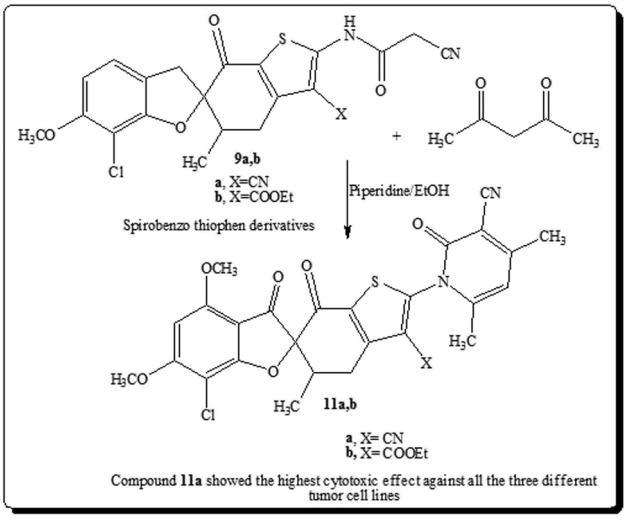
Research Article

Karam A. El-Sharkawy*, Mohammed AlBratty, Hassan A. AlHazmi, Asim Najmi

Design, synthesis, and cytotoxicity evaluation of novel thiophene, pyrimidine, pyridazine, and pyridine: Griseofulvin heterocyclic extension derivatives

https://doi.org/10.1515/chem-2022-0225
received September 9, 2022; accepted October 12, 2022

Abstract: Griseofulvin, an antifungal drug, has also shown good antiproliferative activity previously. This study was aimed to synthesize heterocyclic extension derivatives of griseofulvin and test them against cancer cell lines. Griseofulvin was hydrolyzed to afford griseofulvic acid (**1**) followed by hybridization with important heterocyclic moieties. Initially, the active methylene group of the 1,3-cyclohexanedione moiety in **1** was utilized to synthesize fused thiophene derivatives (**4a** and **b**) by reacting with malononitrile or ethyl cyanoacetate together with elemental sulfur. Compounds **4a** and **b** were further converted to fused pyrimidine derivatives (**5a–d**) using ethyl isothiocyanate or phenyl isothiocyanate. Compound **1** was also reacted with aryldiazonium chlorides to synthesize compounds **6a** and **b**, which were used to prepare fused thiophene derivatives (**7a**–**d**). The resulting thiophenes (**7a**–**d**) underwent cyclization to produce fused pyridazine derivatives (**8a**–**d**). In addition, fused pyridine derivatives (**10a** and **b**) were also prepared by the reaction of **4a** and **b** with ethyl cyanoacetate using two different catalytic bases. The first was triethylamine to form **10a** and **b** in two steps via **9a** and **b**, and the second was sodium ethoxide to afford **10a** and **b** in one step. Finally, **9a** and **b** underwent cyclization in the presence of acetylacetone to yield compounds **11a** and **b**. The structures of synthesized compounds were confirmed using IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry techniques. The synthesized compounds were subjected to cytotoxic screening against three tumor cell lines and presented good to excellent cytotoxic profiles. Compounds **4a** and **11a** showed significant inhibitory activity against the three cell lines compared to the standard drug doxorubicin.



Graphical abstract

Keywords: thiophene, pyrimidine, pyridazine and pyridine, griseofulvin, cytotoxic activity

*** Corresponding author: Karam A. El-Sharkawy,** Pharmaceutical Chemistry Department, Pharmacy College, Jazan University, P.O. Box 114, Postal Code 45142, Jazan, Saudi Arabia, e-mail: karamsyn@yahoo.com, tel: +966-559686517
Mohammed AlBratty, Asim Najmi: Pharmaceutical Chemistry Department, Pharmacy College, Jazan University, P.O. Box 114, Postal Code 45142, Jazan, Saudi Arabia
Hassan A. AlHazmi: Pharmaceutical Chemistry Department, Pharmacy College, Jazan University, P.O. Box 114, Postal Code 45142, Jazan, Saudi Arabia; Substance Abuse & Toxicology Research Center, Jazan University, P.O. Box 114, Postal Code 45142, Jazan, Saudi Arabia

1 Introduction

Griseofulvin is one of the most important antifungal drugs used clinically [1–3]; however, recent studies showed the extent of its activity against resistant cancer cells [4] and

its efficacy as inhibitors of centrosomal clustering in cancer cells [5]. In addition, griseofulvin has been reported to possess antibacterial activity, metabolic stability, and efficacy against multiple myeloma and toenail onychomycosis [6–9]. On the other hand, spiro-benzothiophene derivatives also showed a specific property as anticonvulsant agents [10]. Spiro derivatives containing thiophene ring have been shown to have promising antimicrobial activity [11], whereas the spiro-benzofuran derivatives were found to have inhibitory effects on the human peptidyl prolyl *cis*/*trans* isomerase Pin1 [12] and fungal growth [13]. Also, spiro-furanone derivatives showed considerable properties as antitumor agents [14] and antimicrobial agents [15]. In this study, novel thiophene, pyrimidine, pyridazine, and pyridine heterocyclic derivatives were prepared as extensions of the griseofulvin drug, which could have improved antiproliferative activities against various cancer cell lines.

2 Experimental

2.1 Material and methods

2.1.1 Chemicals and reagents

All the chemicals and reagents in this study were provided from Sigma Aldrich (UK).

2.1.2 Instruments

The melting points of the synthesized compounds were measured in open capillaries and were uncorrected. Elemental analyses were performed on a Yanaco CHNS Corder elemental analyzer (Tokyo, Japan). IR spectra were evaluated using KBr discs on a Pye Unicam SP-1000 spectrophotometer (Cambridge, UK). ^{1}H NMR and ^{13}C NMR spectra were determined on a Varian EM 390–200 MHz instrument with CD_3SOCD_3 as the solvent and TMS as an internal standard material, the chemical shifts were expressed as δ ppm. Mass spectra were recorded on Kratos (75 eV) MS equipment (Germany).

2.2 Synthesis

2.2.1 Synthesis of compound: 7-chloro-4,6-dimethoxy-2′-methyl-3*H*-spiro-[benzofuran-2,1′-cyclohexane]-3,4′,6′-trione (1)

Griseofulvin (7.056 g, 0.02 mol) was dissolved in glacial acetic acid (40 mL) followed by heating on a water bath at 70°C. To the solution, 2 N aqueous sulfuric acid (10 mL) was added and the clear solution thus obtained was heated further with continuous stirring for 2 h. Product was separated as white precipitate. The reaction mixture was cooled to room temperature and poured on to cold water (100 mL). The solid obtained was filtered, washed with methanol (50 mL) followed by diethyl ether (25 mL), and dried.

Compound **1**: Off-white fine crystals from methanol, yield 74%, 5.000 g, m.p. 261–263°C as reported before [5,16]. IR (KBr): υ/cm^{-1} = 3,046 (CH aromatic), 2,987, 2,965, 2,954 ($3CH_3$), 2,843, 2,833 ($2CH_2$), 1,781, 1,767, 1,715 (3C═O), 1,654 (C═C), 1,112 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.1 (d, 3H, CH_3), 3.08, 3.13 (2s, 6H, $2OCH_3$), 3.78 (d, J = 2.55 Hz, CH_2), 4.23 (s, 2H, CH_2), 5.12 (m, 1H, CH), 6.78 (s, 1H, benzene ring). ^{13}C NMR: δ = 17.2, 38.3, 41.7 (CH_3, $2OCH_3$), 44.3, 46.1 ($2CH_2$), 56.1 (CH), 88.3 (CO), 118.1, 119.3, 121.5, 123.7, 131.2 (benzene C), 160.3, 163.5, 170.7 (3C═O). MS (relative intensity) m/z: 338 (M^+, 27.2%). Calcd for $C_{16}H_{15}ClO_6$ (338.74): C, 56.73; H, 4.46%. Found: C, 56.95; H, 4.21%.

2.2.2 General procedure for the synthesis of compounds: 2-amino-7′-chloro-4′,6′-dimethoxy-5-methyl-3′,7-dioxo-5,7-dihydro-3′*H*,4*H*-spiro [benzo[*b*]thiophene-6,2′-benzofuran]-3-carbonitrile (4a) and ethyl 2-amino-7′-chloro-4′,6′-dimethoxy-5-methyl-3′,7-dioxo-5,7-dihydro-3′*H*,4*H*-spiro [benzo[*b*]thiophene-6,2′-benzofuran]-3-carboxylate (4b)

Either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.131 g, 0.0 l mol) with elemental sulfur (0.32 g, 0.01 mol) were added to the solution of griseofulvic acid (**1**) (3.38 g, 0.0 l mol) in ethanol (100 mL) containing triethylamine (1 mL). The reaction mixture in both cases was heated under reflux for 4 h and poured onto ice–water mixture containing few drops of HCl. The solid product formed was collected by filtration.

Compound **4a**: Faint brown crystals from ethanol, yield 63%, 2.591 g, m.p. 125–127°C. IR (KBr): υ/cm^{-1} = 3,258–3,198 (NH_2), 3,055 (CH aromatic), 2,990, 2,972, 2,948 ($3CH_3$), 2,856 (CH_2), 2,231 (CN), 1,703, 1,677 (2C═O), 1,646 (C═C), 1,121 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.23 (d, 3H, CH_3), 2.25, 2.38 (2s, 6H, $2OCH_3$), 2.67 (d, 2H, J = 2.72 Hz, CH_2), 4.38 (s, 2H, D_2O exchangeable, NH_2), 5.32 (m, 1H, CH), 6.62 (s, 1H, benzene ring). ^{13}C NMR: δ = 19.4, 36.7, 40.1 (CH_3, $2OCH_3$)), 44.8 (CH_2), 60.6 (CH), 90.7 (CO), 117.6 (CN), 120.1, 122.5, 123.7, 126.8, 128.7, 130.9, 132.6, 134.1 (benzene C, thiophene C), 154.3, 157.5 (2C═O). MS (relative intensity) m/z: 418 (M^+, 18.2%). Calcd for $C_{19}H_{15}ClN_2O_5S$ (418.85): C, 54.48; H, 3.61; N, 6.69; S, 7.66%. Found: C, 54.76; H, 3.44; N, 6.97; S, 7.38%.

Compound **4b**: Faint brown crystals from ethanol, yield 54%, 2.515 g, m.p. 212–214°C. IR (KBr): υ/cm^{-1} = 3,312–3,122 (NH_2), 3,061 (CH aromatic), 2,988–2,912 ($4CH_3$), 2,876, 2,856 ($2CH_2$), 1,751, 1,708, 1,685 (3C═O), 1,652 (C═C), 1,128 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.17 (d, 3H, CH_3), 2.31 (t, 3H, J = 3.12 Hz, CH_3), 2.66, 2.83 (2s, 6H, $2OCH_3$), 2.97 (d, 2H, J = 2.42 Hz, CH_2), 3.83 (q, 2H, J = 3.12 Hz, CH_2), 4.54 (s, 2H, D_2O exchangeable, NH_2), 5.28 (m, 1H, CH), 6.71 (s, 1H, benzene ring). ^{13}C NMR: δ = 17.3, 19.7, 21.4, 25.4 ($2CH_3$, $2OCH_3$),), 33.1, 36.2 ($2CH_2$), 63.9 (CH), 85.4 (CO), 119.1, 121.4, 123.3, 125.9, 128.9, 131.2, 134.7 (benzene C, thiophene C), 153.3, 154.9, 161.5 (3C═O). MS (relative intensity) m/z: 465 (M^+, 24.6%). Calcd for $C_{21}H_{20}ClNO_7S$ (465.90): C, 54.14; H, 4.33; N, 3.01; S, 6.88%. Found: C, 53.89; H, 4.11; N, 2.77; S, 6.61%.

2.2.3 General procedure for the synthesis of compounds: 7′-chloro-3-ethyl-4-imino-2-mercapto-4′,6′-di-methoxy-6-methy-3′-oxo-6,8-dihydro-3′*H*,5*H*,8*H*-spiro[benzo-4,5-thieno[2,3-*d*] pyrimidine-7,2′-benzofuran]-8-one (5a), 7′-chloro-2-mercapto-4-imino-4′,6′-dimethoxy-6-methyl-3-phenyl-3′-oxo-6, 8-dihydro-3′*H*,5*H*,8*H*-spiro [benzo-4,5-thieno[2,3-*d*] pyrimidine-7,2′-benzofuran]-8-one (5b), 7′-chloro-3-ethyl-2-mercapto-4′,6′-dimethoxy-6-methyl-3′,4-dioxo-6,8-dihydro-3′*H*,5*H*,8*H*-spiro[benzo-4,5-thieno [2,3-*d*]pyrimidine-7,2′-benzofuran]-8-one (5c), and 7′-chloro-4′,6′-2-mercapto-dimethoxy-6-methyl-3-phenyl-3′,4-dioxo-6,8-dihydro-3′*H*, 5*H*, 8*H*-spiro[benzo-4,5-thieno[2,3-*d*] pyrimidine-7,2-benzofuran]-8-one (5d)

Either ethyl isothiocyanate (0.087 g, 0.001 mol) or phenyl isothiocyanate (0.135 g, 0.001 mol) was added to either solution of compound **4a** (0.419 g, 0.001 mol) or compound **4b** (0.466 g, 0.001 mol) in 1,4-dioxane (40 mL) containing 0.5 mL of triethylamine. The reaction mixture was refluxed for 5 h, cooled, and poured onto ice/water mixture containing few drops of HCl. The separated solid in each case was collected by filtration.

Compound **5a**: Off-white crystals from ethanol, yield 57%, 0.289 g, m.p. 195–197°C. IR (KBr): υ/cm^{-1} = 3,219 (NH), 3,044 (CH aromatic), 2,995–2,895 ($4CH_3$), 2,848–2,823 ($2CH_2$), 2,583 (SH), 1,758, 1,710 (2C═O), 1,661 (C═N), 1,646 (C═C), 1,105 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.11 (d, 3H, CH_3), 1.84 (t, 3H, J = 2.58 Hz, CH_3), 2.83, 3.07 (2s, 6H, $2OCH_3$), 3.12 (d, 2H, J = 2.68 Hz, CH_2), 3.31 (q, 2H, J = 2.58 Hz, CH_2), 4.1 (s, 1H, D_2O exchangeable, SH), 5.17 (m, 1H, CH), 6.54 (s, 1H, benzene ring), 8.58 (s, 1H, D_2O-exchangeable, NH). ^{13}C NMR: δ = 17.6, 19.3, 35.1, 36.3 ($2CH_3$, $2OCH_3$), 37.5, 42.3 ($2CH_2$), 56.6 (CH), 83.9 (CO), 120.7, 121.9, 124.2, 126.3, 128.5, 129.3, 130.8, 132.7, 133.8, 134.1, 136.2, (benzene C, thiophene C, pyrimidine C), 159.1, 160.4 (2C═O). MS (relative intensity) m/z: 505 (M^+, 15.6%). Calcd for $C_{22}H_{20}ClN_3O_5S_2$ (505.99): C, 52.22; H, 3.98; N, 8.30; S, 12.67%. Found: C, 52.50; H, 4.23; N, 8.56; S, 12.95%.

Compound **5b**: Off-white crystals from ethanol, yield 67%, 0.371 g, m.p. 147–149°C. IR (KBr): υ/cm^{-1} = 3,264 (NH), 3,053 (CH aromatic), 2,983–2,926 ($3CH_3$), 2,856 (CH_2), 2,568 (SH), 1,745, 1,687 (2C═O), 1,659 (C═N), 1,652 (C═C), 1,126 (C–O). ^{1}H NMR (DMSO-d_6) δ = 0.98 (d, 3H, CH_3), 2.65, 2.93 (2s, 6H, $2OCH_3$), 3.32 (d, 2H, J = 2.82 Hz, CH_2), 4.48 (s, 1H, D_2O exchangeable, SH), 5.66 (m, 1H, CH), 6.73–7.32 (m, 6H, benzene rings), 9.23 (s, 1H, D_2O exchangeable, NH). MS (relative intensity) m/z: 554 (M^+, 24.8%), 477 (M^+, 17.4%). Calcd for $C_{26}H_{20}ClN_3O_5S_2$ (554.04): C, 56.36; H, 3.64; N, 7.58; S, 11.58%. Found: C, 56.08; H, 3.37; N, 7.83; S, 11.84%.

Compound **5c**: Creamy-white crystals from ethanol, yield 48%, 0.244 g, m.p. 131–133°C. IR (KBr): υ/cm^{-1} = 3,063 (CH aromatic), 2,987–2,934 ($4CH_3$), 2,863–2,811 ($2CH_2$), 2,571 (SH), 1,767, 1,729, 1,674 (3C═O), 1,666 (C═N), 1,645 (C═C), 1,123 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.33 (d, 3H, CH_3), 1.75 (t, 3H, J = 4.62 Hz, CH_3), 2.98, 3.23 (2s, 6H, $2OCH_3$), 3.34 (d, 2H, J = 2.28 Hz, CH_2), 3.69 (q, 2H, J = 4.62 Hz, CH_2), 4.58 (s, 1H, D_2O exchangeable, SH), 5.37 (m, 1H, CH), 6.67 (s, 1H, benzene ring). MS (relative intensity) m/z: 506 (M^+, 15.7%). Calcd for $C_{22}H_{19}ClN_2O_6S_2$ (506.98): C, 52.12; H, 3.78; N, 5.53; S, 12.65%. Found: C, 52.40; H, 4.06; N, 5.81; S, 12.93%.

Compound **5d**: Off-white crystals from ethanol, yield 74%, 0.410 g, m.p. 167–169°C. IR (KBr): υ/cm^{-1} = 3,043 (CH aromatic), 2,961–2,906 ($3CH_3$), 2,873 (CH_2), 2,602 (SH), 1,765, 1,712, 1,673 (3C═O), 1,655 (C═N), 1,643 (C═C), 1,117 (C–O). ^{1}H NMR (DMSO-d_6) δ = 0.85 (d, 3H, CH_3), 2.74, 2.98 (2s, 6H, $2OCH_3$), 3.41 (d, 2H, J = 3.18 Hz, CH_2), 5.12 (s, 1H, D_2O exchangeable, SH), 5.78 (m, 1H, CH), 6.98–7.43 (m, 6H, benzene rings). MS (relative intensity) m/z: 555 (M^+, 17.5%). Calcd for $C_{26}H_{19}ClN_2O_6S_2$ (555.02): C, 56.26; H, 3.45; N, 5.05; S, 11.55%. Found: C, 56.55; H, 3.72; N, 5.34; S, 11.27%.

2.2.4 General procedure for the synthesis of compounds: 7-chloro-4,6-dimethoxy-6′-methyl-3′-(2-phenylhydrazono)-3*H*-spiro[benzofuran-2,1′-cyclohexane]-2′,3,4′-trione (6a) and 7-chloro-3′-[2-(chlorohexa-1,3,5-triynyl)hydrazono]-4,6-dimethoxy-6′-methyl-3*H*-spiro-[benzofuran-2,1′-cyclohexane]-2′,3,4′-trione (6b)

Griseofulvic acid (**1**) (1.016 g, 0.003 mol) in ethanol (50 mL) was added to either benzenediazonium chloride or 4-chlorobenzenediazonium chloride (0.003 mol) [prepared by adding an

aqueous sodium nitrite solution (0.207 g, 0.003 mol) to a cold solution of either aniline (0.285 g, 0.003 mol) or 4-chloroaniline (0.382 g, 0.003 mol) in appropriate amount of glacial acetic acid (20 mL) at (0–5°C) with continuous stirring] at (0–5°C) in the presence of sodium acetate (0.246 g, 0.003 mol), with continuous stirring. The reaction mixture was stirred at room temperature for an additional 3 h and the solid product so formed, in each case, was collected by filtration.

Compound **6a**: Buff crystals from ethanol, yield 70%, 0.929 g, m.p. 191–193°C. IR (KBr): υ/cm^{-1} = 3,288 (NH), 3,063 (CH aromatic), 2,982, 2,969, 2,952 ($3CH_3$), 2,856 (CH_2), 1,777, 1,753, 1,708 (3C═O), 1,656 (C═C), 1,117 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.32 (d, 3H, CH_3), 2.65, 2.87 (2s, 6H, $2OCH_3$), 3.64 (d, 2H, J = 2.58 Hz, CH_2), 5.32 (m, 1H, CH), 6.88–7.41 (m, 6H, benzene rings), 9.72 (s, 1H, D_2O exchangeable, NH). MS (relative intensity) m/z: 442 (M^+, 22.7%). Calcd for $C_{22}H_{19}ClN_2O_6$ (442.85): C, 59.67; H, 4.32; N, 6.33%. Found: C, 59.38; H, 4.08; N, 6.06%.

Compound **6b**: Buff crystals from ethanol, yield 59%, 0.844 g, m.p. 217–219°C. IR (KBr): υ/cm^{-1} = 3,178 (NH), 3,056 (CH aromatic), 2,976, 2,953, 2,942 ($3CH_3$), 2,863 (CH_2), 1,771, 1,745, 1,712 (3C═O), 1,653 (C═C), 1,121 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.15 (d, 3H, CH_3), 2.67, 2.85 (2s, 6H, $2OCH_3$), 4.12 (d, 2H, J = 2.44 Hz, CH_2), 5.44 (m, 1H, CH), 7.12–7.38 (m, 5H, benzene rings), 9.92 (s, 1H, D_2O exchangeable, NH). MS (relative intensity) m/z: 477 (M^+, 18.2%). Calcd for $C_{22}H_{18}Cl_2N_2O_6$ (477.29): C, 55.36; H, 3.80; N, 5.87%. Found: C, 55.59; H, 4.06; N, 5.58%.

2.2.5 General procedure for the synthesis of compounds: 2-amino-7′-chloro-4′,6′-dimethoxy-7-methyl-3′,5-dioxo-4-(2-phenylhydrazono)-5,7-dihydro-3′*H*,4*H*-spiro [benzo[*b*]thiophene-6,2′-benzofuran]-3-carbonitrile (7a), ethyl 2-amino-7′-chloro-4′,6′-dimethoxy-7-methyl-3′,5-dioxo-4-(2-phenylhydrazono)-5,7-dihydro-3′*H*,4*H*-spiro[benzo [*b*]thiophene-6,2′-benzofuran]-3-carboxylate (7b), 2-amino-7′-chloro-4-[2-(chloro-hexa-1,3,5-triynyl) hydrazono]-4′,6′-dimethoxy-7-methyl-3′,5-dioxo-5,7-dihydro-3′*H*, 4H-spiro[benzo[*b*] thiophene-6,2′-benzo-furan]-3-carbonitrile (7c), and ethyl 2-amino-7′-chloro-4-[2-(chloro-hexa-1,3,5-triynyl) hydrazono] 4′,6′-dimethoxy-7-methyl-3′,5-dioxo-5,7-dihydro-3′ *H*,4*H*-spiro[benzo[*b*]thiophene-6,2′-benzofuran]-3-carboxylate (7d)

The solution of either compound **6a** (0.664 g, 0.00l5 mol) or **6b** (0.715 g, 0.00l5 mol) in ethanol (40 mL) containing triethylamine (1 mL) was added to either malononitrile (0.099 g, 0.0015 mol) or ethyl cyanoacetate (0.169 g, 0.00l5 mol) containing elemental sulfur (0.048 g, 0.0015 mol). The reaction mixture in both cases was heated under reflux for 5 h and poured onto ice–water mixture containing few drops of HCl. The solid product in each case was collected by filtration.

Compound **7a**: Pale brown crystals from ethanol, yield 76%, 0.596 g, m.p. 231–233°C. IR (KBr): υ/cm^{-1} = 3,387–3,181 (NH_2, NH), 3,055 (CH aromatic), 2,977, 2,961, 2,953 ($3CH_3$), 2,233 (CN), 1,745, 1,721 (2C═O), 1,656 (C═C), 1,111 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.18 (d, J = 3.28 Hz, 3H, CH_3), 2.73, 3.22 (2s, 6H, $2OCH_3$), 4.53 (s, 2H, D_2O exchangeable NH_2), 5.65 (m, 1H, CH), 7.14–7.38 (m, 6H, benzene rings), 9.43 (s, 1H, D_2O-exchangeable, NH). MS (relative intensity) m/z: 522 (M^+, 29.4%). Calcd for $C_{25}H_{19}ClN_4O_5S$ (522.96): C, 57.42; H, 3.66; N, 10.71; S, 6.13%. Found: C, 57.71; H, 3.93; N, 10.43; S, 5.88%.

Compound **7b**: Brown crystals from ethanol, yield 63%, 0.539 g, m.p. 250–252°C. IR (KBr): υ/cm^{-1} = 3,298–3,150 (NH_2, NH), 3,058 (CH aromatic), 2,988–2,933 ($4CH_3$), 1,762, 1,738, 1,717 (3C═O), 1,658 (C═C), 1,123 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.27 (d, J = 4.12 Hz, 3H, CH_3), 1.76 (t, 3H, J = 3.15 Hz, CH_3), 2.76, 3.12 (2s, 6H, $2OCH_3$), 3.25 (q, 2H, J = 3.15 Hz, CH_2), 5.12 (s, 2H, D_2O exchangeable NH_2), 5.78 (m, 1H, CH), 7.24–7.54 (m, 6H, benzene rings), 8.34 (s, 1H, D_2O exchangeable, NH). MS (relative intensity) m/z: 570 (M^+, 16.3%). Calcd for $C_{27}H_{24}ClN_3O_7S$ (570.01): C, 56.89; H, 4.24; N, 7.37; S, 5.63%. Found: C, 57.14; H, 3.96; N, 7.09; S, 5.35%.

Compound **7c**: Brown crystals from ethanol, yield 68%, 0.569 g, m.p. 188–190°C. IR (KBr): υ/cm^{-1} = 3,355–3,156 (NH_2, NH), 3,061 (CH aromatic), 2,983–2,944 ($3CH_3$), 2,224 (CN), 1,753, 1,714 (2C═O), 1,653 (C═C), 1,118 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.05(d, J = 3.34 Hz, 3H, CH_3), 2.66, 2.88 (2s, 6H, $2OCH_3$), 4.41 (s, 2H, D_2O exchangeable NH_2), 5.45 (m, 1H, CH), 7.18–7.51 (m, 5H, benzene rings), 9.88 (s, 1H, D_2O exchangeable, NH). MS (relative intensity) m/z: 557 (M^+, 12.8%). Calcd for $C_{25}H_{18}Cl_2N_4O_5S$ (557.41): C, 53.87; H, 3.25; N, 10.05; S, 5.75%. Found: C, 53.61; H, 3.03; N, 10.32; S, 5.47%.

Compound **7d**: Brown crystals from ethanol, yield 55%, 0.498 g, m.p. 160–162°C. IR (KBr): υ/cm^{-1} = 3,342–3,164 (NH_2, NH), 3,068 (CH aromatic), 2,971–2,928 ($4CH_3$), 1,770, 1,742, 1,715 (3C═O), 1,661 (C═C), 1,117 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.37 (d, J = 3.36 Hz, 3H, CH_3), 1.68 (t, 3H, J = 3.45 Hz, CH_3), 2.65, 2.88 (2s, 6H, $2OCH_3$), 3.34 (q, 2H, J = 3.45 Hz, CH_2), 4.75 (s, 2H, D_2O exchangeable NH_2), 5.64 (s, 1H, CH), 7.11–7.43 (m, 5H, benzene rings), 9.67 (s, 1H, D_2O exchangeable, NH). MS (relative intensity) m/z: 604 (M^+, 29.6%). Calcd for $C_{27}H_{23}Cl_2N_3O_7S$ (604.46): C, 53.65; H, 3.84; N, 6.95; S, 5.30%. Found: C, 53.38; H, 3.57; N, 7.21; S, 5.58%.

2.2.6 General procedure for the synthesis of compounds: 4′-amino-7-chloro-3′-imino-4,6-dimethoxy-6′-methyl-2′-phenyl-2′,3′-dihydro-3*H*-spiro[benzofuran-2,7′-thieno[4,3,2-*de*]cinnoline]-3,8′(6′*H*)-dione (8a), 4′-amino-7-chloro-4,6-dimethoxy-6′-methyl-2′-phenyl-3*H*-spiro[benzofuran-2,7′-thieno[4,3,2-*de*]cinnoline]-3,3′,8′(2′*H*,6′*H*)-trione (8b), 4′-amino-7-chloro-2′-(chlorohexa-1,3,5-triynyl)-3′-imino-4,6-dimethoxy-6′-methyl-2′,3′-dihydro-3*H*-spiro-[benzofuran-2,7′-thieno[4,3,2-*de*] cinnoline]-3,8′(6′*H*)-dione (8c), and 4′-amino-7-chloro-2′-(chlorohexa-1,3,5-triynyl)-4,6-dimethoxy-6′-methyl-3*H*-spiro[benzofuran-2,7′-thieno[4,3,2-*de*]cinnoline]-3,3′,8′(2′*H*,6′*H*)-trione (8d)

Catalytic amount of piperidine (1 mL) was added to the solution of either compound **7a** (0.522 g, 0.001 mol), **7b** (0.570 g, 0.001 mol), **7c** (0.557 g, 0.001 mol), or **7d** (0.604 g, 0.001 mol) in absolute ethanol (50 mL). The reaction mixture in each case was heated under reflux for 6 h and the progress was monitored under TLC. Upon completion of the reaction, the reaction mixture was poured onto ice/water containing few drops of HCl and the separated solid in each case was filtered and dried.

Compound **8a**: Pale brown crystals from 1,4-dioxane, yield 59%, 0.308 g, m.p. 188–190°C. IR (KBr): υ/cm^{-1} = 3,323–3,124 (NH_2, NH), 3,063 (CH aromatic), 2,982, 2,955, 2,941 ($3CH_3$), 1,749, 1,717 (2C═O), 1,654 (C═C), 1,119 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.31(d, J = 2.92 Hz, 3H, CH_3), 2.89, 3.15 (2s, 6H, $2OCH_3$), 4.67 (s, 2H, D_2O exchangeable NH_2), 5.45 (m, 1H, CH), 7.27–7.56 (m, 6H, benzene rings), 9.34 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR: δ = 18.7, 43.6, 54.1 (CH_3, $2OCH_3$), 65.1 (CH), 86.5 (CO), 120.9, 121.8, 123.1, 125.5, 127.6, 128.4, 130.3, 132.5, 133.8, 134.2, 136.6, 137.8, 139.2, 140.8, 141.3 (benzene C, thiophene C, furan, C pyridazine C), 159.9, 164.3 (2C═O). MS (relative intensity) m/z: 522 (M^+, 31.8%). Calcd for $C_{25}H_{19}ClN_4O_5S$ (522.96): C, 57.42; H, 3.66; N, 10.71; S, 6.13%. Found: C, 57.17; H, 3.38; N, 10.96; S, 6.41%.

Compound **8b**: Pale brown crystals from 1,4-dioxane, yield 66%, 0.345 g, m.p. 261–263°C. IR (KBr): υ/cm^{-1} = 3,372–3,276 (NH_2), 3,057 (CH aromatic), 2,988, 2,961, 2,953 ($3CH_3$), 1,735, 1,712, 1,673 (3C═O), 1,656 (C═C), 1,124 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.43 (d, J = 2.98 Hz, 3H, CH_3), 2.63, 2.87 (2s, 6H, $2OCH_3$), 4.38 (s, 2H, D_2O exchangeable NH_2), 5.31 (m, 1H, CH), 7.32–7.48 (m, 6H, benzene rings). ^{13}C NMR: δ = 20.5, 40.7, 43.9 (CH_3, $2OCH_3$), 58.1 (CH), 92.3 (CO), 120.2, 122.3, 123.7, 124.6, 127.5, 129.1, 130.8, 131.7, 133.5, 134.6, 135.7, 136.4, 138.1, 141.3, 142.6 (benzene C, thiophene C, furan C, pyridazine C), 161.4, 163.5, 164.7 (3C═O). MS (relative intensity) m/z: 523 (M^+, 38.2%). Calcd for $C_{25}H_{18}ClN_3O_6S$ (523.94): C, 57.31; H, 3.46; N, 8.02; S, 6.12%. Found: C, 57.59; H, 3.28; N, 8.31; S, 6.38%.

Compound **8c**: Brown crystals from 1,4-dioxane, yield 62%, 0.345 g, m.p. 207–209°C. IR (KBr): υ/cm^{-1} = 3,351–3,223 (NH_2, NH), 3,060 (CH aromatic), 2,986, 2,963, 2,952 ($3CH_3$), 1,756, 1,729 (2C═O), 1,657 (C═C), 1,126 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.12 (d, J = 3.14 Hz, 3H, CH_3), 2.31, 2.45 (2s, 6H, $2OCH_3$), 4.37 (s, 2H, D_2O exchangeable NH_2), 5.39 (m, 1H, CH), 7.13–7.43 (m, 5H, benzene rings), 8.28 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR: δ = 19.2, 41.2, 43.5 (CH_3, $2OCH_3$), 62.2 (CH), 89.1 (CO), 118.7, 120.6, 122.4, 123.6, 125.9, 126.8, 130.1, 131.7, 132.7, 133.8, 135.4, 137.3, 138.9, 140.7, 141.9 (benzene C, thiophene C, furan, C pyridazine C), 163.8, 167.5 (2C═O). MS (relative intensity) m/z: 557 (M^+, 22.7%). Calcd for $C_{25}H_{18}Cl_2N_4O_5S$ (557.41): C, 53.87; H, 3.25; N, 10.05; S, 5.75%. Found: C, 53.59; H, 3.51; N, 10.32; S, 5.48%.

Compound **8d**: Pale brown crystals from 1,4-dioxane, yield 71%, 0.396 g, m.p. 228–230°C. IR (KBr): υ/cm^{-1} = 3,263–3,138 (NH_2), 3,048 (CH aromatic), 2,971, 2,963, 2,941 ($3CH_3$), 1,747, 1,722, 1,668 (3C═O), 1,652 (C═C), 1,121 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.25 (d, J = 2.48 Hz 3H, CH_3), 2.23, 2.41 (2s, 6H, $2OCH_3$), 4.48 (s, 2H, D_2O exchangeable NH_2), 5.44 (m, 1H, CH), 7.52–7.69 (m, 5H, benzene rings). ^{13}C NMR: δ = 21.2, 44.8, 47.2 (CH_3, $2OCH_3$), 55.5 (CH), 87.2 (CO), 121.4, 122.9, 123.8, 125.5, 126.8, 128.3, 129.8, 131.4, 133.8, 134.7, 135.3, 137.8, 139.6, 140.5, 142.4 (benzene C, thiophene C, furan, C, pyridazine C), 160.5, 163.7, 167.6 (3C═O). MS (relative intensity) m/z: 558 (M^+, 25.3%). Calcd for $C_{25}H_{17}Cl_2N_3O_6S$ (558.39): C, 53.77; H, 3.07; N, 7.53; S, 5.74%. Found: C, 53.48; H, 3.33; N, 7.25; S, 5.97%.

2.2.7 General procedure for the synthesis of compounds: *N*-(7′-chloro-3-cyano-4′,6′-dimethoxy-5-methyl-3′,7-dioxo-5,7-dihydro-3′*H*,4*H*-spiro[benzo[*b*]thiophene-6,2′-benzofuran]-2-yl)-2-cyanoacetamide (9a) and ethyl 7′-chloro-2-(2-cyanoacetamido)-4′,6′-dimethoxy-5-methyl-3′,7-dioxo-5,7-dihydro-3′*H*,4*H*-spiro[benzo[*b*]thiophene-6,2′-benzofuran]-3-carboxylate (9b)

Either solution of compound **4a** (0.419 g, 0.001 mol) or compound **4b** (0.466 g, 0.001 mol) in absolute ethanol (50 mL) containing 0.5 mL of triethylamine, ethyl cyanoacetate (0.113 g, 0.001 mol) was added. The reaction mixture was heated under reflux for 4 h. The progress of the reaction was monitored using TLC. Upon completion of

the reaction, the contents were poured onto ice/water containing few drops of HCl and the separated solid was collected by filtration and dried.

Compound **9a**: Brown crystals from 1,4-dioxane, yield 55%, 0.267 g, m.p. 222–225°C. IR (KBr): υ/cm^{-1} = 3,317 (NH), 3,066 (CH aromatic), 2,987, 2,953, 2,941 ($3CH_3$), 2,858–2,841 ($2CH_2$), 2,234, 2,226 (2CN), 1,778, 1,732, 1,691 (3C═O), 1,649 (C═C), 1,116 (C–O). ^{1}H NMR (DMSO-d_6) δ = 0.88 (d, J = 3.26 Hz, 3H, CH_3), 2.66, 2.89 (2s, 6H, $2OCH_3$), 3.38 (d, 2H, CH_2), 4.58 (s, 2H, CH_2), 5.66 (m, 1H, CH), 6.97 (s, 1H, benzene ring), 8.53 (s, 1H, D_2O exchangeable, NH). MS (relative intensity) m/z: 485 (M^+, 21.4%). Calcd for $C_{22}H_{16}ClN_3O_6S$ (485.90): C, 54.38; H, 3.32; N, 8.65; S, 6.60%. Found: C, 54.63; H, 3.49; N, 8.91; S, 6.33%.

Compound **9b**: Dark brown crystals from 1,4-dioxane, yield 63%, 0.335 g, m.p. 241–243°C. IR (KBr): υ/cm^{-1} = 3,286 (NH), 3,062 (CH aromatic), 2,991–2,923 ($4CH_3$), 2,862–2,837 ($3CH_2$), 2,221 (CN), 1,797, 1,752, 1,726, 1,680 (4C═O), 1,648 (C═C), 1,114 (C–O). ^{1}H NMR (DMSO-d_6) δ = 0.96 (d, J = 3.22 Hz, 3H, CH_3), 2.16 (t, 3H, J = 3.72 Hz, CH_3), 2.83, 3.23 (2s, 6H, $2OCH_3$), 3.06 (d, 2H, CH_2), 3.35 (s, 2H, CH_2), 3.63 (q, 2H, J = 3.72 Hz, CH_2), 5.53 (m, 1H, CH), 6.86 (s, 1H, benzene ring), 9.12 (s, 1H, D_2O exchangeable, NH). MS (relative intensity) m/z: 532 (M^+, 17.6%). Calcd for $C_{24}H_{21}ClN_2O_8S$ (532.95): C, 54.09; H, 3.97; N, 5.26; S, 6.02%. Found: C, 53.82; H, 3.73; N, 5.53; S, 5.77%.

2.2.8 General procedure for the synthesis of compounds: 4-amino-7′-chloro-4′,6′-dimethoxy-6-methyl-3′,2,8-trioxo-6,8-dihydro-3′*H*,2*H*,5*H*-spiro [benzo-4,5-thieno [2,3-*b*]pyridine-7,2′-benzofuran]-3-carbonitrile (10a) and 7′-chloro-4-hydroxy-4′,6′dimethoxy-6-methyl-3′,2,8-trioxo-6,8-dihydro-3′*H*,2*H*,5*H*-spiro[benzo-4,5-thieno [2,3-*b*]pyridine-7,2-benzo furan]-3-carbonitrile (10b)

Method A: The solution of either compound **9a** (0.485 g, 0.001 mol) or **9b** (0.532 g, 0.001 mol) in abs. ethanol (50 mL) containing 0.5 mL of trimethylamine was heated under reflux for 4 h and poured onto ice/water containing few drops of HCl and the obtained solid in each case was filtered and dried.

Method B: Ethyl cyanoacetate (0.113 g, 0.001 mol) was added to a suspension of either **4a** (0.419 g, 0.001 mol) or **4b** (0.466 g, 0.001 mol), in sodium ethoxide (0.001 mol) [prepared by dissolving sodium metal (0.023 g, 0.001 mol) in absolute ethanol (30 mL)]. The reaction mixture was heated in a boiling water bath for 8 h and then left to cool. The solid product was formed on pouring onto ice/water containing few drops from HCl, which was collected by filtration and dried.

Compound **10a**: Buff crystals from 1,4-dioxane, yield 65%, 0.315 g, m.p. 112–114°C. IR (KBr): υ/cm^{-1} = 3,344–3,172 (NH_2, NH), 3,053 (CH aromatic), 2,993, 2,964, 2,952 ($3CH_3$), 2,861 (CH_2), 2,229 (CN), 1,768, 1,741, 1,681 (3C═O), 1,652 (C═C), 1,119 (C–O). ^{1}H NMR (DMSO-d_6) δ = 0.72 (d, J = 3.74 Hz, 3H, CH_3), 2.23, 2.46 (2s, 6H, $2OCH_3$), 3.58 (d, 2H, CH_2), 4.63 (s, 2H, D_2O exchangeable, NH_2), 5.83 (m, 1H, CH), 6.71 (s, 1H, benzene ring), 8.44 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR: δ = 18.5, 40.2, 41.3 (CH_3, $2OCH_3$), 42.7 (CH_2), 63.4 (CH), 87.6 (CO), 114.9 (CN), 122.9, 125.9, 128.2, 129.7, 130.5, 132.9, 134.1, 135.6, 137.3, 140.2, 142.3, 144.1 (benzene C, thiophene C, furan, C, pyridine C), 162.1, 163.9, 165.4 (3C═O). MS (relative intensity) m/z: 485 (M^+, 26.2%). Calcd for $C_{22}H_{16}ClN_3O_6S$ (485.90): C, 54.38; H, 3.32; N, 8.65; S, 6.60%. Found: C, 54.25; H, 3.17; N, 8.38; S, 6.38%.

Compound **10b**: Buff crystals from 1,4-dioxane, yield 61%, 0.297 g, m.p. 170–172°C. IR (KBr): υ/cm^{-1} = 3,282, 3,136 (OH, NH), 3,058 (CH aromatic), 2,990, 2,968, 2,951 ($3CH_3$), 2,863 (CH_2), 2,227 (CN), 1,753, 1,738, 1,672 (3C═O), 1,646 (C═C), 1,128 (C–O). ^{1}H NMR (DMSO-d_6) δ = 0.93 (d, J = 3.56 Hz, 3H, CH_3), 2.14, 2.42 (2s, 6H, $2OCH_3$), 3.62 (d, 2H, CH_2), 5.35 (m, 1H, CH), 6.98 (s, 1H, benzene ring), 8.11 (s, 1H, D_2O exchangeable, OH), 8.98 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR: δ = 17.8, 42.2, 43.4 (CH_3, $2OCH_3$), 48.9 (CH_2), 57.3 (CH), 88.8 (CO), 117.2 (CN), 121.8, 124.5, 127.6, 128.3, 131.6, 133.2, 134.5, 135.8, 138.4, 141.6, 142.8, 145.6 (benzene C, thiophene C, furan C, pyridine C), 165.4, 167.2, 168.5 (3C═O). MS (relative intensity) m/z: 486 (M^+, 16.6%). Calcd for $C_{22}H_{15}ClN_2O_7S$ (486.88): C, 54.27; H, 3.11; N, 5.75; S, 6.59%. Found: C, 54.55; H, 3.34; N, 5.98; S, 6.83%.

2.2.9 General procedure for the synthesis of compounds: 1-(7′-chloro-3-cyano-4′,6′-dimethoxy-5-methyl-3′,7-dioxo-5,7-dihydro-3′*H*,4*H*-spiro[benzo[*b*]thiophene-6,2′-benzofuran]-2-yl)-4,6-di-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (11a) and ethyl 7′-chloro-2-(3-cyano-4,6-di-methyl-2-oxopyridin-1(2*H*)-yl)-4′,6′-dimethoxy-5-methyl-3′,7-dioxo-5,7-dihydro-3′*H*,4*H*-spiro[benzo[*b*]thiophene-6,2′-benzofuran]-3-carboxylate (11b)

Acetylacetone (0.1 g, 0.001 mol) was added to a solution of either compound **9a** (0.485 g, 0.001 mol) or **9b** (0.532 g, 0.001 mol) in absolute ethanol (50 mL) containing piperidine (1 mL). The reaction mixture in each case was heated under reflux for 7 h and then poured onto ice/water

containing few drops of HCl and the solid thus obtained in each case was collected by filtration.

Compound **11a**: Pale brown crystals from 1,4-dioxane, yield 69%, 0.378 g, m.p. 180–182°C. IR (KBr): υ/cm^{-1} = 3,053 (CH aromatic), 2,991–2,926 ($5CH_3$), 2,841 (CH_2), 2,238, 2,223 (2CN), 1,772, 1,756, 1,683 (3C═O), 1,646 (C═C), 1,114 (C–O). ^{1}H NMR (DMSO-d_6) δ = 0.95–1.26 (2s, d, 9H, $2OCH_3$, CH_3), 2.21, 2.44 (2s, 6H, $2CH_3$), 3.44 (d, J = 3.38 Hz, 2H, CH_2), 5.57 (m, 1H, CH), 6.84 (m, 2H, benzene ring, pyridine ring). MS (relative intensity) m/z: 549 (M^+, 15.6%). Calcd for $C_{27}H_{20}ClN_3O_6S$ (549.98): C, 58.96; H, 3.67; N, 7.64; S, 5.83%. Found: C, 58.71; H, 3.44; N, 7.37; S, 5.55%.

Compound **11b**: Pale brown crystals from 1,4-dioxane, yield 63%, 0.376 g, m.p. 131–133°C. IR (KBr): υ/cm^{-1} = 3,060 (CH aromatic), 2,982–2,893 ($6CH_3$), 2,852–2,833 ($2CH_2$), 2,233 (CN), 1,779, 1,743, 1,688, 1,652 (4C═O), 1,643 (C═C), 1,117 (C–O). ^{1}H NMR (DMSO-d_6) δ = 1.15–1.37 (2s, d, 9H, $2OCH_3$, CH_3), 2.12 (t, 3H, J = 3.64 Hz, CH_3), 2.72, 3.18 (2s, 6H, $2CH_3$), 3.13 (d, J = 3.16 Hz, 2H, CH_2), 3.81 (q, 2H, J = 3.64 Hz, CH_2), 5.34 (m, 1H, CH), 6.66 (m, 2H, benzene ring, pyridine ring). MS (relative intensity) m/z: 597 (M^+, 31.4%). Calcd for $C_{29}H_{25}ClN_2O_8S$ (597.04): C, 58.34; H, 4.22; N, 4.69; S, 5.37%. Found: C, 58.61; H, 3.94; N, 4.38; S, 5.11%.

2.3 Pharmacology

2.3.1 *In vitro* cytotoxicity assay

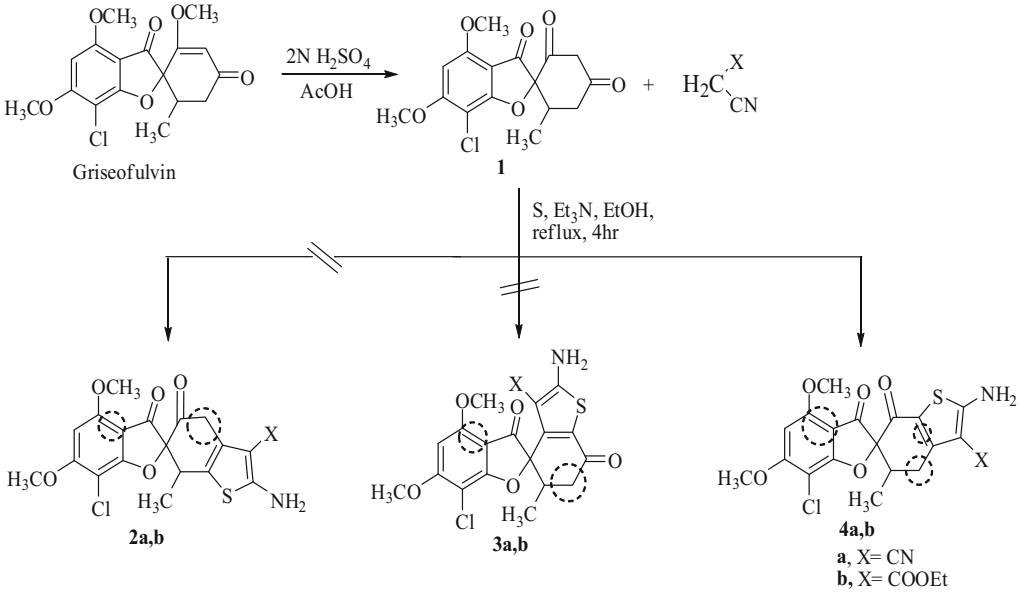
All the reagents and chemicals used for the study, such as doxorubicin, cisplatin, and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich (USA). Three different human tumor cell lines were used in the study including SF-268 (CNS cancer cell), MCF-7 (breast adenocarcinoma cell), and NCI-H460 (non-small lung cancer cell). The MCF-7 was afforded from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and NCI-H460 and SF-268 cells were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt).

The cytotoxic activity was evaluated using the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [17]. The cell suspensions were diluted to 10^5 cells mL^{-1}, appropriately prepared and distributed in plates of culture with 96 wells (225 µL in each well), and then incubated at 37°C in a humid atmosphere with 5% CO_2. After 24 h, 25 µL of either newly synthesized compounds **1**, **4a** and **b**, **5a**–**d**, **6a** and **b**, **7a**–**d**, **8a**–**d**, **9a** and **b**, **10a** and **b**, and **11a** and **b** or the reference drugs (doxorubicin and cisplatin) were added to each well. The plates were incubated again at 37°C for 72 h. Then, 25 µL of MTT solution (5 mg mL^{-1}) was added to each well, and the mixture was incubated further at 37°C for 2 h. Finally, the culture medium containing excess MTT was aspirated and 100 µL of DMSO was added to each well to dissolve the formazan crystals [18]. The optical density of the wells was measured at 540 nm and compared with the control (cells with medium only). The data represented the mean of experiments in triplicate and were expressed as mean ± standard deviation [19]. The concentration at which 50% survival of cells was observed (IC_{50}); the effective concentration causing 50% decrease in cell viability (EC_{50}) and their 95% confidence intervals were determined from nonlinear regression.

3 Results and discussion

3.1 Chemistry

In continuation to our previous studies on the synthesis of heterocyclic compounds with promising biological activities [20–23], this study was aimed to synthesize griseofulvin derivatives containing another heterocyclic moiety that could improve binding to the receptor and have better efficacy and selectivity. Griseofulvin drug was converted to griseofulvic acid (**1**), which was made to react with either malononitrile or ethylcyanoacetate in the presence of elemental sulfur and triethylamine to afford compounds **4a** and **b**. The structures of compounds **4a** and **b** were confirmed using spectroscopic techniques. The FT-IR spectrum of compound **4a** indicated the presence of two stretching vibration bands indicating two α,β-unsaturated carbonyl groups at υ_{max} = 1,703 and 1,677 cm^{-1}. Also, ^{13}C NMR spectrum showed two peaks at δ = 154.3 and 157.5 ppm showing the presence two α,β-unsaturated carbonyl groups. Thus, compounds **2a** and **b**, which contained only one α,β-unsaturated carbonyl group, were ruled out. Furthermore, ^{1}H NMR spectrum indicated the presence of one doublet at δ = 2.67 ppm due to the presence of 2H of γ-methylene of carbonyl group, which was further confirmed by ^{13}C NMR spectrum, which revealed one peak at δ = 44.8 ppm due to γ-methylene of the carbonyl group. Therefore, compounds **3a** and **b** containing α-methylene of the carbonyl group were excluded (Scheme 1). Thienopyrimidine derivatives **5a**–**d** were synthesized via the reaction of compounds **4a** and **b** with either ethylisothiocyanate or phenylisothiocyanate. The structures of compounds **5a**–**d**



Scheme 1: Synthetic routes of compounds **1** and **4a** and **b**; formation of **2a** and **b** and **3a** and **b** was ruled out in the spectral analysis.

were confirmed by elemental analysis and spectral data. In case of compound **5a**, the ^{13}C NMR spectrum revealed four peaks at δ = 17.6, 19.3, 35.1, and 36.3 ppm due to the presence of both two $-CH_3$ and two $-OCH_3$ groups; two peaks at δ = 37.5 and 42.3 ppm corresponding to two $-CH_2$ groups; one peak at δ = 56.6 ppm for one –CH group; one peak at δ = 83.9 ppm for one –CO group; eleven peaks at δ = 120.7, 121.9, 124.2, 126.3, 128.5, 129.3, 130.8, 132.7, 133.8, 134.1, and 136.2 ppm corresponding to benzene, thiophene, and pyrimidine; and two peaks at δ = 159.1 and 160.4 ppm indicating the presence of 2C=O groups. Arylhydrazono derivatives **6a** and **b** were afforded via reaction of griseofulvic acid (**1**) with aryldiazonium chlorides and the structures of synthesized compounds (**6a** and **b**) were established by spectral data. ^{1}H NMR spectrum of compound **6a** indicated the presence of a doublet at δ = 1.32 ppm, which could be assigned to the $-CH_3$ group; two singlets at δ = 2.65 and 2.87 ppm indicating the presence of two $-OCH_3$ groups; a doublet at δ = 3.64 ppm corresponding to one $-CH_2$ group; a multiplet at δ = 5.32 ppm representing the presence of one –CH group; a multiplet at δ = 6.88–7.41 ppm for the six protons of benzene rings; and a singlet at δ = 9.72 ppm indicating the presence of 1H of the –NH group. Hydrazono derivatives **6a**–**d** were further reacted with either malononitrile or ethylcyanoacetae in the presence of elemental sulfur and trimethylamine to prepare fused thiophene derivatives **7a**–**d**. The structures of these compounds (**7a**–**d**) were also confirmed using elemental and spectral data. The ^{1}H NMR spectrum of compound **7a** showed the presence of a doublet at δ = 1.18 ppm corresponding to the $-CH_3$ group; two singlets at δ = 2.73 and 3.22 ppm, indicating the presence of two $-OCH_3$ groups; a singlet at δ = 4.53 ppm corresponding to the two protons of $-NH_2$ group; a multiplet at δ = 5.65 ppm representing the presence of –CH group; a multiplet at δ = 7.14–7.38 ppm showing the presence of six protons of benzene rings; and a singlet at δ = 9.43 ppm indicating the presence of the single proton of –NH group. Furthermore, fused pyridazine derivatives **8a**–**d** were synthesized via internal cyclization of compounds **7a**–**d**. The structure elucidation of compounds **8a**–**d** was made using the elemental and spectral analysis. The FT-IR spectrum of compound **8a** indicated the absence of stretching vibration band of the –CN group confirming the cyclization (Schemes 2 and 3).

Fused benzo-thienopyridine derivatives **10a** and **b** were afforded by the reaction of compounds **4a** and **b** with ethylcyanoacetate using two different methods. The first one utilized triethylamine in a two-step process via formation of cyanoacetamido-fused thiophene derivatives **9a** and **b**, whereas the second method utilized sodium ethoxide in a single step. The structures of compounds **10a** and **b** were also confirmed using analytical and spectral data. The ^{1}H NMR spectrum of compound **10a** showed the presence of a doublet at δ = 0.72 ppm corresponding to the $-CH_3$ group; two singlets at δ = 2.23 and 2.46 ppm indicating the presence of two $-OCH_3$ groups; a doublet δ = 3.58 ppm corresponding to the $-CH_2$ group; a singlet at

δ = 4.63 ppm indicating the presence of $-NH_2$ group; a multiplet at δ = 5.83 ppm representing the presence of –CH group; a singlet at δ = 6.71 ppm due to one proton of benzene ring; and a singlet at δ = 8.44 ppm showing the presence of –NH group. Finally, compounds **11a** and **b** were prepared via reaction of cyanoacetamido-fused thiophene derivatives **9a** and **b** with acetylacetone. The structures of compounds **11a** and **b** were confirmed using analytical techniques. The ^{1}H NMR spectrum of compound **11a** showed the presence of two singlets and a doublet at δ = 0.95–1.26 ppm corresponding to the three $-CH_3$ groups; two singlets at δ = 2.21 and 2.44 ppm indicating the presence of two $-OCH_3$ groups; a doublet at δ = 3.44 ppm corresponding to the $-CH_2$ group; a multiplet at δ = 5.57 ppm indicating the presence of one –CH group; and a multiplet at δ = 6.84 ppm representing the presence of two protons of benzene and pyridine ring. The mass spectrum revealed m/z at 549 $[M]^+$ further confirming the synthesis of compounds.

3.2 Growth inhibition of human tumor cell lines

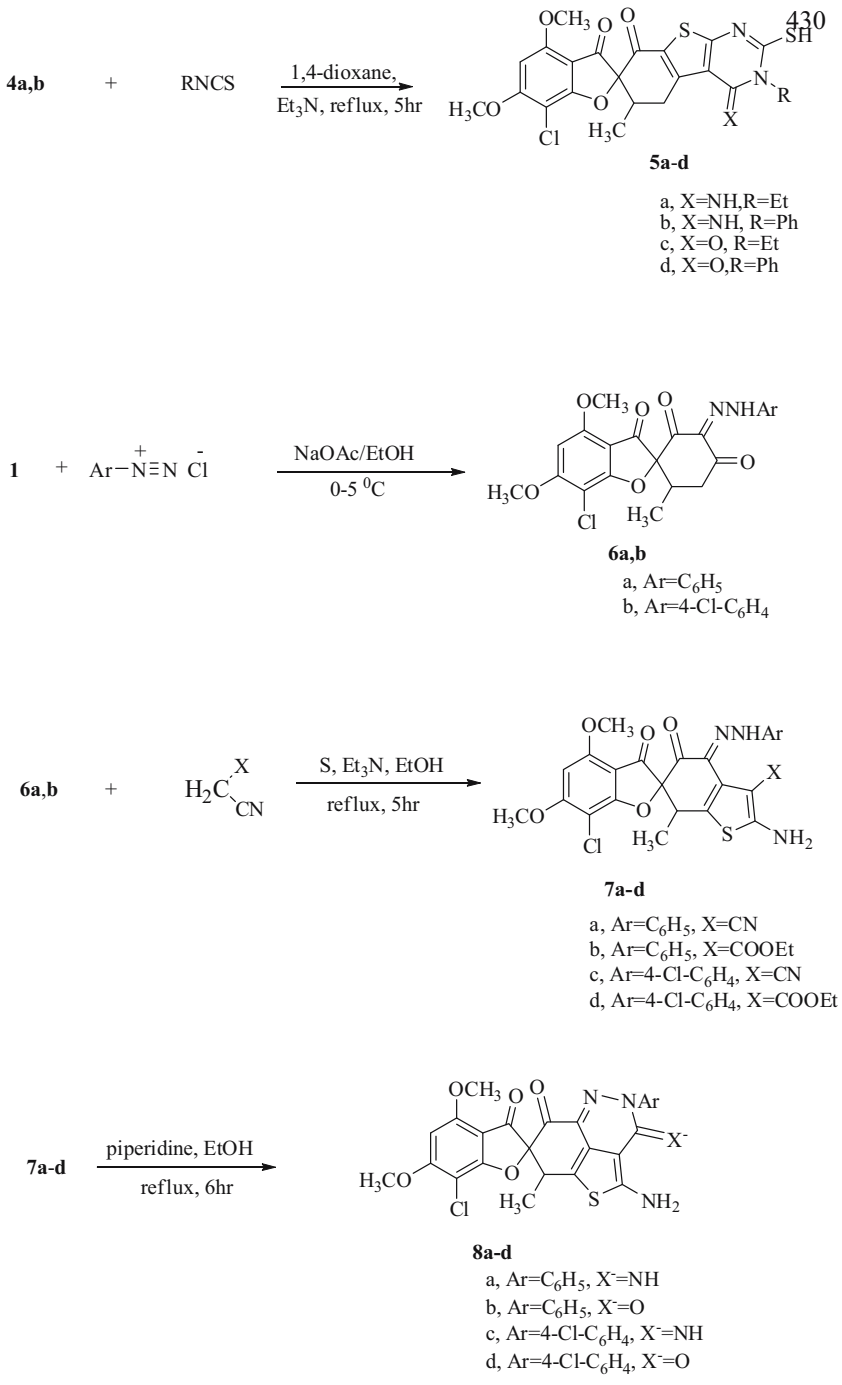
The inhibitory effects of the synthesized compounds **1–11a** and **b** on the growth of human tumor cell lines were evaluated *in vitro*. Three human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7), non-small-cell lung cancer (NCI-H460), and CNS cancer (SF-268) were used and the growth inhibition was measured after a continuous exposure of test compounds for 72 h. All the synthesized compounds were able to inhibit the growth of the tested human tumor cell lines in a dose-dependent manner. The obtained results (Table 1) indicated that the fused thiophene derivatives containing pyridine moiety (**11a**) showed highest efficacy against all three tumor cell lines (SF-268: 94.6%; MCF-7: 92.4%; NCI-H460: 93.5%) comparable to the reference drug, doxorubicin (SF-268: 87.5%; MCF-7: 89.7%; NCI-H460: 93.7%). Furthermore, fused thiophene derivatives of griseofulvin (**4a**) also showed marked inhibitory activities against all three tumor cell lines; non-small-cell lung cancer (NCI-H460: 92.9%), breast adenocarcinoma (MCF-7: 88.5%), and CNS cancer (SF-268: 91.3%) in comparison to other test compounds. On the other hand, compound **4b** (SF-268: 84.3%; MCF-7: 87.5%; NCI-H460: 82.4%), fused thiophene derivatives containing pyrimidine moiety (**5a**–**c**) (SF-268: 76.9–88.1%; MCF-7: 78.1–84.8%; NCI-H460: 75.3–83.6%), cyanoacetamido fused thiophene derivative **9a** (SF-268: 77.4%; MCF-7: 89.3%; NCI-H460: 81.1%), and fused thienopyridine derivative **10a** (SF-268: 83.5%; MCF-7: 82.6%; NCI-H460: 86.3%) also displayed good inhibitory effects against the three cancer cell lines. The remaining compounds **1**, **5d**, **6a** and **b**, **7a**–**d**, **8a**–**d**, **9b**, **10b** and **11b** showed moderate growth inhibitory effects (SF-268: 22.6–71%; MCF-7: 22.9–70.7%; NCI-H460: 25.3–59.2%).

Table 1: Cell growth inhibition percentage of compounds **1–11a** and **b** at 25 μg mL^{-1} on the three human tumor cell lines

Comp. no.	SF-268[a] (%)	MCF-7[a] (%)	NCI-H460[a] (%)
1	35.2 ± 6.5	38.8 ± 6.6	59.2 ± 3.7
4a	91.3 ± 1.3	88.5 ± 0.1	92.9 ± 1.4
4b	84.3 ± 2.3	87.5 ± 2.2	82.4 ± 4.2
5a	82.9 ± 1.5	84.8 ± 0.4	83.4 ± 0.5
5b	88.1 ± 2.1	87.9 ± 2.3	83.6 ± 3.7
5c	76.9 ± 4.6	78.1 ± 6.9	75.3 ± 1.9
5d	71.0 ± 5.9	70.7 ± 1.3	51.7 ± 0.9
6a	54.4 ± 3.7	55.7 ± 2.7	39 ± 3.1
6b	22.6 ± 4.8	22.9 ± 2.7	25.3 ± 1.7
7a	31.7 ± 8.9	49.2 ± 5.6	29.6 ± 0.8
7b	31.5 ± 1.9	37.3 ± 7.5	26.3 ± 1.2
7c	39.1 ± 9.2	37.7 ± 8.5	42.8 ± 9.1
7d	40.3 ± 8.4	41.9 ± 7.2	33.8 ± 8.5
8a	34.1 ± 7.2	28.3 ± 5.4	26.1 ± 7.9
8b	33.4 ± 5.6	31.3 ± 4.2	32.5 ± 8.2
8c	25.1 ± 8.4	27.2 ± 8.1	35.1 ± 7.1
8d	29.5 ± 7.9	35.9 ± 6.3	43.7 ± 9.7
9a	77.4 ± 2.6	89.3 ± 1.6	81.1 ± 2.7
9b	58.1 ± 1.5	60.4 ± 2.9	41.8 ± 5.5
10a	83.5 ± 0.9	82.6 ± 1.2	86.3 ± 1.9
10b	61.1 ± 1.2	49.5 ± 1.7	57.6 ± 0.7
11a	94.6 ± 2.2	92.4 ± 0.9	93.5 ± 1.3
11b	63.1 ± 1.6	62.4 ± 0.6	43.8 ± 0.2
Doxorubicin	87.5 ± 1.7	89.7 ± 0.7	93.7 ± 1.8

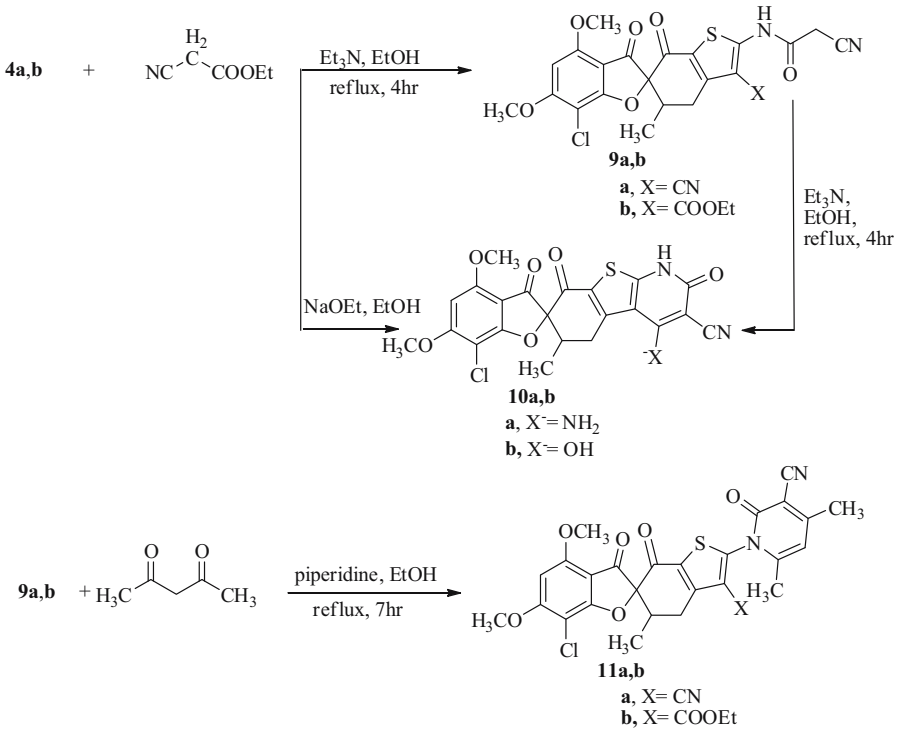
[a]Percentage inhibition ± standard deviation against CNS cancer (SF-268) cell line, breast adenocarcinoma (MCF-7) and non-small-cell lung cancer (NCI-H460).

Upon comparing the fused thiophene derivatives containing pyridinone moiety **11a** and **b**, it was evident that compound **11a** showed highest efficacy, which was comparable to the reference standard drug (doxorubicin). This was due to the presence of the cyanide group in **11a** instead of ethylcarboxylate in **11b**, which further increased the cytotoxicity. Also, upon comparing the fused thiophene derivatives of griseofulvin (**4a** and **b**), it was observed that compound **4a** showed better efficacy than **4b**, which was again due to the presence of cyanide group in **4a**. Upon analyzing the structure–activity relationship of fused thiophene derivatives containing pyrimidine moiety (**5a**–**d**), it was noticed that the compounds **5a** and **5c** showed similar inhibitory effects; however, the most active compound in



Scheme 2: Synthetic routes for compounds **5a–d**, **6a** and **b**, **7a–d**, and **8a–d**.

this group was **5b**. This was due to the presence of both imino and phenyl groups on pyrimidine ring in **5b**. While comparing compounds **9a** and **b**, it was found that compound **9a** showed better inhibition than compound **9b**, which was due to the presence of cyanide group increasing the cytotoxicity. Similarly, when the structures and activities of compounds **10a** and **b** were compared, it was observed that compound **10a** showed better inhibition than the compound **10b**, which was due to the presence of amino group in **10a**. On regarding and comparing the rest of compounds **1**, **5d**, **6a** and **b**, **7a–d**, and **8a–d**, it was clear that they have moderate growth inhibitory effects, but compound **5d** can be considered the more effective one in this group; it may be due to the presence of the thienopyrimidinone group.



Scheme 3: Synthetic routes for the compounds **9a** and **b, 10a** and **b**, and **11a** and **b**.

3.3 Cytotoxicity evaluation of selected compounds

The percentages of cell growth inhibition by all tested compounds (**1–11a** and **b**) were measured and the data are shown in Table 1. Compounds showing at least 75% inhibition in all tested cell lines at 25 μg mL^{-1} concentration (**4a** and **b**, **5a–c**, **9a**, **10a**, **11a**) were selected for further evaluation. The half maximal inhibitory concentration (IC_{50}) values (μM) were determined for these compounds and the results are displayed in Table 2. The highest cytotoxic activity against all three different cell lines was displayed by the fused thiophene derivatives containing pyridine moiety (**11a**), whereas compounds **4b**, **5b**, **5c**,

Table 2: Cytotoxic activity expressed as IC_{50} of most active compounds against tumor cell lines

Comp. no.	Cell line IC_{50} (μg mL^{-1})			
	SF-268	**MCF-7**	**NCI-H460**	**BGMK**
4a	4.97 (3.01–7.16)	16.62 (13.5–21.31)	ND	ND
4b	3.82 (3.12–4.81)	10.68 (5.56–17.87)	11.49 (9.13–14.42)	8.26 (7.45–9.64)
5a	12.61 (11.91–13.57)	4.58 (3.80–5.64)	15.63 (13.20–18.57)	6.55 (5.12–8.65)
5b	4.73 (4.61–4.83)	7.26 (6.95–9.13)	ND	4.21 (2.84–5.92)
5c	0.10 (0.08–0.16)	12.18 (10.60–14.07)	10.64 (10.04–11.43)	4.78 (3.33–6.92)
9a	0.66 (0.58–0.82)	12.27 (10.37–14.56)	14.25 (10.72–19.06)	8.18 (5.76–13.59)
10a	0.90 (0.8–1.1)	11.33 (10.23–12.56)	13.80 (11.83–16.03)	5.93 (5.4–6.5)
11a	0.50 (0.5–0.6)	1.13 (1.03–1.36)	1.60 (1.23–1.93)	22.83 (22.3–23.4)
Doxorubicin	0.35 (0.18–0.45)	0.42 (0.18–0.95)	0.64 (0.42–0.83)	>49.2
Cisplatin	ND	ND	ND	9.3 (6.66–12.04)

Data are presented as half maximal inhibitory concentration (IC_{50}) values and 95% confidence intervals obtained by nonlinear regression for all cell lines. Doxorubicin was used as positive control. Compounds with an IC_{50} value lower than 5 μg mL^{-1} against at least one cell line were considered active; SF-268 = CNS cancer cell line, MCF-7 = breast adenocarcinoma cell line, NCI-H460 = non-small-cell lung cancer cell line, ND = not determined.

9a, and **10a** were found to have good-to-moderate cytotoxic effects against all tested tumor cell lines. Compounds showing least cytotoxic activity against all three cell lines were fused thiophene derivatives of griseofulvin (**4a**) and fused thiophene derivatives containing pyrimidine-imine moiety (**5a**). Overall, the tested compounds presented consistent cytotoxicity against the tested tumor cell lines, although they displayed remarkable inhibitory effects against the buffalo green monkey kidney cell growth (BGMK) used to assess the selectivity.

When tested against the CNS cancer cell line (SF-268), it was found that the fused thiophene derivatives containing pyrimidine-one moiety (**5c**) had cytotoxic activity higher than the remaining compounds. Furthermore, compounds **9a**, **10a**, and **11a** also showed good cytotoxicities; whereas compounds **4a**, **4b**, **5a**, and **5b** had moderate cytotoxic properties. Similarly, fused thiophene derivatives containing pyridine moiety **11a** showed highest inhibition against the breast adenocarcinoma (MCF-7) cells and non-small-cell lung cancer (NCI-H460) cell lines. The remaining compounds **4a** and **5b** did not show any cytotoxic effects on the non-small-cell lung cancer (NCI-H460) (Table 2).

4 Conclusion

In this study, griseofulvic acid was successfully converted to several quadricyclic and pentacyclic derivatives and the structure of each derivative was characterized spectroscopically. Antitumor testing of the synthesized derivatives provided noteworthy examples of compounds, which were active against different human cancer cell lines including non-small cell lung cancer (NCI-H460), breast adenocarcinoma (MCF-7), and CNS cancer (SF-268). Compounds 4a and 11a showed significant inhibitory activity against the three cell lines comparable to the standard drug doxorubicin. All the tested compounds proved to be superior to doxorubicin for cytotoxic effects against the BGMK cell lines.

Acknowledgments: The authors would like to thank the research groups working at both the National Research Center, Dokki, Egypt, and the National Cancer Institute for recording the antitumor evaluation for the synthesized products. Moreover, the staff working at Substance Abuse Research Center (SARC) in Pharmacy College, Jazan University, are greatly appreciated for their kind revisions for the analytical and spectral data.

Funding information: This work was supported by Substance Abuse Research & Toxicology Center (SARTC) at Jazan University for carrying out the analytical and spectral data and National Cancer Institute in Egypt for recording the antitumor evaluation of the newly synthesized compounds.

Author contributions: Conceptualization: K.A.E.-S. and H.A.A. conceived the research formulations and information of the synthesized compounds and wrote the article. Formal analysis: M.A. performed and established the structures by applying different spectral data. Data curation: M.A. performed to integrate and maintain the obtained research data. Writing – review & editing: K.A.E.-S. and A.N. prepared, wrote, and revised the article for publication.

Conflict of interest: Authors declare no conflict of interest.

Informed consent: Not applicable.

Ethical approval: Not applicable.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Al-Obaidi H, Kowalczyk RM, Kalgudi R, Zariwala MG. Griseofulvin solvate solid dispersions with synergistic effect against fungal biofilms. Colloids Surf B. 2019;184:110540. doi: 10.1016/j.colsurfb.2019.110540.

[2] Aggarwal N, Goindi S. Preparation and evaluation of antifungal efficacy of griseofulvin loaded deformable membrane vesicles in optimized guinea pig model of Microsporum canis-Dermatophytosis. Int J Pharm. 2012;437(1–2):277–87. doi: 10.1016/j.ijpharm.2012.08.015.

[3] Gupta AK, Adam P, Dlova N, Lynde CW, Hofstader S, Morar N, et al. Therapeutic op-tions for the treatment of tinea capitis caused by Trichophyton species: griseofulvin versus the new oral antifungal agents, terbinafine, itraconazole, and fluconazole. Pediatr Dermatol. 2001;18(5):433–8. doi: 10.1046/j.1525-1470.2001.01978.x.

[4] Liéby-Muller F, Le Baliner QH, Grisoni S, Fournier E, Marion F. Synthesis and activities towards resistant cancer cells of sulfone and sulfoxide griseofulvin derivatives. Bioorg Med Chem Lett. 2015;25:2078–81. doi: 10.1016/j.bmcl.2015.03.081.

[5] Rønnest MH, Rebacz B, Markworth L, Terp AH, Larsen TO, Krämer A, et al. Synthesis and structure–activity relationship of griseofulvin analogues as inhibitors of centrosomal clustering in cancer cells. J Med Chem. 2009;52(10):3342–7. doi: 10.1021/jm801517j.

[6] Geronikaki A, Kartsev V, Petrou A, Akrivou MG, Vizirianakis IS, Chatzopoulou FM, et al. Antibacterial activity of griseofulvin analogues as an example of drug repurposing. Int J Antimicrob Agents. 2020;55(3):105884. doi: 10.1016/j.ijantimicag.2020.105884.

[7] Petersen AB, Andersen NS, Konotop G, Md Hanafiah NH, Raab M, Krämer SA, et al. Synthesis and formulation studies of griseofulvin analogues with improved solubility and metabolic stability. Eur J Med Chem. 2017;130:240–7. doi: 10.1016/j.ejmech.2017.02.055.

[8] Kim Y, Alpmann P, Blaum-Feder S, Krämer S, Endo T, Lu D, et al. In vivo efficacy of griseofulvin against multiple myeloma. Leuk Res. 2011;35(8):1070–3. doi: 10.1016/j.leukres.2010.10.008.

[9] Faergemann J, Anderson C, Hersle K, Hradil E, Nordin P, Kaaman T, et al. Double-blind, paral-lel-group comparison of terbinafine and griseofulvin in the treatment of toenail onychomycosis. J Am Acad Dermatol. 1995;32(5):750–3. doi: 10.1016/0190-9622(95) 91454-4-.

[10] Zaher AF, Khalil NA, Ahmed EM. Synthesis and anticonvulsant activity of new 3′-aryl-7 -bromo-spiro[[1] benzothio-phene-3,2′-[1,3]thiazolidine]-2,4′-dione derivatives. Orient J Chem. 2010;26(4):1241–8.

[11] Kanagaraju G, Thangamani A. Design and synthesis of spiro derivatives containing a thiophene ring and evaluation of their anti-microbial activity. Orient J Chem. 2014;30(4):1619–30. doi: 10.13005/ojc/300421.

[12] Braun M, Hessamian-Alinejad A, de Lacroix BF, Alvarez BH, Fischer G. Novel spiroannulated 3-benzofuranones. synthesis and inhibition of the human peptidyl prolyl cis/trans isomerase Pin1. Molecules. 2008;13:995–1003. doi: 10.3390/molecules13040995.

[13] Yang C, Li J, Zhou R, Chen X, Gao Y, He Z. Facile synthesis of spirooxindole-pyrazolines and spirobenzo-furanone-pyrazolines and their fungicidal activity. Org Biomol Chem. 2015;13(17):4869–78. doi: 10.1039/c5ob00258c.

[14] Abou-Elmagd WSI, Hashem AI. Synthesis and antitumor activity evaluation of some novel-fused and spiro heterocycles derived from a 2(3H) furanone derivative. J Heterocycl Chem. 2016;53(1):202–8. doi: 10.1002/jhet.2401.

[15] Chen C, He L. Advances in research of spirodienone and its derivatives: Biological activities and synthesis methods. Eur J Med Chem. 2020;203:112577. doi: 10.1016/j.ejmech.2020.112577.

[16] Walker T, Process of preparing griseofulvic acid. US Pat. 3102122, 27 Aug. 1963

[17] Alley MC, Scudiero DA, Monsk A, Hursey MI, Czerwinski MJ, Fine DL, et al. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res. 1988;48:589–601.

[18] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity as-says. J Immunol Methods. 1983;16:55–63. doi: 10.1016/0022-1759(83)90303-4.

[19] Berridge MV, Tan AS, McCoy KD, Wang R. The biochemical and cellular basis of cell proliferation assays that use tetrazolium salts. Biochemica. 1996;4:14–9.

[20] Mohareb RM, Abdo NYM, EL-Sharkawy KA. New approaches for the uses cyclohexan-1,4-dione for the synthesis of 5,6,7,8-tetrahydro-benzo-[4,5]thieno[2,3-b]pyridine derivatives used as potential anti-prostate cancer agents and Pim-1 ki-nase inhibitors. Anti-Cancer Agents Med Chem. 2018;18(12):1736–49. doi: 10.2174/1871520618666180604091135.

[21] Mohareb RM, EL-Sharkawy KA, Al Farouk FO. Synthesis, cytotoxicity against cancer and normal cell lines of novel hydrazide–hydrazone derivatives bearing 5H-chromen-5-one. Med Chem Res. 2019;28:1885–900. doi: 10.1007/s00044-019-02421-6.

[22] AlBratty MM, El-Sharkawy KA, AlHazmi HA. Design, synthesis and biological evaluation of pyrimidine-based deriva-tives as antitumor agents. Rev Roum Chim. 2020;65(3):227–38. doi: 10.33224/rrch.2020.65.3.02.

[23] AlBratty M, El-Sharkawy KA, Alam MS. Synthesis and antitumor activity of some novel thiophene, pyrimidine, coumarin, pyrazole and pyridine derivatives. Acta Pharmaceut. 2017;67(1):15–33. doi: 10.1515/acph-2017-0004.