5-((Thiophen-2-yl)-1,3,4-thiadiazole derivatives: synthesis, molecular docking and in vitro cytotoxicity evaluation as potential anticancer agents

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Background: Nowadays, cancer is an important public health problem in all countries. Limitations of current chemotherapy for neoplastic diseases such as severe adverse reactions and tumor resistance to the chemotherapeutic drugs have been led to a temptation for focusing on the discovery and development of new compounds with potential anticancer activity. The importance of thiophene and thiadiazole rings as scaffolds present in a wide range of therapeutic agents has been well reported and has driven the synthesis of a large number of novel antitumor agents.

Methods: A series of new 1,3,4-thiadiazoles were synthesized by heterocyclization of N-(4-nitrophenyl)thiophene-2-carboxyhydrazonoyl chloride with a variety of hydrazine-carbodithioate derivatives. The mechanisms of these reactions were discussed and the structure of the new products was elucidated via spectral data and elemental analysis. All the new synthesized compounds were investigated for in vitro activities against human hepatocellular carcinoma (HepG-2) and human lung cancer (A-549) cell lines compared with cisplatin standard anticancer drug. Moreover, molecular docking using MOE 2014.09 software was also carried out for the high potent compound 20b with the binding site of dihydrofolate reductase (DHFR, PDB ID (3NU0)).

Results: The results showed that compound 20b has promising activities against HepG-2 and A-549 cell lines (IC50 value of 4.37±0.7 and 8.03±0.5 μM, respectively) and the results of molecular docking supported the biological activity with total binding energy equals −1.6 E (Kcal/mol).

Conclusion: Overall, we synthesized a new series of 1,3,4-thiadiazoles as potential antitumor agents against HepG-2 and A-549 cell lines.

Keywords: hydrazonoyl chlorides, hydrazine-carbodiithioates, 1,3,4-thiadiazoles, molecular docking, anticancer activity

Introduction
Cancer is a leading cause of death worldwide. Lung and liver cancers cause the most cancer deaths each year.1 Cancer chemotherapy has been one of the major advances in the field of medicine in the last few decades. However, drugs administered for chemotherapy have a narrow therapeutic index and therefore, there is a high incidence of unwanted side effects.2,3 The development of new antitumor agents is one of the most pressing research areas in medicinal chemistry and medicine. The importance of thiophene and thiadiazole rings as scaffolds present in a wide range of therapeutic agents has been well reported and has driven the synthesis of a large number of novel antitumor agents.

Heterocyclic derivatives possessing the thiophene ring have diverse and wide range of biological activities, including analgesic, antimicrobial, anti-inflammatory, antirhinitis,
antidepressant, anticonvulsant, anticancer, and antidermatitis activities. In cancer, thiophene derivatives have been shown to exhibit cytotoxicity in several types of cancer cells such as leukemia, ovarian, glioma, renal, and lung. These studies have showed that thiophene derivatives induced cell cycle arrest and apoptosis and affected tubulin polymerization. Studies have shown that antiprotozoal compounds might have anticancer properties in vitro and animal experiments. Literature survey revealed that 1,3,4-thiadiazole derivatives have many pharmacological activities, such as antifungal, antibacterial, anti-inflammatory, analgesic, antileishmanial, anticancer, antihypertension, central nervous system (CNS) depressant, antioxidant, molluscidic, antiabetic, diuretic, antihypertensive, anticonvulsant, and antitubercular activities. Also, many drugs containing 1,3,4-thiadiazole nucleus are known, such as methazolamide, megazol, and acetazolamide. Recently, several pharmacophores containing 1,3,4-thiadiazoles have been reported with potential antitumor activity (Figure 1). Different mechanisms of action were attributed to antitumor activity of 1,3,4-thiadiazole ring, such as inhibited RNA and DNA syntheses specifically without affecting protein synthesis, phosphodiesterase-7, histone deacetylase, inhibition of carbonic anhydrase, or as adenosine A3 receptor antagonists. It has been proved that 1,3,4-thiadiazole-based compounds treat several cancers in vitro and in vivo by targeting the uncontrolled DNA replication/cell division, which is a hallmark of neoplastic diseases. Moreover, the heteroatoms of the thiadiazole can form interactions with biological targets, including key kinases that take part in tumorigenesis. These results prompted us to screen the anticancer activity of the newly prepared 1,3,4-thiadiazoles against 2 cell lines, human hepatocellular carcinoma and human lung cancer cell lines.

Encouraged by these facts and in continuation of our previous works in synthesis of bioactive heterocyclic compounds, it was planned to synthesize 1,3,4-thiadiazoles incorporating the thiophene ring using N-thiophene-2-carboxyhydrazonoyl chloride derivative as versatile building blocks, as promising antitumor agents.

**Experimental**

Nuclear magnetic resonance (NMR) spectra were measured on a Varian Mercury VX-300 NMR spectrometer operating at 300 MHz and run in deuterated dimethylsulfoxide (DMSO-$d_6$). Chemical shifts were related to that of the solvent. Infrared (IR) spectra were measured on Shimadzu FTIR 8101 PC infrared spectrophotometers in KBr discs. Melting points were measured on an electrothermal IA 9000 series digital melting point apparatus. Elemental analyses were measured by using an Elementar Vario LIII CHNS analyzer. Mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer at 70 eV.

**Synthesis of 1,3,4-thiadiazol derivatives 4, 7, 9, 11, 13, 15, and 17**

Triethylamine (0.1 g, 1 mmol) was added while stirring to a mixture of ([1,1′-biphenyl]-4,4′-diyl)bis(2-oxopropanehydrazonoyl chloride) (1) (0.390 g, 1 mmol) and the appropriate hydrazinecarbodithioates (2, 5, 8, 10, 12, 14, and 16) in ethanol (30 mL) at room temperature for 60 min. The solid was collected and crystallized from the proper solvent. The products 4, 7, 9, 11, 13, 15, and 17 prepared together with their physical constants are given below.
2-((4-Methylbenzylidene)hydrazono)-3-(4-nitrophenyl)-5-(thiophen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (4)

Orange solid, (73% yield), melting point (mp) 203°C–205°C (ethanol/dimethylformamide [EtOH/DMF]); IR (KBr) ν_max 1,596 (C=N), 2,923, 3,104 (C-H) cm⁻¹; ¹H NMR (DMSO-d₆) δ 7.23 (s, 1H, CH=S), 7.24–7.26 (m, 5H, Ar-H), 7.75 (d, 1H, Ar-H), 7.79 (d, J=7.8 Hz, 2H, Ar-H), 7.81 (d, J=7.8 Hz, 2H, Ar-H), 8.21 (d, 1H, Ar-H), 8.51 (s, 1H, CH=S); MS m/z (%) 471 (M⁺, 3), 393 (1), 315 (1), 287 (31), 233 (35), 190 (43), 167 (46), 117 (15), 111 (15), 78 (100), 63 (10), 51 (11), 34 (15). Anal. Calcd. for C₁₉H₁₅N₅S (448.48): C, 53.56; H, 2.70; N, 18.74. Found: C, 53.69; H, 2.57; N, 18.53%

2-(Cyclopentylidenehydrazono)-3-(4-nitrophenyl)-5-(thiophen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (13)

Yellow solid, (60% yield), mp 218°C–220°C; (EtOH/Dioxane); IR (KBr) ν_max 1,588 (C=N), 2,956 (C-H), 3,442 (NH) cm⁻¹; ¹H NMR (DMSO-d₆) δ 7.18–7.20 (m, 4H, 2CH₂), 7.28–7.29 (m, 4H, 2CH₂), 7.47 (t, 1H, Ar-H), 7.50 (d, 1H, Ar-H), 7.69 (d, 1H, Ar-H), 8.18 (d, 1H, Ar-H), 8.40–8.42 (m, 4H, Ar-H); MS m/z (%) 439 (M⁺, 1), 310 (2), 281 (14), 252 (23), 192 (41), 180 (7), 165 (7), 152 (18), 135 (11), 118 (17), 103 (41), 90 (21), 77 (100), 63 (15), 51 (25). Anal. Calcd. for C₁₉H₁₅N₅S (385.46): C, 52.97; H, 3.92; N, 18.17. Found: C, 53.73; H, 3.76; N, 17.19%

2-(Cycloheptylidenehydrazono)-3-(4-nitrophenyl)-5-(thiophen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (15)

Yellow solid, (70% yield), mp 200°C–203°C; (EtOH/DMF); IR (KBr) ν_max 1,594 (C=N), 2,917, 3,103 (C-H) cm⁻¹; ¹H NMR (DMSO-d₆) δ 7.21 (d, 1H, Ar-H), 7.45 (d, 1H, Ar-H), 7.88–8.32 (m, 4H, Ar-H), 8.44 (d, J=9 Hz, 2H, Ar-H), 8.69 (d, J=9 Hz, 2H, Ar-H), 10.86 (s, br, 1H, NH); MS m/z (%) 448 (M⁺, 2), 333 (3), 239 (5), 186 (4), 134 (6), 119 (10), 106 (75), 91 (39), 78 (100), 63 (10), 51 (11), 43 (15). Anal. Calcd. for C₂₀H₁₂N₇O₃S (448.48): C, 53.56; H, 2.70; N, 18.74. Found: C, 53.69; H, 2.57; N, 18.53%

3-((3-(4-Nitrophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2(3H)-yldene)hydrazono) indolin-2-one (11)

Yellow solid, (77% yield), mp 180°C–182°C; (EtOH/DMF); IR (KBr) ν_max 1,602 (C=C), 1,715 (C=O), 2,977, 3,084 (C-H), 3,423 (NH) cm⁻¹; ¹H NMR (DMSO-d₆) δ 6.64 (t, 1H, Ar-H), 7.21 (d, 1H, Ar-H), 7.45 (d, 1H, Ar-H), 7.88–8.32 (m, 4H, Ar-H), 8.44 (d, J=9 Hz, 2H, Ar-H), 8.69 (d, J=9 Hz, 2H, Ar-H), 10.86 (s, br, 1H, NH); MS m/z (%) 448 (M⁺, 2), 333 (3), 239 (5), 186 (4), 134 (6), 119 (10), 106 (75), 91 (39), 78 (100), 63 (10), 51 (11), 43 (15). Anal. Calcd. for C₂₀H₁₂N₇O₃S (448.48): C, 53.56; H, 2.70; N, 18.74. Found: C, 53.69; H, 2.57; N, 18.53%.
General procedure for the synthesis of 1,3,4-thiadiazoles 20a–c and 23a–c
A mixture of hydrazonoyl chloride 1 (0.28 g, 1 mmol) and thiosemicarbazone 18 (or 21) (1 mmol) in 20 mL dioxane containing triethylamine (TEA) (0.1 g, 1 mmol) was refluxed until all the starting materials were consumed (4–8 h, as monitored by thin layer chromatography [TLC]). The solid, which was formed after cooling, was filtered off, washed with ethanol, dried, and recrystallized from the suitable solvent to give 1,3,4-thiadiazoles 20a–c and 23a–c. The products 20a–c and 23a–c and their physical constants are listed below.

2-(Benzyldienehydrazono)-3-(4-nitrophenyl)-5-(thiophen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (20a)
Yellow solid, (71% yield), mp 170°C–172°C (EtOH); IR (KBr) ν_max 1,595 (C=N), 2,916, 3,100 (C-H) cm⁻¹; ¹H NMR (DMSO-d₆) δ 7.05 (t, 1H, Ar-H), 7.21 (d, 1H, Ar-H), 7.35 (m, 10H, Ar-H), 8.57 (s, 1H, CH=N); MS m/z (%) 435 (M⁺, 2), 348 (36), 234 (8), 217 (9), 197 (7), 172 (72), 133 (38), 119 (13), 95 (19), 77 (44), 63 (48), 53 (47), 40 (100). Anal. Calcd. for C_{20}H_{15}N_2O_S_2 (407.47): C, 54.91; H, 3.46; N, 16.01. Found: C, 56.12; H, 3.14; N, 16.97%.

2-(Benzyldienehydrazono)-3-(4-nitrophenyl)-5-(thiophen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (20b)
Yellow solid, (69% yield), mp 188°C–190°C (dioxane); IR (KBr) ν_max IR (KBr) ν_max 1,607 (C=N), 2,917, 3,099 (C-H) cm⁻¹; ¹H NMR (DMSO-d₆) δ 3.80 (s, 3H, CH₂); MS m/z (%) 421 (M⁺, 100), 392 (10), 351 (6), 317 (11), 225 (12), 197 (11), 162 (25), 135 (25), 122 (100), 108 (25), 95 (35), 78 (13), 56 (6). Anal. Calcd. for C_{20}H_{15}N_2O_S_2 (417.47): C, 56.12; H, 3.14; N, 16.97%.

2-(3-Chlorobenzylidene)hydrazono)-3-(4-nitrophenyl)-5-(thiophen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (20c)
Yellow solid, (73% yield), mp 190°C–192°C (EtOH/DMF); IR (KBr) ν_max 1,593 (C=N), 2,965, 3,022 (C-H) cm⁻¹; ¹H NMR (DMSO-d₆) δ 7.05–8.42 (m, 11H, Ar-H), 8.48 (s, 1H, CH=N); MS m/z (%) 443 (M⁺, 1), 441 (M⁺, 3), 364 (1), 219 (3), 161 (6), 133 (4), 111 (8), 97 (11), 83 (25), 78 (10), 71 (55), 69 (28), 57 (100), 45 (21). Anal. Calcd. for C_{19}H_{16}ClN_2O_S_2 (441.91): C, 51.64; H, 2.74; N, 15.85. Found: C, 51.69; H, 2.53; N, 15.72%.

3-(4-Nitrophenyl)-2-((1-phenylethylidene)hydrazono)-5-(thiophen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (23a)
Yellow solid, (76% yield), mp 213°C–215°C (EtOH/DMF); IR (KBr) ν_max 1,598 (C=N), 2,916, 3,057 (C-H) cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.30 (s, 3H, CH₃), 6.92–8.39 (m, 12H, Ar-H); MS m/z (%) 421 (M⁺, 2), 342 (60), 262 (6), 215 (58), 188 (100), 145 (97), 132 (52), 123 (18), 92 (73), 78 (26), 64 (12). Anal. Calcd. for C_{15}H_{13}N_2O_S (421.50): C, 56.99; H, 3.59; N, 16.62. Found: C, 57.82; H, 3.50; N, 16.41%.

3-(4-Nitrophenyl)-5-((1-(p-tolyl)ethylidene)hydrazono)-2,3-dihydro-1,3,4-thiadiazole (23b)
Yellow solid, (75% yield), mp 180°C–182°C (DMF); IR (KBr) ν_max 1,612 (C=N), 3,071 (C-H) cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.34 (s, 3H, CH₃), 2.46 (s, 3H, CH₃), 7.12–8.40 (m, 11H, Ar-H); MS m/z (%) 435 (M⁺, 2), 412 (38), 348 (41), 304 (14), 262 (21), 216 (73), 172 (60), 168 (86), 139 (53), 113 (23), 90 (62), 81 (56), 68 (47), 43 (100). Anal. Calcd. for C_{30}H_{21}N_2O_S_2 (435.52): C, 57.91; H, 3.39; N, 16.08. Found: C, 58.73; H, 3.14; N, 15.90%.

2-((1-(4-Methoxyphenyl)ethylidene)hydrazono)-3-(4-nitrophenyl)-5-(thiophen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (23c)
Yellow solid, (73% yield), mp 180°C–182°C (EtOH/DMF); IR (KBr) ν_max 1,602 (C=N), 2,911, 3,087 (C-H) cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.27 (s, 3H, CH₃), 3.80 (s, 3H, OCH₃), 6.93–8.42 (m, 11H, Ar-H); MS m/z (%) 451 (M⁺, 37), 434 (9), 392 (10), 351 (6), 317 (11), 225 (12), 197 (11), 162 (25), 135 (25), 122 (100), 108 (25), 95 (35), 78 (13), 56 (6). Anal. Calcd. for C_{30}H_{21}N_2O_S_2 (451.52): C, 55.66; H, 3.79; N, 15.51. Found: C, 56.23; H, 3.35; N, 15.32%.

Anticancer activity
Evaluation of cytotoxic effects of certain chemical compound
Mammalian cell lines
A-549 cells (human lung cancer cell line), HepG-2 cells (human hepatocellular carcinoma) were obtained from VACSER A Tissue Culture Unit.

Chemicals used
Dimethyl sulfoxide (DMSO), crystal violet, and trypan blue dye were purchased from Sigma (St Louis, MO, USA).
Fetal bovine serum, DMEM, Roswell Park Memorial Institute-1640, HEPES buffer solution, L-glutamine, gentamicin, and 0.25% Trypsin-EDTA were purchased from Lonza (Lonza Group, Basel, Switzerland).

Crystal violet stain (1%)
It was composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with double-distilled water (ddH₂O) and filtered through a Whatmann No. 1 filter paper.

Cell line propagation
The cells were propagated in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, and 50 μg/mL gentamicin (Pfizer, New York, NY, USA). All cells were maintained at 37°C in a humidified atmosphere with 5% CO₂ and subcultured 2 times a week.

Cytotoxicity evaluation using viability assay
For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10⁴ cells per well in 100 μL of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial 2-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO₂ for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for 48 h at 37°C, various concentrations of sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method.

In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 min. The stain was removed and the plates were rinsed using tap water until all excess stain was removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates was measured after gently shaking on Microplate reader (TECAN, Inc., Morrisville, NC, USA), using a test wavelength of 490 nm. All results were

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Scheme 1  Synthesis of 1,3,4-thiadiazole derivatives 4 and 7.
Abbreviations: EtOH, ethanol; TEA, triethylamine.
Scheme 2 Synthesis of 1,3,4-thiadiazole derivatives 7a,b.
Abbreviations: EtOH, ethanol; TEA, triethylamine.

Scheme 3 Synthesis of 1,3,4-thiadiazole derivatives 13, 15, and 17.
Abbreviations: EtOH, ethanol; TEA, triethylamine.

Scheme 4 Synthesis of 1,3,4-thiadiazole derivatives 20a–c.
Abbreviation: TEA, triethylamine.
corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated. The optical density was measured with the microplate reader (Sunrise; TECAN, Inc.) to determine the number of viable cells and the percentage of viability was calculated as \[1 \pm \frac{(\text{OD}_t - \text{OD}_c)}{\text{OD}_c}\times100\%\] where \(\text{OD}_t\) is the mean optical density of wells treated with the tested sample and \(\text{OD}_c\) is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (\(\text{IC}_{50}\)), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose–response curve for each concentration using Graphpad Prism software (GraphPad Software, San Diego, CA, USA).

Molecular modeling

The docking study was performed using the MOE 2014.010 software. The crystal structure of the enzyme dihydrofolate reductase (DHFR, Protein Data Bank Identifier [PDB ID: 3NU0]) was downloaded out from Protein Data Bank website. Regularization and optimization for protein and ligand were performed. A compound score was assigned according to its fit in the binding pocket of ligand and its mode of binding. The performance of the docking method was evaluated by re-docking the crystal ligand into the assigned active DHFR enzyme to determine a root-mean-square deviation value.

Results and discussion

Chemistry

N-Thiophene-2-carbohydrazonoyl chloride derivative 1 was reacted with methyl 2-benzylidenehydrazine-1-carbodithioate (2) and methyl 2-((1,3-diphenyl-1H-pyrazol-4-yl)

![Scheme 5 Synthesis of 1,3,4-thiadiazole derivatives 23a–c. Abbreviation: TEA, triethylamine.](image)

| Tested compounds | Tumor cell lines | Tumor cell lines |
|------------------|------------------|------------------|
|                  | A-549            | HepG-2           |                  | A-549            | HepG-2           |
| 4                | 132.9±7.4        | 281±5.8          | 20a              | 11.6±0.6         | 86.6±2.9         |
| 7                | 30.6±2.8         | 35.9±3.1         | 20b              | 4.37±0.7         | 8.03±0.5         |
| 9                | 122.6±8.1        | 123.8±9.7        | 20c              | 80.4±10.8        | 91.7±9.1         |
| 11               | 23.2±0.9         | 22.4±1.8         | 23a              | 32.1±1.4         | 72.6±8.1         |
| 13               | 68.5±6.7         | 70.1±10.2        | 23b              | 108.3±7.5        | 316.7±19.8       |
| 15               | 158.9±12.1       | 175.7±9.8        | 23c              | 146.2±11.8       | 137.2±7.9        |
| 17               | 48.1±5.6         | 68.9±8.2         | Cisplatin        | 0.95±0.9         | 1.40±1.1         |

Note: The in vitro inhibitory activity of compounds 4, 7, 9, 11, 13, 15, 17, 20a–c, and 23a–c against tumor cell lines are expressed as mean ± SD.
methylene)hydrazine-1-carbothioate (5) afforded 2-((4-methylbenzylidene) hydrazono)-3-(4-nitrophenyl)-5-(thiopen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (4) and 2-(((1,3-diphenyl-1H-pyrazol-4-yilmethylene)hydrazono)-3-(4-nitrophenyl)-5-(thiopen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (7) respectively. The structures of 4 and 7 were confirmed by their elemental analyses and spectral data (MS, IR, and 1H-NMR). For example, 1H NMR spectrum of compound 4 revealed 2 singlet signals at δ 2.36 and 8.53 ppm assignable to methyl (CH3) and methine (CH=N) protons, respectively, in addition to the 11 aromatic protons. 13C-NMR spectrum revealed 1 signal at δ21.0 ppm assignable to methyl carbon, in addition to the signals of 15 aromatic carbons. The formation of the products 4 and 7 takes place via reaction of hydrazonoyl chloride with an equivalent amount of each of compound 2 and 5 by loss of 1 mole of hydrogen chloride to form thiobenzotriazolate derivatives 3 and 6, which cyclizes to give the final products of thiadiazoles 4 and 7 via elimination of 1 mole of thiomethanol (Scheme 1).

In a similar manner, compound 1 was reacted with methyl 2-benzylidenehydrazine-1-carbothioate (8) and methyl 2-(2-oxoindolin-3-ylidene)hydrazine-1-carbothioate (10) afforded 2-((1-(naphthalen-2-yl)ethylidene) hydrazono)-3-(4-nitrophenyl)-5-(thiopen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (9) and 3-((3-(4-nitrophenyl)-5-(thiopen-2-yl)-1,3,4-thiadiazol-2(3H)-ylidene)hydrazono)indolin-2-one (11) as shown in Scheme 2. The structures of 9 and 11 were confirmed by their spectral data (MS, IR, and 1H-NMR) and elemental analyses. The 1H NMR spectrum of compound 11 revealed characteristic signals at the expected chemical shifts related to the aromatic protons in addition to broad singlet signal at δ=10.86 ppm assignable to the NH. Its IR spectrum revealed a band at νmax =3,423 cm⁻¹ (NH). The mass spectra of compounds 9 and 11 gave molecular ion peaks at the exact m/z values (discussed in the “Experimental” section).

Also, methyl cyclocarbodithioates 12, 14, and 16 were reacted with hydrazonoyl chloride 1 in ethanolic triethylamine afforded corresponding 1,3,4-thiadiazole derivatives 21–23 (Scheme 3).

Our work was extended to investigate the reactivity of the hydrazonoyl halide 1 toward the thiosemicarbazone derivatives derived from aldehydes 18a–c to prepare another series of 1,3,4-thiadiazoles, thus, reaction of compound 1 with aryldienethiosemicarbazones 18a–c, yielded the respective 1,3,4-thiadiazoles 20a–c (Scheme 4). The structures of products 20a–c were confirmed by their elemental analyses and spectral data. The formation of 20a–c takes place via reaction of hydrazonoyl chloride 1 with thiosemicarbazones by loss of HCl to form of thiohydrazonate, which cyclizes to give the thiadiazole 20a–c via elimination of NH₃.

Similarly, compound 1 was reacted with thiosemicarbazone derivatives, which were derived from aromatic ketones 21a–c, afforded the 1,3,4-thiadiazoles 23a–c (Scheme 5). The structure of products 23a–c was confirmed by their spectral data and elemental analyses.

### Antitumor activity

The antitumor activity of the products 4, 7, 9, 11, 13, 15, 17, 20a–c, and 23a–c was investigated against 2 carcinoma cell lines, human hepatocellular carcinoma and human lung cancer cell lines, in comparison with cisplatin as anticancer standard drug using colorimetric MTT assay. IC₅₀ (the concentration of test compounds required to kill 50% of cell population) was determined from the dose–response curve. The activity was expressed as IC₅₀ values (µM) ± SD from 3 replicates.

![Figure 2](image-url)
In vitro inhibitory activity of compounds
The in vitro growth inhibitory activities of the compounds depend on the structural skeleton and electronic environment of the molecules and all the evaluated compounds showed activity in a concentration-dependent manner.

The descending order of activity of the synthesized compounds toward the A-549 cell line was as follows: $20b > 20a > 11 > 7 > 23a > 17 > 13 > 20c$ while the descending order of inhibitory activity of the tested compounds toward the HepG-2 was as follows: $20b > 11 > 7 > 17 > 13 > 23a > 20a > 20c$ (Table 1).

Examination of the structure activity relationship
Generally, the in vitro inhibitory activity of the tested thiadiazoles against A-549 cell lines is more than HepG-2 cell lines.

Compound $20b$ was the most active against A-549 and HepG-2 (IC$_{50}$ value of 4.37±0.7 and 8.03±0.5 μM, respectively) while IC$_{50}$ of cisplatin = 0.95±0.90 and 1.40±1.1 μM, respectively (Figure 2).

Compounds 4, 9, 15, 23b, and 23c were inactive against A-549 and HepG-2 (IC$_{50}$ value >100 μM). The other compounds moderately inhibited activity (IC$_{50}$ =12.4–91.7 μM).

For 1,3,4-thiadiazole derivatives $20a–c$ and $23a–c$, compounds $20b$ and $23b$ (with methoxy group as electron-donating group on aryl moiety) have promising antitumor activity than compounds $20c$ and $23c$ (with chlorine atom as electron-withdrawing group on aryl moiety).

Also, the selected compounds were evaluated for their cytotoxic effects on normal (Vero) cells. In general, the results showed that all the tested compounds showed high degree of selectivity in activity hence the 50% cytotoxic concentration (CC$_{50}$) required to inhibit the normal cell line (Vero) was far away from those measured against the tested tumor cell lines confirming the high activity of these compounds (Table 2).

Molecular docking
The MOE 2014.010 package software was used to analyze all binding energies and docking poses between compound $20b$ and the enzyme DHFR to evaluate the affinity of compound...
Figure 4 Three-dimensional representation showed interactions of compound 20b and the DHFR enzyme pocket amino acids.
Abbreviation: DHFR, dihydrofolate reductase.

Figure 5 Two-dimensional representation showed interactions of native inhibitor MTX and the DHFR enzyme pocket amino acids.
Abbreviations: DHFR, dihydrofolate reductase; MTX, methotrexate.
Table 3  Bioactivity and ADME toxicity

| Properties                        | Compound 20b |
|-----------------------------------|--------------|
| Molecular weight                  | 437.49 g/mol |
| No. of H-bond acceptors           | 6            |
| No. of H-bond donors              | 0            |
| No. of rotatable bonds            | 6            |
| Topological polar surface area (TPSA) | 154.07 Å²  |
| Log Kp (skin permeation)          | -5.11 cm/s   |
| Gastrointestinal (GI) absorption  | Low          |
| Pan-assay interference structure (PAINS) | 0 alert     |
| Lipinski                          | Yes; 0 violation |
| Lead likeness                     | No; 2 violations: MW >350, XLOGP3 >3.5 |
| Synthetic accessibility           | 3.89         |

Abbreviations: ADME, absorption, distribution, metabolism, and excretion; MW, molecular weight.

20b according to its binding energy with the enzyme. From Figures 3 and 4, which represent all the binding energies of this compound, it is clear that the total binding energy of compound 20b equals $-1.6 \text{ E} (\text{kcal/mol})$, showing good affinity with the DHFR enzyme by forming 4 pi-hydrogen interactions with binding energy $-1.4 \text{ E} (\text{kcal/mol})$, 1 hydrogen acceptor interaction with binding energy $-0.2 \text{ E} (\text{kcal/mol})$, and 1 pi–pi interaction with almost zero binding energy. From Figure 5, we concluded that NH of compound native making H-bond with lle (94) amino acid of the target enzyme, NH of compound native making H-bond with Asp (27) amino acid of the target enzyme, dioxygen in ring of compound native making H-bond with Leu (54), Lys (32), and Arg (57) amino acid of the target enzyme all of these bonds giving good affinity of compound 20b to the interested enzyme.

Bioactivity and ADME toxicity

A computational study on compound 20b was carried out using Swiss pdb and molinspiration web basis, including prediction of pharmacokinetic properties, toxicity, and bioactivity studies as shown in Table 3.

Conclusion

In this paper, new series of 1,3,4-thiadiazole derivatives were synthesized and investigated for their in vitro antitumor activity against human lung cancer cell lines and human hepatocellular carcinoma cell lines, and the results obtained exploring the high potency of compound 20b compared with the employed cisplatin standard (IC$_{50}$ value of 4.37±0.7 and 8.03±0.5 μM). Moreover, molecular docking using MOE 2014.09 software was also carried out for the highly potent compound 20b and the results supported the biological activity.

Author contributions

All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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