Drug Design, Development and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

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

This article was published in the following Dove Press journal:
Drug Design, Development and Therapy

Sobhi M Gomha[1]
Mastoura M Edrees[2,3]
Zeinab A Muhammad[2]
Ahmed AM El-Reedy[4]

[1]Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt; [2]Department of Organic Chemistry, National Organization for Drug Control and Research (NODCAR), Giza, Egypt; [3]Department of Chemistry, Faculty of Science, King Khalid University, Abha, Saudi Arabia; [4]Department of Basic and Applied Science, Faculty of Oral and Dental Medicine, Nahda University, Beni-Suef, Egypt

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-carbohydrazonoyl 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-carbodithioates, 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,

Correspondence: Sobhi M Gomha
Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt
Tel +20 23 740 0304
Fax +20 2 568 5799
Email s.m.gomha@gmail.com

submit your manuscript | www.dovepress.com
Dovepress
http://dx.doi.org/10.2147/DDDT.S165276

antidepressant, anticonvulsant, anticancer, and antidermatitis activities.[4–9] In cancer, thiophene derivatives have been shown to exhibit cytotoxicity in several types of cancer cells such as leukemia, ovarian, glioma, renal, and lung.[10,11] 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.[10–13] Literature survey revealed that 1,3,4-thiadiazole derivatives have many pharmacological activities, such as antifungal, antibacterial, anti-inflammatory, analgesic, antileishmanial, anticancer, antihepatitis B viral, central nervous system (CNS) depressant, antioxidant, molluscicidal, antidiabetic, diuretic, antihypertensive, anticonvulsant, and antitubercular activities.[14–23] 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).[24–34] 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,[35] phosphodiesterase-7,[36] histone deacetylase,[37] inhibition of carbonic anhydrase,[32] or as adenosine A3 receptor antagonists.[38] 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.[39–42] 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,[43–50] it was planned to synthesize 1,3,4-thiadiazoles incorporating the thiophene ring using *N*-thiophene-2-carbohydrazonoyl 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** (1 mmol) 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.

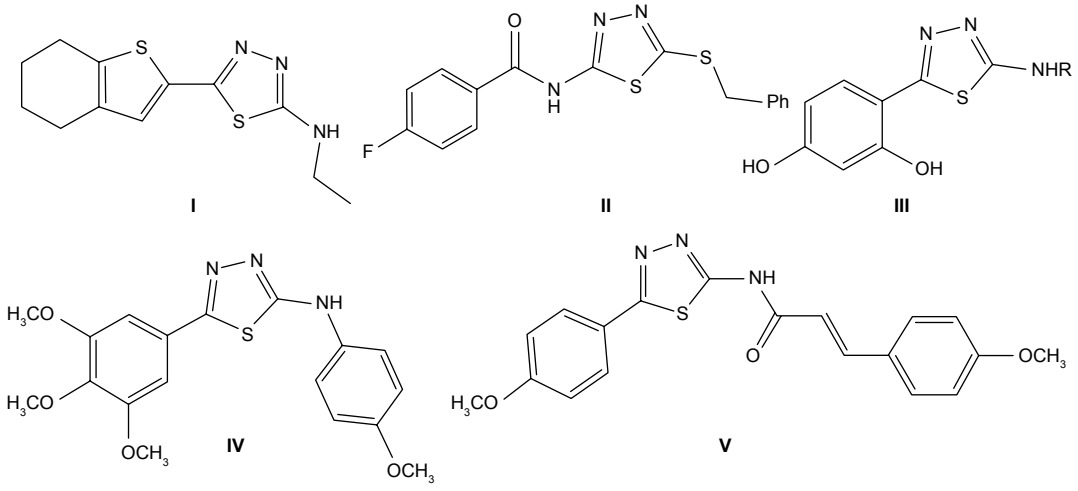


Figure 1 Anticancer activity of 1,3,4-thiadiazoles (**I–V**).

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}; ^{1}H NMR (DMSO-d_6) δ2.36 (s, 3H, CH_3), 7.22–7.30 (m, 3H, Ar-H), 7.69–7.75 (m, 3H, Ar-H), 7.88 (d, 1H, Ar-H), 8.35–8.43 (m, 4H, Ar-H), 8.53 (s, 1H, CH=N); ^{13}C-NMR (DMSO-d_6): δ21.0 (CH_3), 120.3, 120.6, 120.7, 124.6, 127.6, 128.4, 129.3, 130.7, 131.1, 131.3, 137.2, 140.7, 144.0, 146.2, 156.4 (Ar-C and C=N); MS m/z (%) 421 (M^+, 2), 286 (4), 237 (4), 193 (9), 168 (8), 139 (18), 135 (100), 113 (6), 111 (16), 92 (17), 77 (26), 59 (15). Analyzed and calculated for $C_{20}H_{15}N_5O_2S_2$ (421.50): C, 56.99; H, 3.59; N, 16.62. Found: C, 56.83; H, 3.29; N, 16.43%.

2-(((1,3-Diphenyl-1H-pyrazol-4-yl)methylene) hydrazono)-3-(4-nitrophenyl)-5-(thiophen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (7)

Yellow solid, (75% yield), mp 220°C–222°C; IR (KBr) ν_{max} 1,593 (C=N), 2,912, 3,104 (C-H) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ7.23 (t, 1H, Ar-H), 7.39 (d, 1H, Ar-H), 7.49–7.88 (m, 11H, Ar-H), 7.95 (d, *J*=7.8 Hz, 2H, Ar-H), 8.36 (d, *J*=7.8 Hz, 2H, Ar-H), 8.51 (s, 1H, CH=N), 8.95 (s, 1H, pyrazole-H5); MS m/z (%) 549 (M^+, 2), 477 (6), 296 (13), 237 (12), 184 (16), 157 (7), 118 (19), 105 (100), 93 (17), 77 (98), 65 (26), 60 (62). Anal. Calcd. for $C_{28}H_{19}N_7O_2S_2$ (549.63): C, 61.19; H, 3.48; N, 17.84. Found: C, 61.25; H, 3.35; N, 17.72%.

2-((1-(Naphthalen-2-yl)ethylidene)hydrazono)-3-(4-nitrophenyl)-5-(thiophen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (9)

Yellow solid, (70% yield), mp 193°C–195°C; (EtOH/DMF); IR (KBr) ν_{max} 1,590 (C=N), 2,920, 3,105 (C-H) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ2.67 (s, 3H, CH_3), 7.24 (t, 1H, Ar-H), 7.56 (d, 1H, Ar-H), 7.79 (d, 1H, Ar-H), 7.83–8.91 (m, 7H, Ar-H), 8.21 (d, 1H, Ar-H), 8.41 (s, 1H, Ar-H), 8.45 (d, 2H, Ar-H); MS m/z (%) 471 (M^+, 3), 298 (16), 264 (19), 236 (13), 207 (12), 168 (15), 139 (88), 118 (24), 111 (48), 105 (100), 91 (32), 77 (73), 60 (60), 51 (35). Anal. Calcd. for $C_{24}H_{17}N_5O_2S_2$ (471.55): C, 61.13; H, 3.63; N, 14.85. Found: C, 61.35; H, 3.45; N, 14.71%.

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

Yellow solid, (77% yield), mp 180°C–182°C; (EtOH/DMF); IR (KBr) ν_{max} 1,602 (C=N), 1,715 (C=O), 2,977, 3,084 (C-H), 3,423 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ6.84 (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 $C_{20}H_{12}N_6O_3S_2$ (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}; ^{1}H NMR (DMSO-d_6) δ1.78–1.80 (m, 4H, $2CH_2$), 2.58–2.62 (m, 4H, $2CH_2$),7.22 (t, 1H, Ar-H), 7.69 (d, 1H, Ar-H), 7.87 (d, 1H, Ar-H), 8.35–8.43 (m, 4H, Ar-H); MS m/z (%) 385 (M^+, 1), 310 (2), 281 (14), 252 (23), 192 (4), 180 (7), 165 (7), 152 (18), 135 (11), 118 (17), 103 (41), 90 (21), 77 (100), 63 (15), 51 (25). Anal. Calcd. for $C_{17}H_{15}N_5O_2S_2$ (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); ^{1}H NMR (DMSO-d_6) δ1.57–1.67 (m, 4H, $2CH_2$), 1.1.68–1.72 (m, 4H, $2CH_2$), 2. 47–2.78 (m, 4H, $2CH_2$), 7.22 (t, 1H, Ar-H), 7.70 (d, 1H, Ar-H), 7.87 (d, 1H, Ar-H), 8.39 (m, 4H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ24.3, 29.7, 32.4 (CH_2), 119.6, 120.6, 120.7, 124.7, 128.4, 130.5, 130.6, 143.2, 144.3, 160.5, 172.6 (Ar-C and C=N); MS m/z (%) 413 (M^+, 2), 346 (2), 294 (3), 196 (12), 187 (14), 163 (21), 159 (48), 145 (39), 123 (83), 115 (86), 109 (32), 95 (100), 75 (60), 63 (24), 44 (12). Anal. Calcd. for $C_{19}H_{19}N_5O_2S_2$ (413.52): C, 55.19; H, 4.63; N, 16.94. Found: C, 55.34; H, 4.32; N, 16.56%.

2-((3,4-Dihydronaphthalen-1(2H)-ylidene) hydrazono)-3-(4-nitrophenyl)-5-(thiophen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (17)

Yellow solid, (75% yield), mp 198°C–200°C; (EtOH/DMF); IR (KBr) ν_{max} 1,591 (C=N), 2,925, 3,093 (C-H) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.56 (m, 2H, CH_2), 2.83 (m, 2H, CH_2), 2.99 (m, 2H, CH_2), 7.23–7.26 (m, 5H, Ar-H), 7.75 (d, 1H, Ar-H), 7.88 (d, 1H, Ar-H), 8.20–8.43 (m, 4H, Ar-H); MS m/z (%) 447 (M^+, 11), 298 (20), 259 (43), 224 (30), 207 (34), 159 (81), 128 (39), 115 (61), 102 (24), 90 (100), 76 (50), 55 (45). Anal. Calcd. for $C_{22}H_{17}N_5O_5S_2$ (447.53): C, 59.04; H, 3.83; N, 15.65. Found: C, 59.21; H, 3.63; N, 15.43%.

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-thiazoles **20a–c** and **23a–c**. The products **20a–c** and **23a–c** and their physical constants are listed below.

2-(Benzylidenehydrazono)-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-d_6) 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 (%) 407 (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 $C_{19}H_{13}N_5O_2S_2$ (407.47): C, 56.01; H, 3.22; N, 17.19. Found: C, 56.12; H, 3.14; N, 16.97%.

2-((4-Methoxybenzylidene)hydrazono)-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-d_6) δ 3.80 (s, 3H, OCH_3), 7.02–8.40 (m, 11H, Ar-H), 8.51 (s, IH, CH=N); MS m/z (%) 437 (M^+, 6), 337 (12), 254 (25), 228 (88), 189 (38), 160 (38), 105 (64), 85 (94), 77 (100), 69 (50), 51 (66). Anal. Calcd. for $C_{20}H_{15}N_5O_3S_2$ (437.49): C, 54.91; H, 3.46; N, 16.01. Found: C, 55.23; H, 3.15; N, 15.70%.

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-d_6) δ7.05–8.42 (m, 11H, Ar-H), 8.48 (s, 1H, CH=N); MS m/z (%) 443 (M^{+2}, 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 $C_{19}H_{12}ClN_5O_2S_2$ (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-d_6) δ2.30 (s, 3H, CH_3), 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 $C_{20}H_{15}N_5O_2S_2$ (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-(thiophen-2-yl)-2-((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), 2,915, 3,071 (C-H) cm^{-1}; ^1H NMR (DMSO-d_6) δ2.34 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 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 $C_{21}H_{17}N_5O_2S_2$ (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-d_6) δ2.27 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 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 $C_{21}H_{17}N_5O_3S_2$ (451.52): C, 55.86; H, 3.79; N, 15.51. Found: C, 55.66; H, 3.56; 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 VACSERA 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, gentamycin, 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 (ddH_2O) and filtered through a Whatmann No. 1 filter paper.

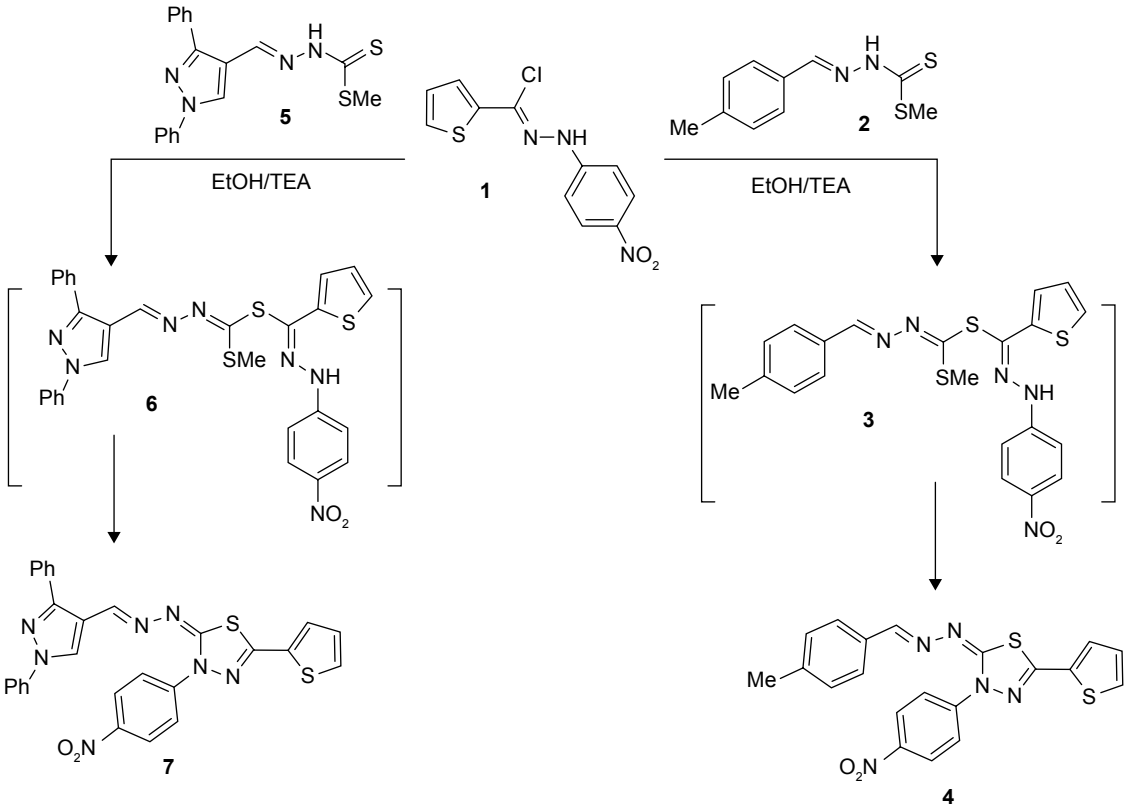
Cell line propagation

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% CO_2 and subcultured 2 times a week.

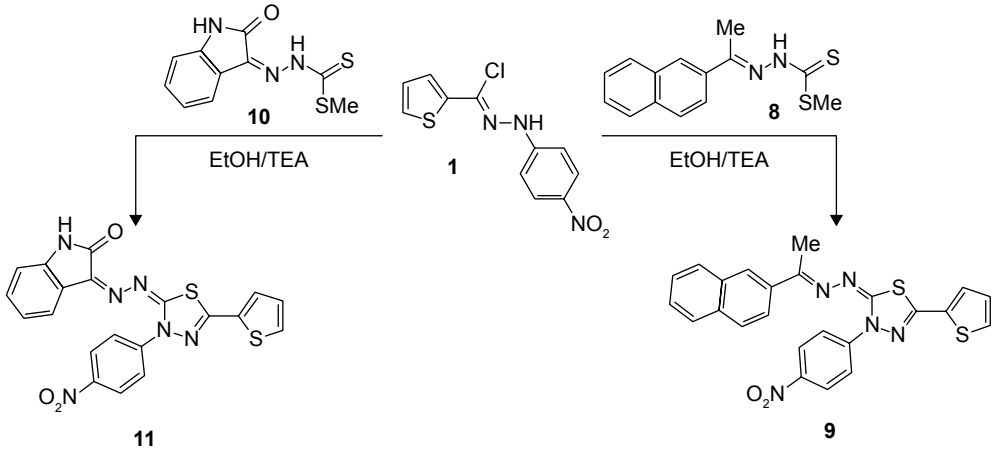
Cytotoxicity evaluation using viability assay

For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10^4 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% CO_2 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.

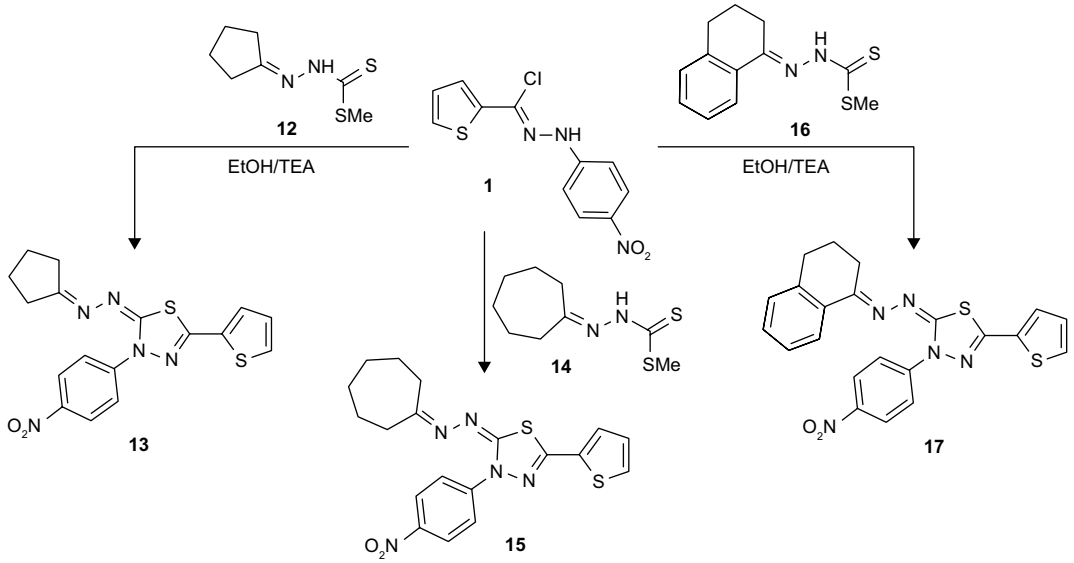
In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 min. The stain was removed and the plates were rinsed using tap water until all excess stain was removed. Glacial acetic acid (30%) was then added to all 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



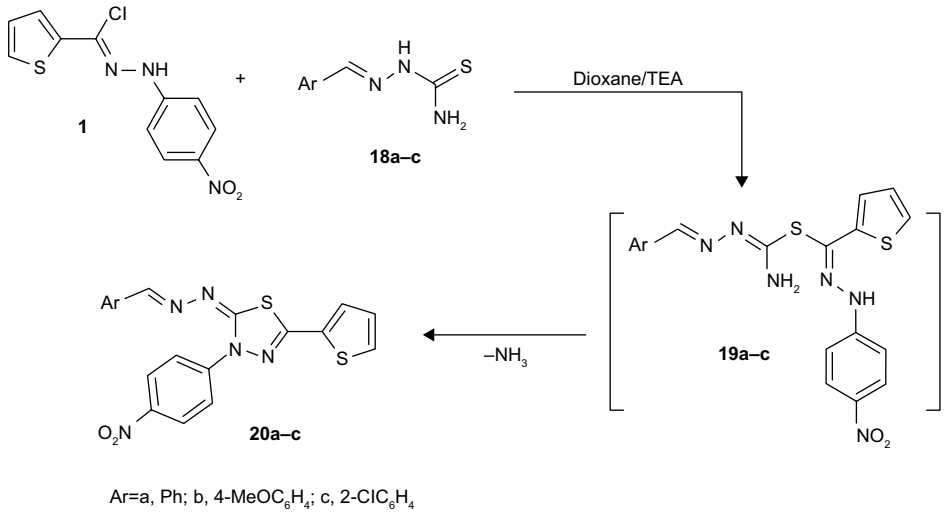
Scheme 1 Synthesis of 1,3,4-thiadiazole derivatives **4** and **7**.
Abbreviations: EtOH, ethanol; TEA, triethylamine.



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

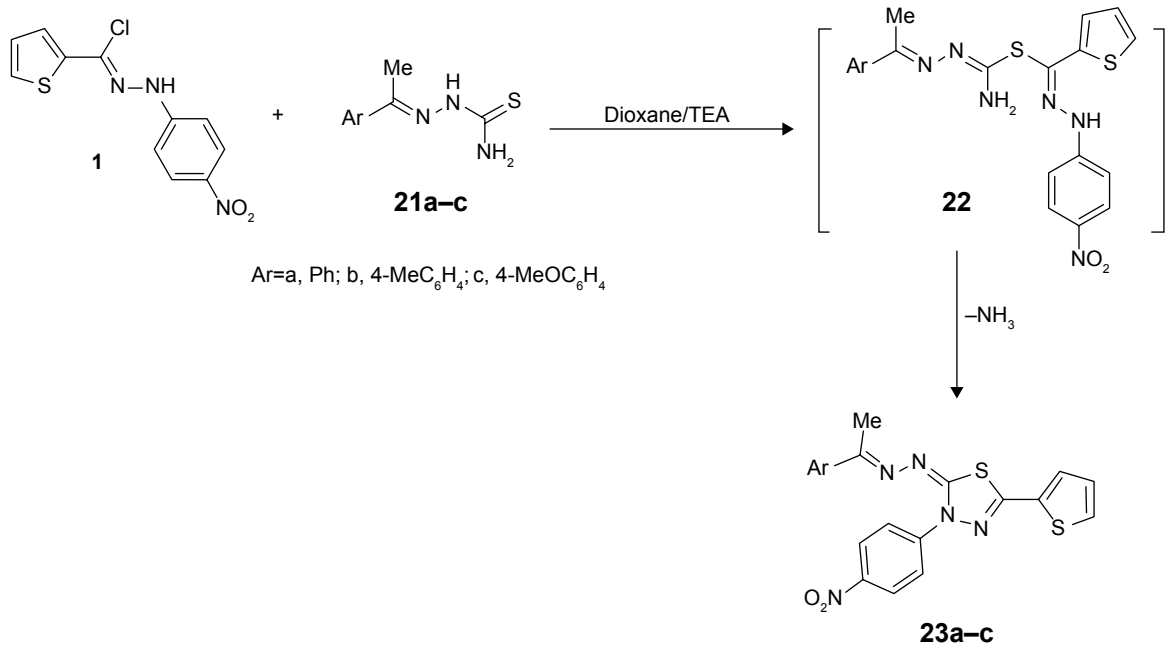


Scheme 3 Synthesis of 1,3,4-thiadiazole derivatives **13, 15**, and **17**.
Abbreviations: EtOH, ethanol; TEA, triethylamine.



Ar=a, Ph; b, 4-MeOC$_6$H$_4$; c, 2-ClC$_6$H$_4$

Scheme 4 Synthesis of 1,3,4-thiadiazole derivatives **20a–c**.
Abbreviation: TEA, triethylamine.



Scheme 5 Synthesis of 1,3,4-thiadiazole derivatives **23a–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 – (ODt/ODc)]×100% where ODt is the mean optical density of wells treated with the tested sample and ODc 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 (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).[51]

Molecular modeling

The docking study was performed using the MOE 2014.010 software.[52–54] 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

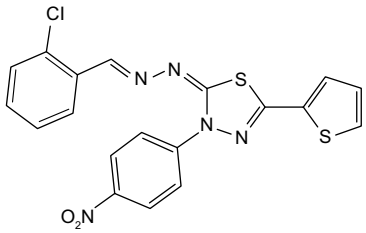
Chemistry

N-Thiophene-2-carbohydrazonoyl chloride derivative **1**[55] was reacted with methyl 2-benzylidenehydrazine-1-carbodithioate (**2**) and methyl 2-((1,3-diphenyl-1*H*-pyrazol-4-yl)

Table 1 The in vitro inhibitory activity of compounds **4, 7, 9, 11, 13, 15, 17, 20a–c**, and **23a–c** against tumor cell lines

Tested compounds	Tumor cell lines		Tested compounds	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.



	20b	Cisplatin
For A-549:	IC_{50}=4.37±0.7 μM	IC_{50}=0.95±0.9 μM
For HepG-2:	IC_{50}=8.30±0.5 μM	IC_{50}=1.40±1.1 μM

Figure 2 The cytotoxic activity of compound **20b** compared to Cisplatin.

methylene)hydrazine-1-carbodithioate (**5**) afforded 2-((4-methylbenzylidene) hydrazono)-3-(4-nitrophenyl)-5-(thiophen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (**4**) and 2-(((1,3-diphenyl-1*H*-pyrazol-4-yl)methylene)hydrazono)-3-(4-nitrophenyl)-5-(thiophen-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 ^{1}H-NMR). For example, ^{1}H NMR spectrum of compound **4** revealed 2 singlet signals at δ 2.36 and 8.53 ppm assignable to methyl (CH_3) and methine (CH=N) protones, respectively, in addition to the 11 aromatic protones. ^{13}C-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 **1** with an equivalent amount of each of compound **2** and **5** by loss of 1 mole of hydrogen chloride to form thiohydrazonate 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-carbodithioate (**8**) and methyl 2-(2-oxoindolin-3-ylidene)hydrazine-1-carbodithioate (**10**) afforded 2-((1-(naphthalen-2-yl)ethylidene) hydrazono)-3-(4-nitrophenyl)-5-(thiophen-2-yl)-2,3-dihydro-1,3,4-thiadiazole (**9**) and 3-((3-(4-nitrophenyl)-5-(thiophen-2-yl)-1,3,4-thiadiazol-2(3*H*)-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 ^{1}H-NMR) and elemental analyses. The ^{1}H 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 ν_{max}=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 arylidenethiosemicarbazones **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 NH_3.

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. IC_{50} (the concentration of test compounds required to kill 50% of cell population) was determined from the dose–response curve. The activity was expressed as IC_{50} values (μM) ± SD from 3 replicates.

Table 2 The in vitro inhibitory activity of compounds **4, 7, 9, 15, 17, 20a–c**, and **23a** against normal cell line (Vero cells)

Tested compounds	Tumor cell lines		Tested compounds	Tumor cell lines	
	A-549	HepG-2		A-549	HepG-2
4	95.7	131.6	**20a**	88.5	112.4
7	74.6	90.4	**20b**	134.5	153.9
9	93.0	123.8	**20c**	148	172
15	124.5	105.3	**23a**	96.4	115.8
17	70.4	53.0	Cisplatin	312	319

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 moderated inhibitory 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

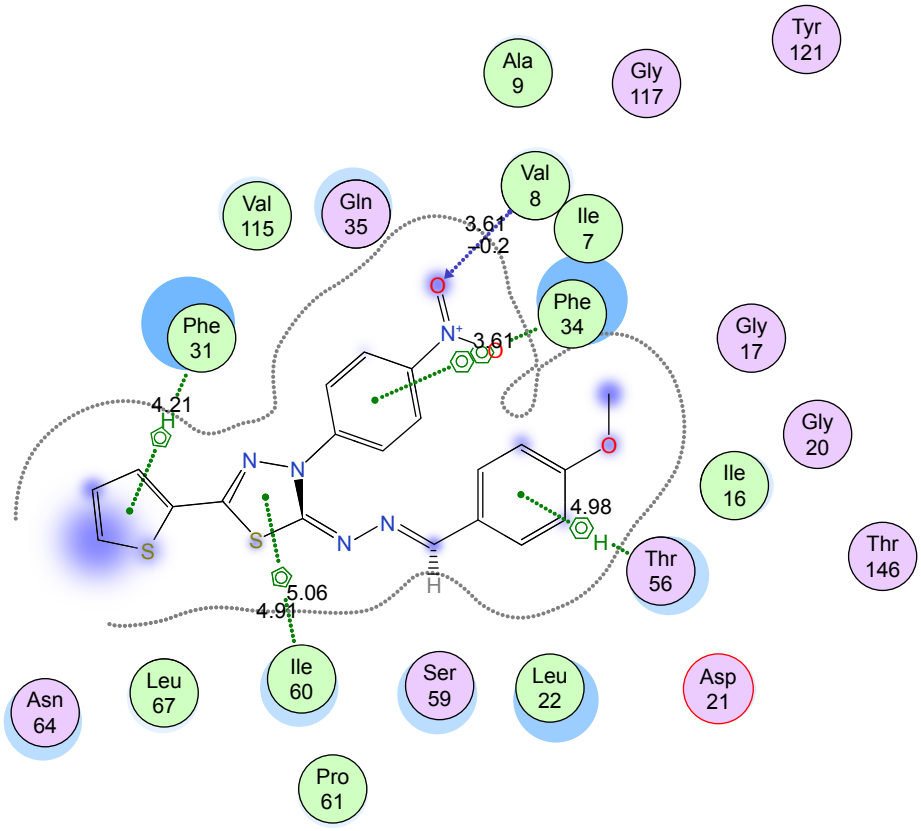


Figure 3 Two-dimensional representation showed interactions of compound **20b** and the DHFR enzyme pocket amino acids.
Abbreviation: DHFR, dihydrofolate reductase.

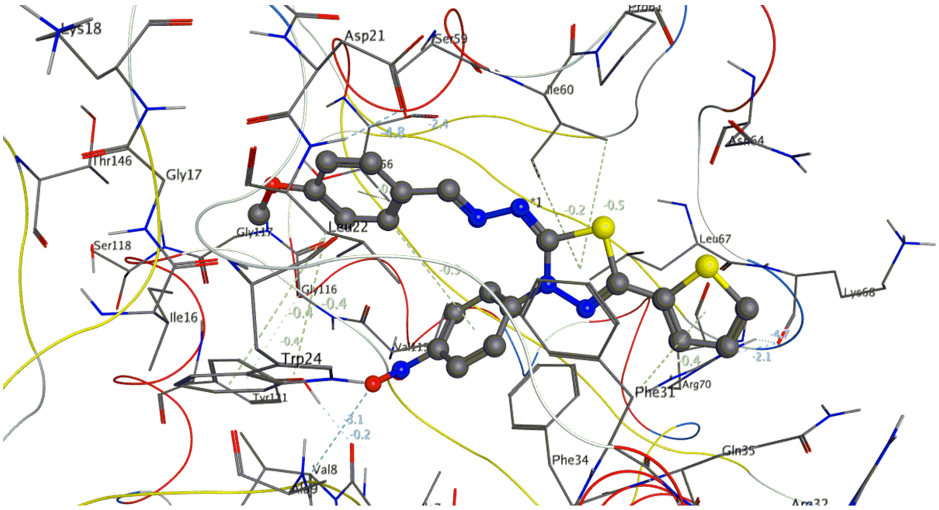


Figure 4 Three-dimensional representation showed interactions of compound **20b** and the DHFR enzyme pocket amino acids.
Abbreviation: DHFR, dihydrofolate reductase.

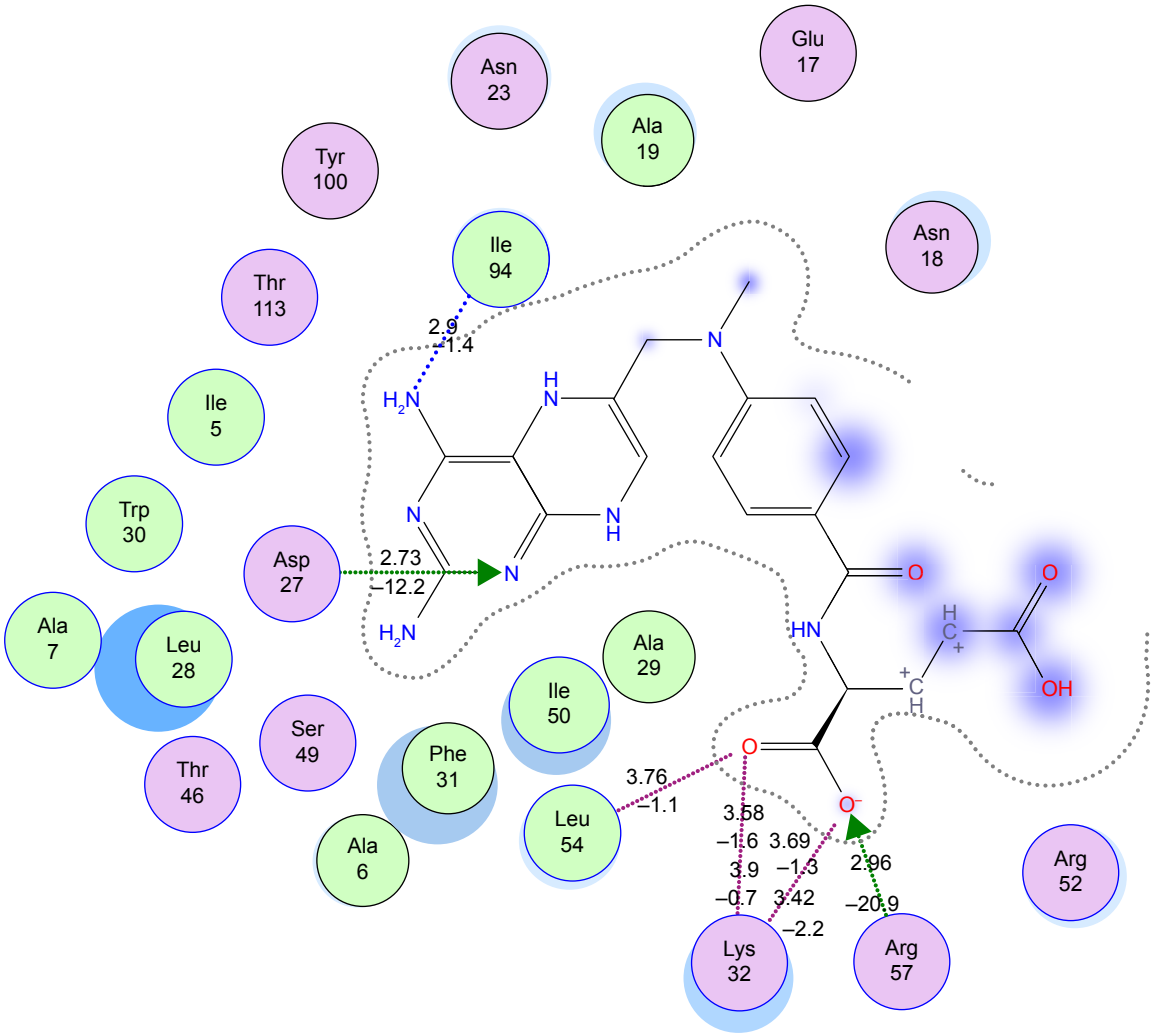


Figure 5 Two-dimensional representation showed interactions of native inhibitor MTX and the DHFR enzyme pocket amino acids.
Abbreviations: DHFR, dihydrofolate reductase; MTX, methotrexate.

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 Å^2
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 E (kcal/mol), showing good affinity with the DHFR enzyme by forming 4 pi-hydrogen interactions with binding energy −1.4 E (kcal/mol), 1 hydrogen acceptor interaction with binding energy –0.2 E (kcal/mol), and 1 pi–pi interaction with almost zero binding energy. From Figure 5, we concluded that NH_2 of compound native making H-bond with IIe (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.

References

1. World Health Organization (WHO) Factsheets. Cancer 2012. Available from: http://www.who.int/mediacentre/factsheets/fs297/en/. Accessed January 14, 2018.
2. Arora A, Scholar EM. Role of tyrosine Kknase inhibitors in cancer therapy. *J Pharmacol Exp Ther*. 2005;3:971–979.
3. Mousavi SH, Tayarani-Najaran Z, Hersey P. Apoptosis: from signalling pathways to therapeutic tools. *Iran J Basic Med Sci*. 2008;11:121–142.
4. Yogeeswari P, Thirumurugan R, Kavya R, Samuel JS, Stables JD, Sriram D. 3-Chloro-2- methylphenyl-substituted semicarbazones: synthesis and anticonvulsant activity. *Eur J Med Chem*. 2004;39:729–734.
5. Shank RP, Doose DR, Streeter AJ, Bialer M. Plasma and whole blood pharmacokinetics of topiramate: the role of carbonic anhydrase. *Epilepsy Res*. 2005;63:103–112.
6. Gomha SM, Edrees MM, Altalbawy FMA. Synthesis and characterization of some new *bis*-pyrazolyl-thiazoles incorporating the thiophene moiety as potent anti-tumor agents. *Inter J Mol Sci*. 2016;17:E1499.
7. Ragavendran JV, Sriram D, Patil S, et al. Design and synthesis of anticonvulsants from a combined phthalimide–GABA–anilide and hydrazone pharmacophore. *Eur J Med Chem*. 2007;42:146–151.
8. Thirumurugan R, Sriram D, Saxena A, Stables J, Yogeeswari P. 2,4-Dimethoxyphenylsemicarbazones with anticonvulsant activity against three animal models of seizures: synthesis and pharmacological evaluation. *Bioorg Med Chem*. 2006;14:3106–3112.
9. Mohareba RM, Abdo NYM. Synthesis and cytotoxic evaluation of pyran, dihydropyridine and thiophene derivatives of 3-acetylcoumarin. *Chem Pharm Bull (Tokyo)*. 2015;63:678–687.
10. Rodrigues KA, Dias CN, Néris PL, et al. 2-Amino-thiophene derivatives present antileishmanial activity mediated by apoptosis and immunomodulation in vitro. *Eur J Med Chem*. 2015;106:1–14.
11. Romagnoli R, Baraldi PG, Salvador MK, et al. Synthesis and biological evaluation of 2-(alkoxycarbonyl)-3-anilinobenzo [b]thiophenes and thieno[2,3-b]pyridines as new potent anticancer agents. *J Med Chem*. 2013;56:2606–2618.
12. Ghorab MM, Bashandy MS, Alsaid MS. Novel thiophene derivatives with sulfonamide, isoxazole, benzothiazole, quinoline and anthracene moieties as potential anticancer agents. *Acta Pharm*. 2014;64:419–431.
13. Takahashi HT, Novello CR, Ueda-Nakamura T, Filho BPD, Mello JCP, Nakamura CV. Thiophene derivatives with antileishmanial activity isolated from aerial parts of Porophyllum ruderale (Jacq.) Cass. *Molecules*. 2011;16:3469–3478.
14. Jain K, Sharma S, Vaidya A, Ravichandran V, Agrawal RK. 1,3,4-Thiadiazole and its derivatives: a review on recent progress in biological activities. *Chem Biol Drug Des*. 2013;81:557–576.
15. Kushwaha N, Kushwaha SKS, Rai AK. Biological activities of thiadiazole derivatives: a review. *Inter J Chem Res*. 2012;4:517–531.
16. Gomha SM, Salah TA, Abdelhamid AO. Synthesis, characterization, and pharmacological evaluation of some novel thiadiazoles and thiazoles incorporating pyrazole moiety as anticancer agents. *Monatsh Chem*. 2015;146:149–158.
17. Gomha SM, Abdel-aziz HM. Synthesis and antitumor activity of 1,3,4-thiadiazole derivatives bearing coumarine ring. *Heterocycles*. 2015;91:583–592.
18. Siddiqui N, Ahuja P, Ahsan W, Pandeya SN, Alam MS. Thiadiazoles: progress report on biological activities. *J Chem Pharm Res*. 2009;1:19–30.

19. Bhattacharya P, Leonard JT, Roy K. Exploring QSAR of thiazole and thiadiazole derivatives as potent and selective human adenosine A3 receptor antagonists using FA and GFA techniques. *Bioorg Med Chem*. 2005;15:1159–1165.
20. Foroumadi A, Kargar Z, Sakhteman A, et al. Synthesis and antimycobacterial activity of some alkyl [5-(nitroaryl)-1,3,4- thiadiazol-2-ylthio] propionates. *Bioorg Med Chem Lett*. 2006;16:1164–1167.
21. Kumar D, Kumar NM, Chang KH, Shah K. Synthesis and anticancer activity of 5-(3-indolyl)-1,3,4-thiadiazoles. *Eur J Med Chem*. 2010;45:4664–4668.
22. Sharma B, Verma A, Prajapati S, Sharma UK. Synthetic methods, chemistry, and the anticonvulsant activity of thiadiazoles. *Int J Med Chem*. 2013:348948.
23. Mathew V, Keshavayya J, Vaidya VP, Giles D. Studies on synthesis and pharmacological activities of 3,6-disubstituted-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles and their dihydro analogues. *Eur J Med Chem*. 2007;42:823–840.
24. Yang XH, Wen Q, Zhao TT, et al. Synthesis, biological evaluation, and molecular docking studies of cinnamic acyl 1,3,4-thiadiazole amide derivatives as novel antitubulin agents. *Bioorg Med Chem*. 2012;20:1181–1187.
25. Matysiak J, Opolski A. Synthesis and antiproliferative activity of N-substituted 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles. *Bioorg Med Chem*. 2006;14:4483–4489.
26. Radi M, Crespan E, Botta G, et al. Discovery and SAR of 1,3,4-thiadiazole derivatives as potent Abl tyrosine kinase inhibitors and cytodifferentiating agents. *Bioorg Med Chem Lett*. 2008;18:1207–1211.
27. Rzeski W, Matysiakb J, Kandefer-Szerszen M. Anticancer, neuroprotective activities and computational studies of 2-amino-1,3,4-thiadiazole based compound. *Bioorg Med Chem*. 2007;15:3201–3207.
28. Gomha SM, Kheder NA, Abdelaziz MR, Mabkhot YN, Alhajoj AM. A facile synthesis and anticancer activity of some novel thiazoles carrying 1,3,4-thiadiazole moiety. *Chem Central J*. 2017;11:25.
29. Gomha SM, Zaki YH, Abdelhamid AO. Utility of 3-acetyl-6-bromo-*2H*-chromen-2-one for synthesis of new heterocycles as potential anticancer agents. *Molecules*. 2015;20:21826–21839.
30. Gomha SM, Salah TA, Hassaneen HME, Abdel-aziz H, Khedr MA. Synthesis, characterization and molecular docking of novel bioactive thiazolyl-thiazole derivatives as promising cytotoxic antitumor drug. *Molecules*. 2016;21:1–17.
31. Chou JY, Lai SY, Pan SL, Jow GM, Chern JM, Guh JH. Investigation of anticancer mechanism of thiadiazole-based compound in human non-small cell lung cancer A549 cells. *Biochem Pharmacol*. 2003;66:115–124.
32. Supuran CT, Scozzafava A. Carbonic anhydrase inhibitors-Part 94. 1,3,4-Thiadiazole-2-sulfonamide derivatives as antitumor agents. *Eur J Med Chem*. 2000;35:867–874.
33. Supuran CT, Brigantl F, Tilli S, Chegwidden WR, Scozzafava A. Carbonic anhydrase inhibitors: sulfonamides as antitumor agents. *Bioorg Med Chem*. 2001;9:703–714.
34. Sun J, Yang YS, Li W, et al. Synthesis, biological evaluation and molecular docking studies of 1,3,4-thiadiazole derivatives containing 1,4-benzodioxan as potential antitumor agents. *Bioorg Med Chem Lett*. 2011;21:6116–6121.
35. Tsukamoto K, Suno M, Igarashi K, Kozai Y, Sugino Y. Mechanism of action of 2,2′-(methylenediimino)bis-1,3,4-thiadiazole (NSC 143019), an antitumor agent. *Cancer Res*. 1975;35:2631–2636.
36. Vergne F, Bernardelli P, Lorthiois E, et al. Discovery of thiadiazoles as a novel structural class of potent and selective PDE7 inhibitors. Part 1: design, synthesis and structure-activity relationship studies. *Bioorg Med Chem Lett*. 2004;14:4607–4613.
37. Rajak H, Agarawal A, Parmar P, et al. 2,5-Disubstituted-1,3,4-oxadiazoles/thiadiazole as surface recognition moiety: design and synthesis of novel hydroxamic acid based histone deacetylase inhibitors. *Bioorg Med Chem Lett*. 2011;21:5735–5738.
38. Jung KY, Kim SK, Gao ZG, et al. Structure-activity relationships of thiazole and thiadiazole derivatives as potent and selective human adenosine A3 receptor antagonists. *Bioorg Med Chem*. 2004;12:613–623.
39. Haider S, Alam MS, Hamid H. 1,3,4-Thiadiazoles: a potent multi targeted pharmacological scaffold. *Eur J Med Chem*. 2015;92:156–177.
40. Kumar D, Vaddula BR, Chang KH, Shah K. One-pot synthesis and anticancer studies of 2-arylamino-5-aryl-1,3,4-thiadiazoles. *Bioorg Med Chem Lett*. 2011;21:2320–2323.
41. Juszczak M, Matysiak J, Szeliga M, et al. 2-Amino-1,3,4-thiadiazole derivative (FABT) inhibits the extracellular signal-regulated kinase pathway and induces cell cycle arrest in human non-small lung carcinoma cells. *Bioorg Med Chem Lett*. 2012;22:5466–5469.
42. Li Y, Geng J, Liu Y, Yu S, Zhao G. Thiadiazole-A promising structure in medicinal chemistry. *Chem Med Chem*. 2013;8:27–41.
43. Gomha SM, Ahmed SA, Abdelhamid AO. Synthesis and cytotoxicity evaluation of some novel thiazoles, thiadiazoles, and pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5(1*H*)-one incorporating triazole moiety. *Molecules*. 2015;20:1357–1376.
44. Gomha SM, Abdallah MA, Mourad MA, Elaasser MM. Application of Mannich and Michael reactions in synthesis of pyridopyrimido[2,1-*b*][1,3,5]thiadiazinones and pyridopyrimido[2,1-b][1,3]thiazinones and their biological activity. *Heterocycles*. 2016;92:688–699.
45. Gomha SM, Eldebss TMA, Abdulla MM, Mayhoub AS. Diphenylpyrroles: novel p53 activators. *Eur J Med Chem*. 2014;82:472–479.
46. Dawood KM, Gomha SM. Synthesis and anti-cancer activity of 1,3,4-thiadiazole and 1,3-thiazole derivatives having 1,3,4-oxadiazole moiety. *J Heterocycl Chem*. 2015;52:1400–1405.
47. Gomha SM, Abbas IM, Elneairy MAA, Elaasser MM, Mabrouk BKA. Synthesis and biological evaluation of novel fused triazolo[4,3-*a*]pyrimidinones. *Turk J Chem*. 2015;39:510–531.
48. Gomha SM, Khalil KD. A convenient ultrasound-promoted synthesis and cytotoxic activity of some new thiazole derivatives bearing a coumarin nucleus. *Molecules*. 2012;17:9335–9347.
49. Gomha SM, Abbas IM, Elaasser MM, Mabrouk BKA. Synthesis, molecular docking and pharmacological study of pyrimido-thiadiazinones and its *bis*-derivatives. *Lett Drug Des Disc*. 2017;14:434–443.
50. Gomha SM, Dawood KM. Synthesis of novel indolizine, pyrrolo[1,2-a]quinoline, and 4,5-dihydrothiophene derivatives via nitrogen ylides and their antimicrobial evaluation. *J Chem Res*. 2014;38:515–519.
51. Gomha SM, Riyadh SM, Mahmmoud EA, Elaasser MM. Synthesis and anticancer activities of thiazoles, 1,3-thiazines, and thiazolidine using chitosan-grafted-poly(vinylpyridine) as basic catalyst. *Heterocycles*. 2015;91:1227–1243.
52. Sharma M, Chauhan PMS. Dihydrofolate reductase as a therapeutic target for infectious diseases: opportunities and challenges. *Future Med Chem*. 2012;4:1335–1365.
53. Daina A, Zoete V. A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. *Chem Med Chem*. 2016;11:1117–1121.
54. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. 2001;46:3–25.
55. Hassaneen HM, Mousa AH, Abeed NM, Shawali AS. Chemistry of C-Heteroarylhydrazidoyl halides. Synthesis and reactions of N-(p-nitrophenyl)-C-(2-thienyl)-formohydrazidoyl halides. *Heterocycles*. 1988;27:695–706.