 ± 0.018 | 0.402 ± 0.011 | 38.2 |
| **7d** | 0.625 ± 0.024 | 0.625 ± 0.098 | 0.469 ± 0.013 | 0.447 ± 0.034 | 45 |
| **7e** | 0.819 ± 0.023 | 0.688 ± 0.033 | 0.375 ± 0.010 | 0.291 ± 0.055 | 40.3 |
| **7f** | 0.812 ± 0.031 | 0.563 ± 0.014 | 0.419 ± 0.016 | 0.364 ± 0.036 | 36.2 |
| **7g** | 0.725 ± 0.015 | 0.606 ± 0.016 | 0.400 ± 0.014 | 0.296 ± 0.026 | 37.9 |
| **7h** | 0.625 ± 0.026 | 0.624 ± 0.020 | 0.344 ± 0.018 | 0.234 ± 0.015 | 36 |
| **7i** | 0.719 ± 0.027 | 0.625 ± 0.032 | 0.563 ± 0.020 | 0.468 ± 0.039 | – |
| **7j** | 0.762 ± 0.011 | 0.700 ± 0.008 | 0.438 ± 0.027 | 0.412 ± 0.011 | 44.3 |
| **8a** | 0.750 ± 0.021 | 0.740 ± 0.012 | 0.438 ± 0.028 | 0.407 ± 0.015 | 45 |
| **8b** | 0.906 ± 0.027 | 0.750 ± 0.032 | 0.500 ± 0.020 | 0.441 ± 0.006 | 50 |
| **8c** | 0.844 ± 0.009 | 0.563 ± 0.031 | 0.469 ± 0.018 | 0.423 ± 0.031 | 42 |
| **8d** | 0.564 ± 0.015 | 0.359 ± 0.098 | 0.308 ± 0.003 | 0.283 ± 0.034 | 16.4 |
| **8e** | 0.615 ± 0.023 | 0.359 ± 0.033 | 0.333 ± 0.017 | 0.292 ± 0.019 | 18.2 |
| **8f** | 0.623 ± 0.032 | 0.419 ± 0.016 | 0.383 ± 0.018 | 0.314 ± 0.015 | 18.4 |
| **8g** | 0.667 ± 0.015 | 0.513 ± 0.007 | 0.256 ± 0.010 | 0.224 ± 0.004 | 26.3 |
| **8h** | 0.513 ± 0.023 | 0.506 ± 0.020 | 0.308 ± 0.018 | 0.289 ± 0.015 | 13.3 |
| **8i** | 0.667 ± 0.024 | 0.395 ± 0.032 | 0.349 ± 0.020 | 0.298 ± 0.015 | 19 |
| **8j** | 0.646 ± 0.019 | 0.395 ± 0.006 | 0.297 ± 0.018 | 0.279 ± 0.031 | 19.7 |
| **9** | 0.844 ± 0.011 | 0.613 ± 0.024 | 0.463 ± 0.012 | 0.428 ± 0.004 | 43.8 |
| **10** | 0.744 ± 0.030 | 0.531 ± 0.014 | 0.431 ± 0.027 | 0.409 ± 0.010 | 33.3 |
| **11** | 0.769 ± 0.032 | 0.688 ± 0.034 | 0.406 ± 0.018 | 0.372 ± 0.006 | 41.7 |
| **12** | 0.694 ± 0.023 | 0.563 ± 0.008 | 0.500 ± 0.013 | 0.451 ± 0.007 | 50 |

[a] Each value is equivalent to the mean of three experiments ± SE

## Molecular modeling and docking

The molecular model of the new compounds was built using standard bond angles and lengths, with the MOE software suite 2007.09. Following geometry optimization, energy minimization was carried out to employing the ConfSearch module in MOE. All molecular mechanics calculations were performed with the Merck Force Field (MMFF94 s). The crystallographic structure of CA II/IX mimic complex was obtained from the Protein DataBank, with PDB ID: 4BCW. Hydrogen was added to

**Table 2** $IC_{50}$ values of compounds **4**, **8d,** and **8h** against HepG2 cell line in combination with γ-radiation

| Cpd. no. | Control | Irradiated (8 Gy) | Compound concentration (μM) + irradiation (8 Gy) | | | | $IC_{50}$ (μM) |
|---|---|---|---|---|---|---|---|
| | | | Surviving fraction (mean ± SE)[a] | | | | |
| | | | 12.5 | 25 | 50 | 100 | |
| **4** | 1.000 | 0.927 ± 0.02* | 0.26 ± 0.08* | 0.25 ± 0.01* | 0.12 ± 0.01* | 0.11 ± 0.01* | 6.5 |
| **8d** | 1.000 | 0.927 ± 0.02* | 0.42 ± 0.01* | 0.34 ± 0.02* | 0.28 ± 0.01* | 0.11 ± 0.01* | 10.7 |
| **8h** | 1.000 | 0.927 ± 0.02* | 0.28 ± 0.02* | 0.25 ± 0.01* | 0.18 ± 0.01* | 0.11 ± 0.01* | 9.1 |

* Significant difference from control group at $p < 0.001$

[a] Each value is equivalent to the mean of three values ± SE

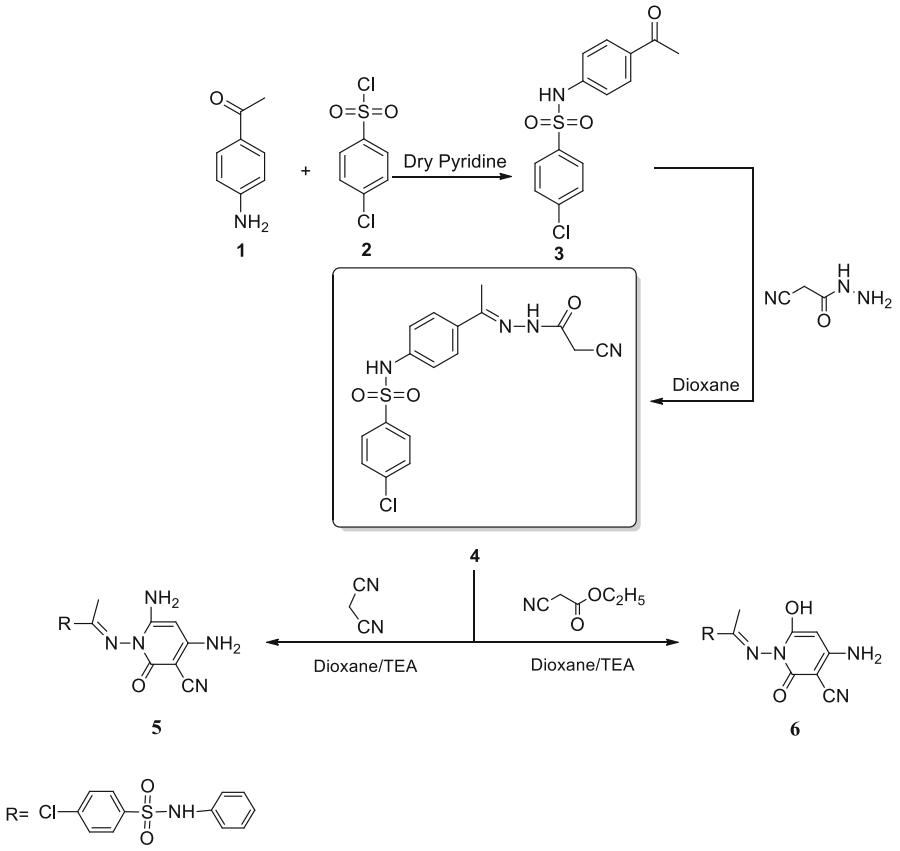
the enzyme and partial charges were deliberated. Validation followed by docking of the new compounds into the active site were carried out, after removing the co-crystallized ligand. The target protein was kept rigid, while the added ligands were left free to explore the conformational space inside the enzyme cavity; 50 separate docking simulations were run using default parameters and the conformations were chosen based on the E conformation, S score data, and appropriate fitting with the relevant amino acids in the binding pocket.

## Results and discussion

### Chemistry

Schemes 1, 2, and 3 showed the synthetic strategies utilized for the synthesis of the target compounds (**5**–**12**) from the starting material 4-Chloro-*N*-(4-(1-(2-(2-cyanoacetyl)hydrazono)ethyl)phenyl) benzenesulfonamide **4** [33]. Reaction of **4** with malononitrile and/or ethyl cyanoacetate in dioxane to give 4-chloro-*N*-(4-(1-((4,6-diamino-3-cyano-2-oxopyridin-1(*2H*)-yl)imino)ethyl)phenyl) benzenesulfonamide **5** and *N*-(4-(1-((4-amino-3-cyano-6-hydroxy-2-oxopyridin-1(*2H*)-yl)imino)ethyl)phenyl)-4-chlorobenzenesulfonamide **6**, respectively (Scheme 1). IR of **5** revealed bands at 3334 and 3283 $cm^{-1}$ for the introduced $NH_2$ groups. $^1$H-NMR displayed up-field singlet at 4.16 ppm assigned to the (CH-pyridone) and two downfield shifted singlets appearing at 10.6 and 10.94 ppm, due to two $NH_2$ protons. $^{13}$C-NMR exhibited a new up-field signal at 88.5 ppm for the (CH-pyridone), 138.3 ppm for (N–C–$NH_2$) and 166.2 ppm for C–$NH_2$. IR of **6** revealed bands at 3420, 3237, and 3210 $cm^{-1}$ for the OH and $NH_2$ groups. $^1$H-NMR displayed an up-field singlet at 4.18 ppm for the (CH-pyridone), and two downfield shifted singlets appearing at 10.62 and 10.96 ppm, for the $NH_2$ and OH protons. $^{13}$C-NMR exhibited a new up-field signal at 75.5 ppm for the (CH-pyridone), 165.3 ppm for the (C–OH) and 166.2 ppm for the (C–$NH_2$).

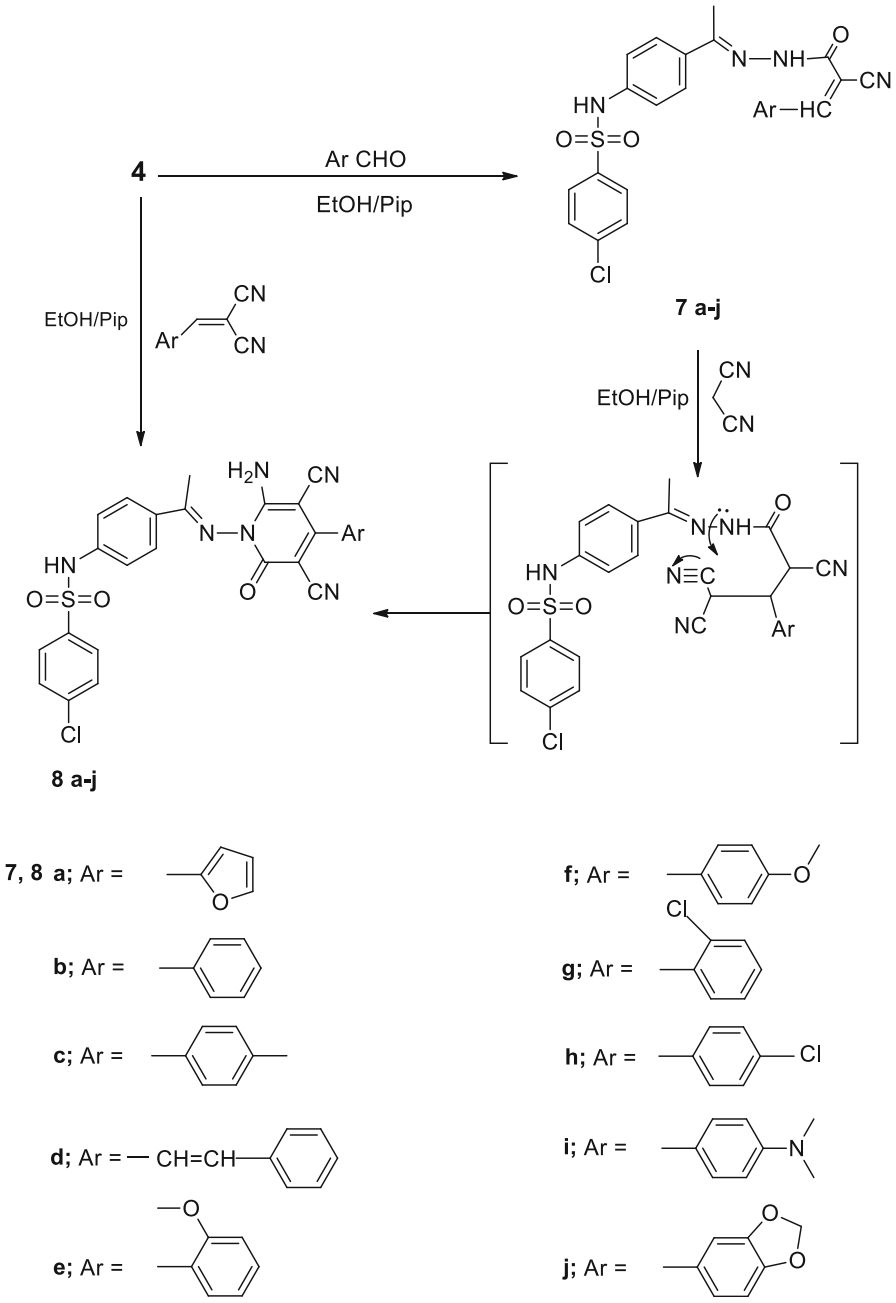
The reaction of **4** with a series of aromatic aldehydes yielded the corresponding hydrazone derivatives **7a**–**j** (Scheme 2), that was further reacted with malononitrile in ethanol containing piperidine to give the corresponding 1,2-dihydropyridone




**Scheme 1** Synthesis of the pyridone derivatives **5**, **6**

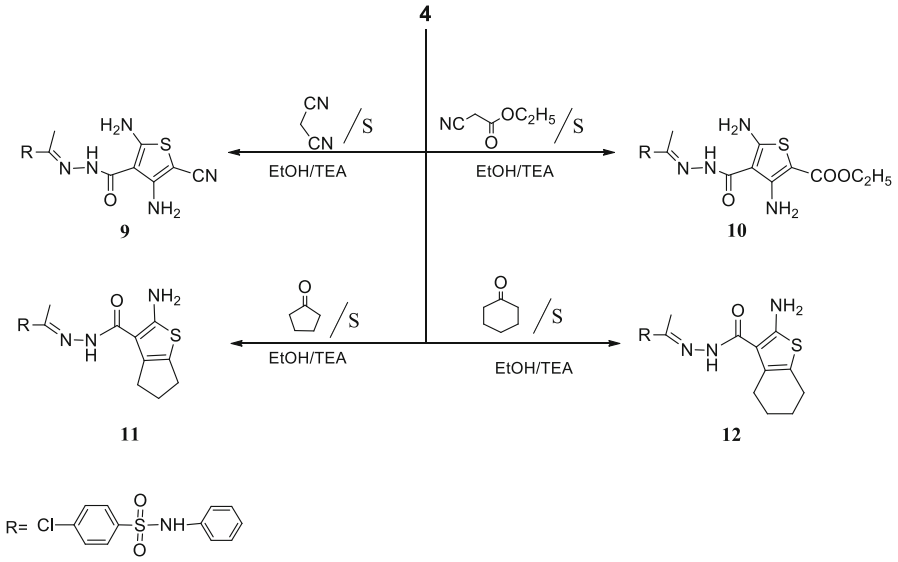
derivatives **8a–j.** Another pathway for the preparation of **8a–j** was carried out through the reaction of **4** with the corresponding arylidene malononitrile in ethanol containing piperidine (Scheme 2). $^{1}$H-NMR of **7a–j** displayed a downfield shifted singlet corresponding to the CH and the disappearance of the $CH_2$ peak of **4**. While, $^{13}$C-NMR exhibited a new signal corresponding to the CH. IR of **8a–j** revealed bands for the $NH_2$ and (2CN) groups at their characteristic regions. $^{1}$H-NMR displayed a downfield shifted singlet attributed to the $NH_2$ protons and the disappearance of $CH_2$ and NH peaks of **4**.

Alternatively, malononitrile and/or ethyl cyanoacetate were reacted with **4** in the presence of elemental sulfur and in absolute ethanol containing triethylamine to yield the thiophene derivatives **9** and **10** (Scheme 3). This reaction goes in parallel to Gewald's thiophene synthesis [34]. IR of **9** and **10** revealed bands for the $NH_2$ groups. $^{1}$H-NMR of **9** displayed two downfield shifted singlets appearing at 7.79 and 10.95 ppm, for the two $NH_2$ protons. $^{13}$C-NMR exhibited new signals at 115.3 ppm for (C-thiophene), 132.2 ppm for (C–$NH_2$) and 166.2 ppm for (S–C–$NH_2$). IR of **10** revealed the band at 1742 $cm^{-1}$ for the (CO) group. $^{1}$H-NMR




**Scheme 2** Synthesis of the hydrazone **7a–j** and pyridone **8a–j** derivatives

displayed up-field triplet at 1.03 ppm for the $CH_3$, quartet at 4.17 ppm for the $CH_2$ and two singlets at 7.79 and 10.95 ppm for the two $NH_2$ protons. $^{13}$C-NMR exhibited two up-field signals at 20.1, 60.3 ppm for the $CH_3$ and $CH_2$ ester,




**Scheme 3** Synthesis of the thiophene and fused thiophene derivatives **9–12**

respectively. Also, new signals at 119.7, 126.0, 132.2, and 163.5 corresponding to (C-thiophene), (C–CO ester), (C–$NH_2$), and (CO ester), respectively. The reaction of **4** with cyclopentanone and cyclohexanone together with elemental sulfur yielded the corresponding *N*-(4-(1-(2-(2-amino-5,6-dihydro-*4H*-cyclopenta*[b]*thiophene-3-carbonyl)hydrazono)ethyl)phenyl)-4-chlorobenzenesulfonamide **11** and *N*-(4-(1-(2-(2-amino-4,5,6,7-tetrahydrobenzo*[b]*thiophene-3-carbonyl)hydrazono)ethyl)phenyl)-4-chlorobenzenesulfonamide **12**, respectively. The reaction proceeded according to the reported method [35] (Scheme 3).

## In vitro anticancer evaluation against human tumor liver cancer (HepG2)

A closure look to the in vitro cytotoxic results of the target compounds in Table 1, it was found that the cyanoacetohydrazone derivative **4** showed the most potent activity ($IC_{50}$ 8 μM). The formation of the 3-cyanopyridone derivatives **5** and **6** provided less active derivatives compared to doxorubicin ($IC_{50}$ 41.2, 50 μM), respectively. Where **5** is more active than its isoster **6**.

The 3-aryl acryloyl hydrazono derivatives **7a–j** also provided either less potent or nearly equipotent derivatives compared to the reference drug, ($IC_{50}$ 36.2–45 μM). Compounds **7a** and **7i** showed no activity, compound **7f** with 3-(4-methoxyphenyl) substitution is the most potent derivative in this series ($IC_{50}$ 36.2 μM).

Concerning the 3,5-dicyanopyridone derivatives **8a–j**, it was found that the 4-aryl substituent greatly affected their activity. Compounds **8d–j** ($IC_{50}$ 16.4, 18.2, 18.4, 26.3, 13.3, 19.0, and 19.7 μM, respectively) showed better activity compared to doxorubicin. The most active derivative is **8h** with a 4-chlorophenyl substituent ($IC_{50}$ 13.3 μM), followed by **8d** with a 4-styrylbenzene substituent ($IC_{50}$ 16.4 μM).

The formation of the thiophene **9**, **10** and the fused thiophene derivatives **11**, **12** resulted in either equipotent or less active derivatives compared to doxorubicin ($IC_{50}$ 33.3–50 μM). The thiophene-3-carboxylate derivative **10** is the most active in this series and nearly equipotent to doxorubicin ($IC_{50}$ 33.3 μM).

## Radiosensitizing evaluation

The radiosensitizing ability of the most active compounds **4**, **8d**, and **8h** was studied. From the results obtained in Table 2, compound **4** showed the $IC_{50}$ value of 8 μM, when the cells were subjected to the compound alone. But, when the cells were subjected to the same concentrations of compound **4** and irradiated with a single dose of 8 Gy γ-radiation, the $IC_{50}$ value was significantly decreased to 6.5 μM. Similarly, compound **8d** showed the $IC_{50}$ value of 16.4 μM before irradiation, that was converted to 10.7 μM after irradiation, and compound **8h** showed the $IC_{50}$ value of 13.3 μM before irradiation, that was decreased to 9.1 μM, after irradiation. The results proved the ability of the synthesized compounds to sensitize cancer cells to the destructive effect of ionizing radiation in order to decrease the dose of the drug and its toxicity as well. The change in $IC_{50}$ (μM) for compounds **4**, **8d,** and **8h** against HepG2 after irradiation is illustrated in Table 2.

## Molecular modeling and docking study

To provide a rationale for the cytotoxic activity of the newly synthesized compounds, a molecular modeling study was carried out on CA II/IX mimic receptor (PDB ID: 4BCW) [36]. The first real CA IX X-ray structure was reported by Alterio et al. (PDB ID: 3IAI) [37], followed by CA II/IX mimic receptor (PDB ID: 4BCW), then another three structures (PDB IDs: 5FL4, 5FL5, and 5FL6) [38]. CA II/IX mimic receptor composed of a dimer, each monomer of which consists of 10-stranded antiparallel sheets forming the core of the molecule [36, 39]. The binding pocket contains a Zn(II) ion, which is crucial for activity, in coordination with His 94, 96, 119, and a water molecule. The essential amino acids in the binding pocket are the proton alternate residues involved in the binding with inhibitors [36]. The active site contains both hydrophobic and hydrophilic regions, at which the different substitutions of the sulfonamide derivative bind to.

Initially, docking validation was performed to ensure the ability of the docking protocol to recognize the active site and reproduce the docking results. A conformational search using an implicit solvent model was achieved for the prepared compounds, followed by geometry refinement of the local minima through a quantum–mechanical (QM) method. Then, docking of the newly synthesized compounds was performed in the CA II/IX mimic active site co-crystallized with a sulfonamide native ligand (*E*)-2-(5-bromo-2-hydroxyphenyl) ethane sulfonic acid, obtained from the Protein DataBank (PDB ID: 4BCW) [36].

Docking of all the synthesized compounds was performed and the results are shown in Table 3. The poses obtained for the new sulfonamide derivatives were found to bind in a co-crystallized ligand-like manner (Figs. 2, 3, 4). herein the findings obtained for the three most active compounds in this study. 3D ligand

**Table 3** Binding interactions and energy scores of all the synthesized compounds inside CA II/IX mimic active site

| Compound | Amino acids | Interacting groups | Length (Å) | Energy score (*S*) (kcal/mol) |
|---|---|---|---|---|
| **Ligand** | Zn | $SO_2$ | 2.12 | −14.04 |
| | Thr 199 | $SO_2$ | 2.71 | |
| **4** | Zn | $SO_2$ | 2.08 | −10.92 |
| | His 94 | $SO_2$ | 3.08 | |
| | His 96 | $SO_2$ | 1.51 | |
| | His 119 | $SO_2$ | 2.70 | |
| | Thr 199 | $SO_2$ | 2.79 | |
| **5** | Zn | $SO_2$ | 1.38 | −9.88 |
| | His 64 | NH | 1.90 | |
| | His 94 | C=N | 3.02 | |
| | His 96 | NH | 2.03 | |
| | His 119 | $SO_2$ | 2.21 | |
| | Thr 199 | $SO_2$ | 2.36 | |
| **6** | Zn | $SO_2$ | 2.53 | −9.12 |
| | His 94 | $SO_2$ | 2.28 | |
| | His 119 | $SO_2$ | 2.51 | |
| | Thr 199 | $SO_2$ | 1.88 | |
| **7a** | Zn | $SO_2$ | 2.22 | −9.01 |
| | His 96 | NH | 2.13 | |
| | His 119 | $SO_2$ | 3.01 | |
| | Thr 199 | $SO_2$ | 2.59 | |
| | Thr 199 | $SO_2$ | 2.52 | |
| **7b** | Zn | $SO_2$ | 2.31 | −8.98 |
| | His 94 | $SO_2$ | 3.10 | |
| | His 96 | $SO_2$ | 1.78 | |
| | His 119 | $SO_2$ | 2.72 | |
| | Thr 199 | $SO_2$ | 2.70 | |
| **7c** | Zn | $SO_2$ | 3.02 | −9.13 |
| | His 96 | NH | 1.73 | |
| | His 119 | $SO_2$ | 2.01 | |
| | Thr 199 | $SO_2$ | 2.54 | |
| **7d** | Zn | $SO_2$ | 1.97 | −9.68 |
| | His 94 | C=N | 2.94 | |
| | His 96 | NH | 2.34 | |
| | His 119 | $SO_2$ | 2.16 | |
| | Thr 199 | $SO_2$ | 2.33 | |
| **7e** | Zn | $SO_2$ | 2.52 | −9.41 |
| | His 94 | $SO_2$ | 3.00 | |
| | His 96 | $SO_2$ | 1.98 | |
| | His 119 | $SO_2$ | 2.12 | |

**Table 3** continued

| Compound | Amino acids | Interacting groups | Length (Å) | Energy score (*S*) (kcal/mol) |
|---|---|---|---|---|
| | Thr 199 | $SO_2$ | 2.79 | |
| **7f** | Zn | $SO_2$ | 2.42 | −8.49 |
| | His 96 | NH | 2.78 | |
| | Thr 199 | $SO_2$ | 2.59 | |
| **7g** | Zn | $SO_2$ | 1.99 | −8.99 |
| | His 96 | NH | 2.54 | |
| | Thr 199 | $SO_2$ | 2.48 | |
| **7h** | Zn | $SO_2$ | 2.79 | −8.88 |
| | His 96 | NH | 1.63 | |
| | Thr 199 | $SO_2$ | 3.05 | |
| **7i** | Zn | $SO_2$ | 3.00 | −9.11 |
| | His 94 | C=N | 2.64 | |
| | His 96 | NH | 2.74 | |
| | His 119 | $SO_2$ | 2.36 | |
| | Thr 199 | $SO_2$ | 2.83 | |
| **7j** | Zn | $SO_2$ | 2.38 | −9.21 |
| | His 94 | $SO_2$ | 2.16 | |
| | His 96 | $SO_2$ | 2.58 | |
| | His 119 | $SO_2$ | 1.76 | |
| | Thr 199 | $SO_2$ | 3.04 | |
| **8a** | Zn | $SO_2$ | 2.58 | −8.41 |
| | His 94 | $SO_2$ | 2.04 | |
| | His 96 | $SO_2$ | 1.76 | |
| | Thr 199 | $SO_2$ | 3.06 | |
| **8b** | Zn | $SO_2$ | 2.89 | −7.99 |
| | Thr 199 | $SO_2$ | 3.01 | |
| **8c** | Zn | $SO_2$ | 2.50 | −8.95 |
| | His 96 | NH | 2.64 | |
| | His 119 | $SO_2$ | 2.38 | |
| | Thr 199 | $SO_2$ | 2.11 | |
| **8d** | Zn | $SO_2$ | 2.38 | −9.98 |
| | Gln 67 | $NH_2$ | 1.50 | |
| | His 64 | NH | 1.77 | |
| | His 94 | C=N | 3.00 | |
| | His 96 | NH | 2.23 | |
| | His 119 | $SO_2$ | 2.71 | |
| | Thr 199 | $SO_2$ | 2.65 | |
| | Thr 199 | $SO_2$ | 2.16 | |
| **8e** | Zn | $SO_2$ | 1.96 | −9.18 |
| | Gln 67 | $NH_2$ | 2.37 | |

**Table 3** continued

| Compound | Amino acids | Interacting groups | Length (Å) | Energy score (*S*) (kcal/mol) |
|---|---|---|---|---|
| | His 96 | $SO_2$ | 1.65 | |
| | Thr 199 | $SO_2$ | 2.12 | |
| **8f** | Zn | $SO_2$ | 2.12 | −9.21 |
| | Gln 67 | $NH_2$ | 2.93 | |
| | His 96 | $SO_2$ | 2.66 | |
| | Thr 199 | $SO_2$ | 2.11 | |
| **8g** | Zn | $SO_2$ | 3.03 | −8.62 |
| | His 94 | $SO_2$ | 2.81 | |
| | His 96 | $SO_2$ | 2.41 | |
| | His 119 | $SO_2$ | 2.75 | |
| | Thr 199 | $SO_2$ | 1.94 | |
| **8h** | Zn | $SO_2$ | 3.01 | −9.22 |
| | His 94 | $SO_2$ | 2.18 | |
| | His 96 | $SO_2$ | 2.47 | |
| | His 119 | $SO_2$ | 2.15 | |
| | Thr 199 | $SO_2$ | 1.69 | |
| **8i** | Zn | $SO_2$ | 3.01 | −8.42 |
| | Gln 67 | $NH_2$ | 2.18 | |
| | His 94 | $SO_2$ | 2.43 | |
| | His 96 | $SO_2$ | 2.14 | |
| | His 119 | $SO_2$ | 2.47 | |
| | Thr 199 | $SO_2$ | 1.62 | |
| **8j** | Zn | $SO_2$ | 2.53 | −9.42 |
| | Gln 67 | $NH_2$ | 2.31 | |
| | His 96 | $SO_2$ | 3.04 | |
| | His 119 | $SO_2$ | 1.77 | |
| | Thr 199 | $SO_2$ | 2.67 | |
| **9** | Zn | $SO_2$ | 1.81 | −7.98 |
| | Gln 67 | $NH_2$ | 2.56 | |
| | His 96 | $SO_2$ | 1.84 | |
| | Thr 199 | $SO_2$ | 2.32 | |
| **10** | Zn | $SO_2$ | 2.48 | −8.35 |
| | His 96 | $SO_2$ | 2.39 | |
| | Thr 199 | $SO_2$ | 2.08 | |
| **11** | Zn | $SO_2$ | 2.82 | −8.97 |
| | Thr 199 | $SO_2$ | 1.76 | |
| **12** | Zn | $SO_2$ | 2.61 | −9.10 |
| | His 96 | NH | 2.57 | |
| | His 119 | $SO_2$ | 2.72 | |
| | Thr 199 | $SO_2$ | 2.27 | |

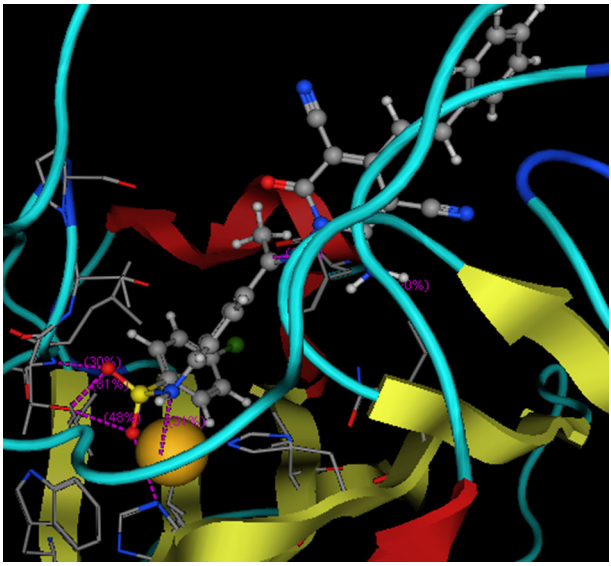


**Fig. 2** 3D docking of **8d** ($S = -9.98$ kcal mol$^{-1}$) in the active site of 4BCW

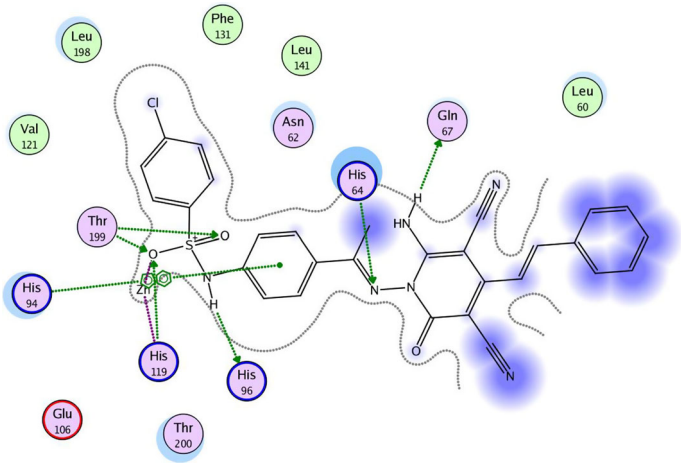



**Fig. 3** 2D ligand interaction of **8d** with the active site amino acids of 4BCW

interactions of compound **8d** (Fig. 2) showed that the compound binds in the same manner as the co-crystallized ligand with a binding score of −9.98 kcal mol$^{-1}$. While, the 2D ligand interactions (Fig. 3) demonstrated that the **8d** binds with the amino acids Thr199, His119, His96, His64, and Gln67, as well as the Zn atom through a network of hydrogen bonds, with an average bond length between 1.77 and 2.71 Å. Simultaneously, the 2D ligand interactions of **8h** (Fig. 4) showed that it binds in the same fashion to the native ligand displaying a set of hydrogen bonds

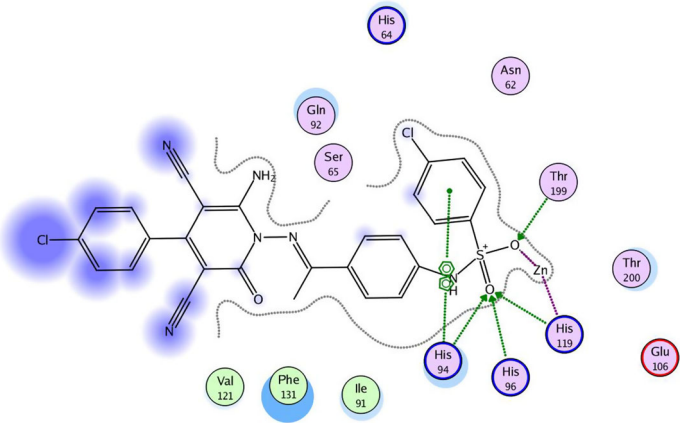



**Fig. 4** 2D ligand interaction of **8h** with the active site amino acids of 4BCW

with the amino acids Thr199, His119, His96, and His94, as well as Zn interactions and binding energy of $-9.22$ kcal mol$^{-1}$ (Table 3).

These remarkable interactions between CA II/IX mimic active site and the new derivatives might explain their cytotoxic activity. Further investigations to explore more the possible mechanism of action of these derivatives are underway.

## Conclusion

In summary, a novel series of sulfonamide derivatives containing pyridone, hydrazone, thiophene, and fused thiophene moieties were synthesized. These compounds were designed and synthesized as potential carbonic anhydrase inhibitors (CAIs) and were evaluated for their anticancer activity against human liver carcinoma cell line (HepG2). Compounds **4**, **8d–j** were found to be more potent than doxorubicin. From the results, we can conclude that the introduction of cyanoacetohydrazone moiety is associated with enhanced anticancer activity and gave the most potent derivative in this study **4** ($IC_{50} = 8.0$ μM). The combination of a sulfonamide with 3,5-dicyanopyridone moiety having different 4-aryl substitution as in compounds **8d** and **8h** gave less potent derivatives than **4**, but still more potent than doxorubicin ($IC_{50} = 16.4$ and 13.3 μM). Moreover, the most active compounds **4**, **8d** and **8h** showed an enhanced radio-sensitizing activity when evaluated for their in vitro anticancer activity in combination with γ-radiation. Also, the molecular modeling showed that all the docked compounds exhibited similar and additional binding interactions as those previously reported by the co-crystallized ligand when docked into CA II/IX mimic active site, which suggests that these compounds may possibly act as CA inhibitors and this may contribute to their anticancer activity.

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