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Synthesis and antitumor evaluation of novel tetrahydrobenzo[4′,5′]thieno[3′,2′:5,6]pyrimido[1,2-*b*]isoquinoline derivatives

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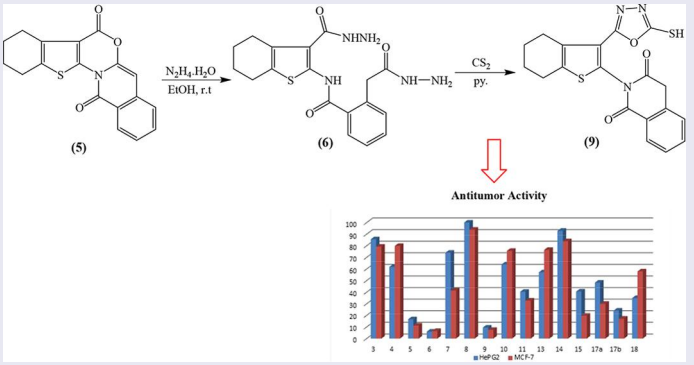
ABSTRACT

In the present study, a novel 8,9,10,11-tetrahydro-7*H*,14*H*-benzo[4′,5′]thieno[2′,3′:4,5]-1,3-oxazino[3,2-*b*]isoquinoline-7,14-dione **5** was prepared by condensation of 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzothiophene with homophthalic anhydride under microwave irradiation, followed by alkaline hydrolysis and cyclization using acetyl chloride. Compound **5** was further allowed to react with different nitrogen nucleophiles to get new tetrahydrobenzothieno-pyrimido isoquinolinone derivatives. The structures of the prepared compounds were elucidated by IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy. The newly prepared compounds were tested *in vitro* against a panel of two human tumor cell lines, namely, hepatocellular carcinoma (liver) HepG2, and mammary gland breast MCF-7. Almost all the tested compounds showed satisfactory activity.

GRAPHICAL ABSTRACT



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Antitumor activity; homophthalic anhydride; microwave irradiation; tetrahydrobenzothieno-pyrimidoisoquinolinone derivatives; tetrahydrobenzothiophene derivatives

Introduction

Thienopyrimidines, being bioisostere and structural analogs of the natural purines, occupy a special position among condensed pyrimidines. Design and synthesis of thienopyrimidine derivatives as potential cancer chemotherapeutic agents have been extensively studied.[1–11] The thienopyrimidine derivatives were reported to exhibit

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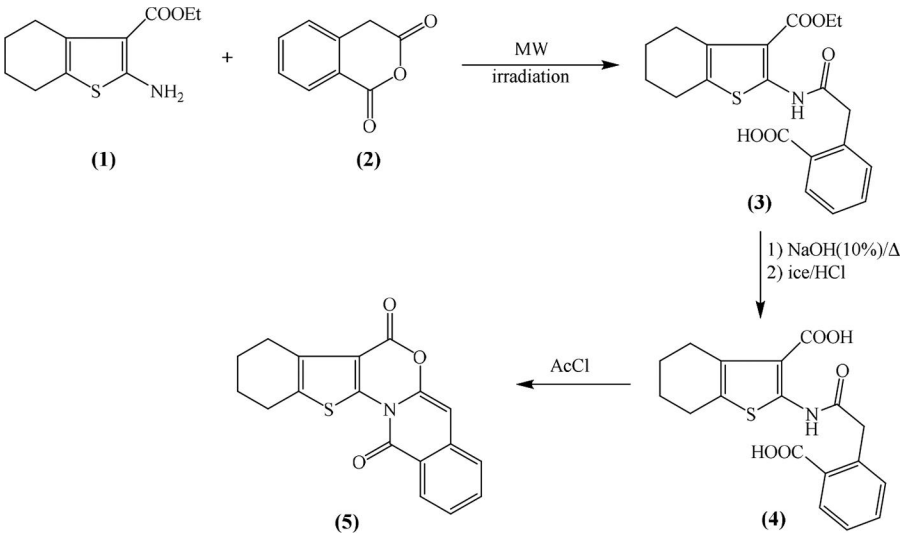
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/lsyc.
Supplemental data [full experimental details and spectroscopic data (IR spectra, ^{1}H-NMR, ^{13}C-NMR, and MS) for compounds **4–11, 13–15, 17a, b, 18**] can be accessed on the publisher's website.

antitumor activity through the inhibition of receptor tyrosine kinases,[3–5] cyclin-dependent kinases,[6–9] or checkpoint kinases.[10,11] A large number of tetrahydrobenzothienopyrimidine derivatives were reported as virucides, bactericides, fungicides, acaricides, anticancer, antimicrobial, antihistaminic, and analgesic activities.[12–22] Moreover, tetrahydrobenzothiophene derivatives were reported as inhibitors of hepatitis C virus NS5B polymerase.[23] In continuation of our efforts in developing heterocycles of biological interest[24–27] and considering the significant role of tetrahydrobenzothiophene derivatives in biological applications, we wish to report here the synthesis of a new series of polyheterocycles containing the thienopyrimidoisoquinoline system that may show biological activities.

Results and discussion

The aim of this study is to describe the synthesis of some new compounds with thienopyrimidoisoquinoline moiety to further assess the pharmacological profile of this class of compounds. The present work deals with the synthesis of these compounds as well as their antitumor activity, for this purpose, we performed the condensation of 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzothiophene **1** with homophthalic anhydride **2** under microwave irradiation to afford the half ester **3** which upon hydrolysis with sodium hydroxide (10%) followed by acidification yielded the corresponding dibasic acid **4** (Scheme 1).

Structural elucidation of the synthesized compounds **3** and **4** was attained by the aid of IR, ^{1}H-NMR, mass spectra, and elemental analyses. Thus, the IR spectrum of compound **3** revealed absorption band characteristic for the presence of NH group at 3237 cm^{-1} and three carbonyl stretching bands for $\nu_{c=o}$(ester) at 1747 cm^{-1}, $\nu_{c=o}$(acid) at 1693 cm^{-1} and $\nu_{c=o}$(amide) at 1664 cm^{-1}. The ^{1}H-NMR spectrum of **3** in dimethyl sulfoxide (DMSO-d_6) showed from low to high field the following signals at δ 13.0 ppm (s, 1H, COOH, exchangeable with D_2O), 10.89 ppm (s, 1H, NH, exchangeable with D_2O),



Scheme 1. Synthesis of 1,3-oxazinoisoquinoline-7,14-dione derivative **5**.

7.98–7.42 ppm (m, 4H, Ar–H), 4.22–4.15 ppm (q, 2H, $COOCH_2CH_3$, $J = 7.2$ Hz), 3.70 ppm (s, 2H, $COCH_2$), 2.67–2.51 ppm (m, 4H, $2CH_2$), 1.69 ppm (m, 4H, $2CH_2$), and 1.26–1.22 ppm (t, 3H, $COOCH_2CH_3$, $J = 7.2$ Hz). Furthermore, the mass spectrum of **3** showed the molecular ion peak at $m/z = 387$ (63.5%) together with the base peak at $m/z = 225$ (100%) characteristic for the radical cation of ethyl 2-amino tetrahydrobenzo thiophene-3-carboxylate.

The corresponding dibasic acid **4** shows the absence of carbonyl stretching band for the ester group in its IR spectrum and retained absorption band for $\nu_{c=o}$ at 1694 cm^{-1} (acid), 1670 cm^{-1} (amide).

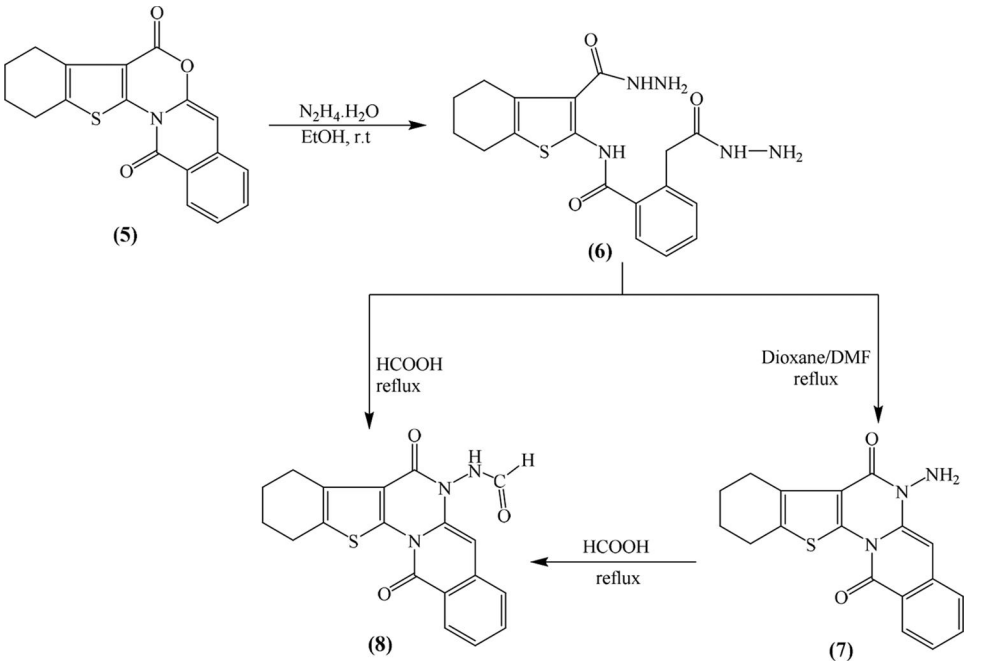
The desired compound 8,9,10,11-tetrahydro-7*H*,14*H*-benzo[4′,5′]thieno[2′,3′:4,5][1,3] oxazine[3,2-*b*]isoquinoline-7,14-dione **5** was obtained as the sole product (thin-layer chromatography (TLC)) upon treatment of the dibasic acid **4** with excess acetyl chloride (Scheme 1). The structure of compound **5** was confirmed by complete analysis of IR, ^{1}H-NMR, and mass spectrum beside the correct elemental analysis. Thus, the IR spectrum shows absorption bands for carbonyl group of oxazinone at 1769 cm^{-1} and cyclic amide at 1675 cm^{-1}. ^{1}H-NMR spectrum (DMSO-d_6) of compound **5** revealed the presence of signals corresponding to aromatic protons (4H) as multiplet in the region at δ 8.27–7.48 ppm another singlet (1H) at δ 6.65 ppm for olefinic proton (C_5-H) and three multiplet integrated for 2H, 2H, and 4H at δ 2.80, 2.74, and 1.80 ppm, respectively.

Heteroannulated 3,1-oxazine-4-ones are of increasing preparative interest due to their reactivity toward nucleophiles. Nucleophilic attack at the carbonyl group of the oxazinone results in ring cleavage amide which may be recyclized to form heterocondensed pyrimidines and pyridines depending on the nucleophile.[28]

Hydrazinolysis of compound **5** using hydrazine hydrate in ethanol at room temperature afforded the 2-substituted amino tetrahydrobenzothiophene-3-carbohydrazide derivative **6**. Surprisingly, 6-amino-8,9,10,11-tetrahydro-7*H*-benzo[4′,5′]thien[3′,2′:5,6]pyrimido[1,2-*b*] isoquinoline-7,14(6*H*)-dione **7** was obtained as the sole product in good yield upon heating compound **6** with dioxane/DMF mixture (Scheme 2). The ^{1}H-NMR (DMSO-d_6) spectrum of compound **7** revealed the presence of signals downfield for aromatic protons (4H) as multiplet in the region of δ 8.10–7.28 ppm, singlet for one olefinic proton (C_5-H) at δ 6.87 ppm, sharp singlet at δ 5.42 ppm integrated for two protons disappeared with D_2O (NH_2) and three multiplet appeared upfield at δ 2.77, 2.58, and 1.69 ppm integrated for 8H with ratio 2:2:4, respectively. Furthermore, the mass spectrum of **7** is a good support for such structure, since it shows the correct molecular ion peak at $m/z = 337$ (100%) which is the base peak.

Refluxing compound **6** and/or compound **7** with formic acid yielded one and the same product which identified using spectral data as 6-formylamino-8,9,10,11-tetrahydro-7*H*-benzo[4′,5′]thieno[3′,2′:5,6]pyrimido[1,2-*b*]isoquinoline-7,14(6*H*)dione **8**. ^{1}H-NMR spectrum of **8** indicates the presence of two singlets at δ 10.99 ppm (disappeared with D_2O) and 8.48 ppm for NH and formyl protons. Moreover, IR spectrum displayed γ_{NH}(sharp) at 3303 cm^{-1} and $\gamma_{c=o}$(aldehydic group) at 1719 cm^{-1} (cf. Exp.). Furthermore, the highest recorded peak in the mass spectrum of **8** at $m/z = 365$ (7.09%) represents the correct molecular ion peak.

The structure of compound **6** gets a further chemical support from the study of its reaction with some electrophilic reagents such as carbon disulfide, triethylorthoformate, and acetic anhydride. Thus, refluxing the dihydrazide derivative **6** with carbon disulfide



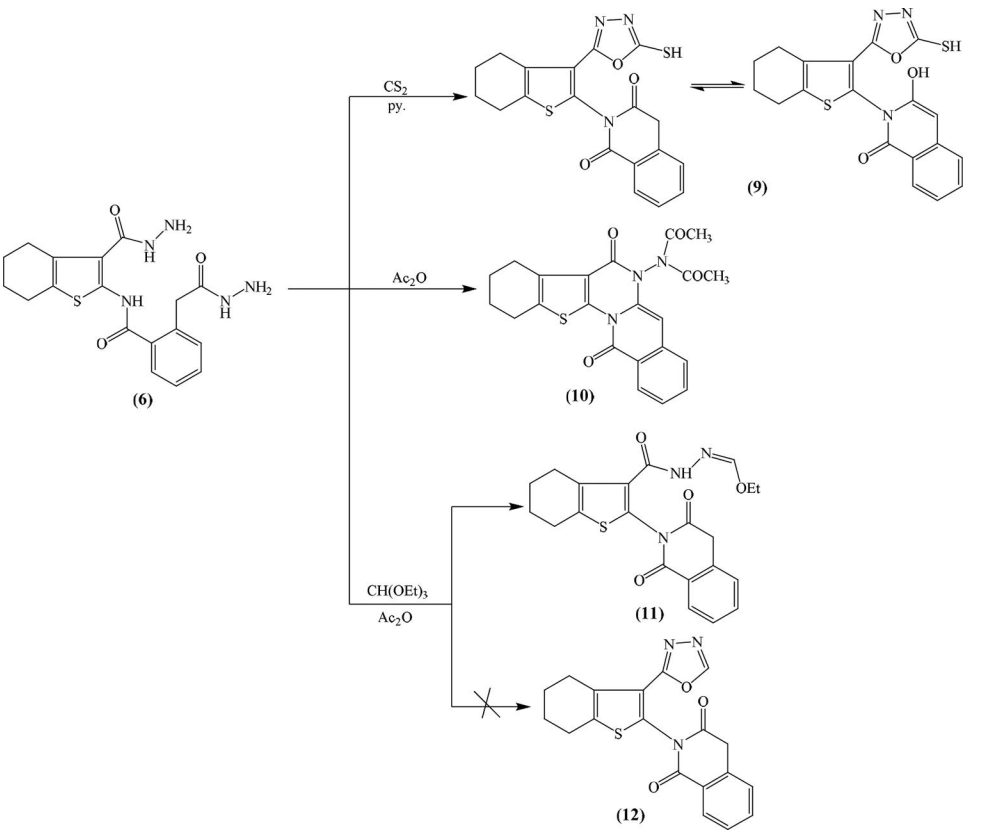
Scheme 2. Hydrazinolysis of compound **5**. *Note*: DMF, dimethylformamide.

in pyridine afforded 2-(3-(5-mercapto-1,3,4,-oxadiazol-2-yl)-4,5,6,7-tetrahydrobenzo[*b*] thiophen-2-yl)isoquinoline-1,3(2*H*,4*H*)-dione **9** (Scheme 3). The molecular weight determination of compound **9** indicates the incorporation of one sulfur atom whose molecular formula is $C_{19}H_{15}N_3O_3S_2$ [$M^+ = 397$ (3.66%)]. Furthermore, IR and ^{1}H-NMR spectra of **9** are completely matched with the assigned structure (cf. Exp.).

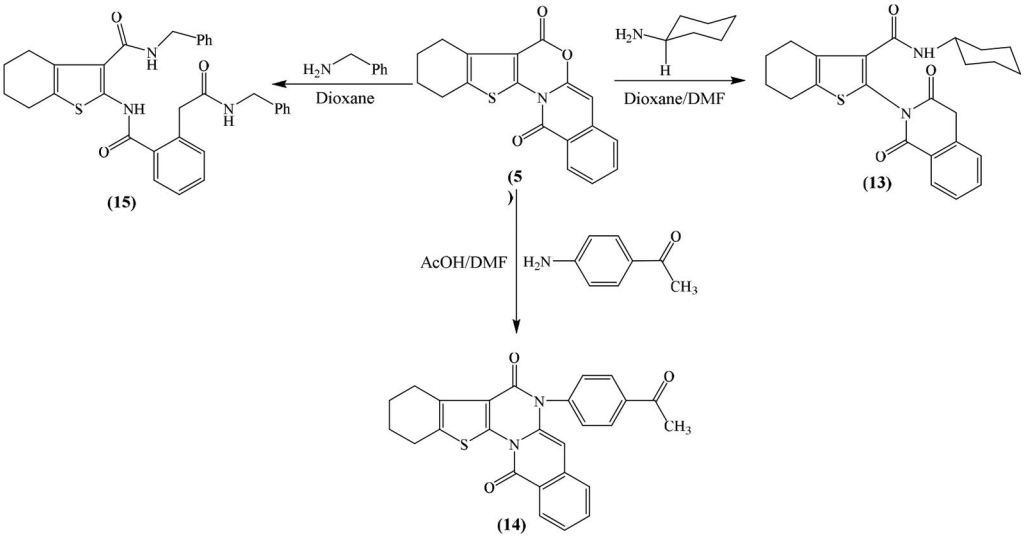
Acylation of compound **6** using freshly distilled acetic anhydride yielded the diacylated product **10** (Scheme 3). Moreover, the reaction of compound **6** with one carbon donor such as triethylorthoformate in the presence of acetic anhydride gave the uncyclized product **11** and no evidence was observed for the cyclized oxadiazole derivative **12** (Scheme 3). ^{1}H-NMR spectrum of the product revealed the existence of triplet and quartet signals characteristic for ethyl protons which are consistent with the uncyclized structure **11** and reject the cyclic structure **12**.

The tendency of 1,3-oxazinoisoquinoline-7,14-dione **5** for undergoing nucleophilic addition with primary and secondary amines was also investigated. Thus, the reaction of compound **5** with cyclohexyl amine in boiling dioxane/DMF afforded the isoquinoline-1,3-dione derivative **13** (Scheme 4). Also, refluxing compound **5** with *p*-aminoacetophenone in DMF in the presence of drops of glacial acetic acid gave the cyclic product **14** (Scheme 4). Structures **13** and **14** were deduced from the analytical and spectral data.

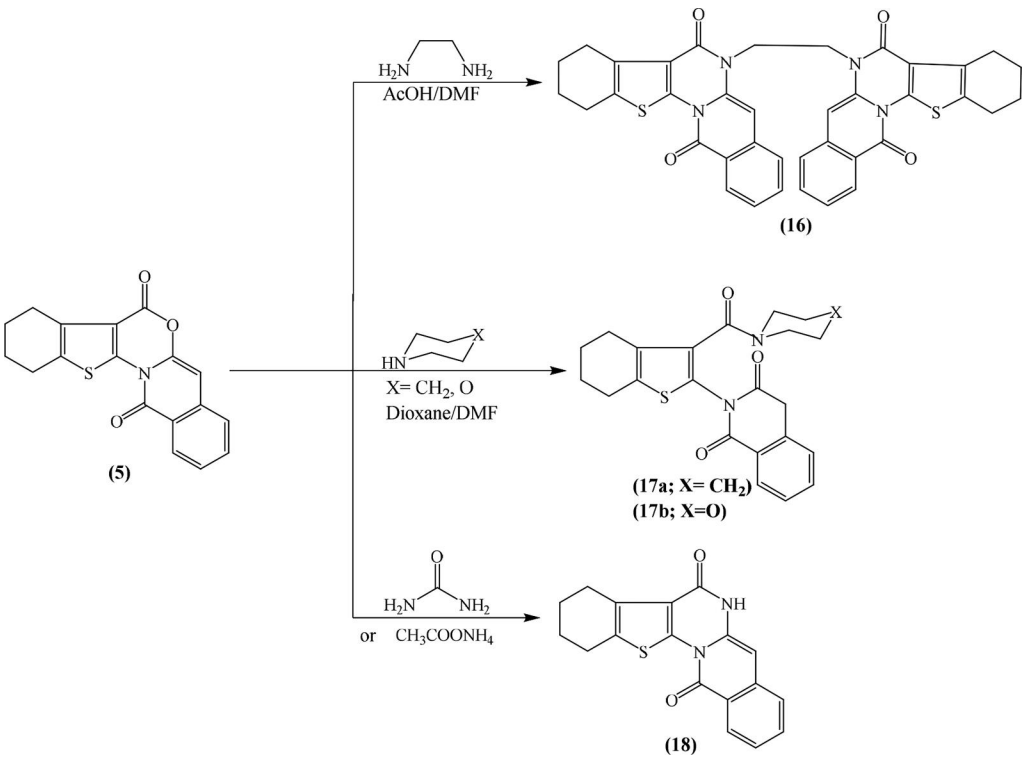
Moreover, the 1,3-oxazino-isoquinoline-7,14-dione derivative **5** undergoes hetero-ring opening when one mole of compound **5** reacted with two moles of benzylamine in boiling dioxane to yield *N*-benzyl-2-(2-(2-(benzylamino)-2-oxoethyl)benzamido)-4,5,6, 7-tetrahydrobenzo[*b*]thiophene-3-carboxamide **15** (Scheme 4). Analytical and spectral data for compound **15** were in agreement with the proposed structure (cf. Exp.).



Scheme 3. Reaction of compound **6** with some electrophilic reagents.



Scheme 4. Reaction of compound 5 with primary amines. *Note*: DMF, dimethylformamide.



Scheme 5. Reaction of compound 5 with ethylene diamine, secondary amines and ammonium acetate. *Note*: DMF, dimethylformamide.

On the other hand, when compound **5** was subjected to react with binucleophile such as ethylene diamine afforded the dimer 6,6′-(ethane-1,2-diyl)bis(8,9,10,11-tetrahydro-7*H*-benzo[4′,5′]thieno[3′,2′:5,6]pyrimido[1,2-*b*] isoquinoline-7,14(6*H*)dione **16** (Scheme 5). Next, we studied the reactivity of the oxazinone **5** with secondary amines such as piperidine and/or morpholine in which we obtain the isoquinolindione derivative **17a, b**, respectively (Scheme 5). Finally, fusion of compound **5** with ammonium acetate and/or urea at 170 °C in oil bath yielded the thienopyrimido[1,2-*b*]isoquinoline-7,14(6*H*)-dione derivative **18** (Scheme 5).

Pharmacological activity

Antitumor activity using in vitro ehrlich ascites assay

Out of the newly synthesized compounds, 15 analogs were selected to be evaluated for their *in vitro* cytotoxic effect against a panel of two human tumor cell lines, namely, hepatocellular carcinoma (liver) HepG2, and mammary gland breast MCF-7 cancer cell lines (Table 1).

In general, the cytotoxic activity of the tested compounds ranged from very strong to weak activity. Compound **6** showed approximately equal activity to doxorubicin as a standard for HepG-2 cell line with IC_{50} 5.95 ± 0.6 and 4.50 ± 0.2 μg mL^{-1}, respectively. The optimal results were observed for compounds **6** and **9** (very strong activity) with IC_{50} 5.95 ± 0.6, 9.49 ± 0.9 μg mL^{-1} for HepG-2 cell line, and with IC_{50} 6.49 ± 0.7, $7.47 \pm$

Table 1. Cytotoxicity (IC_{50}) of the tested compounds on two cell lines.

Compound no.	IC_{50} (μg mL^{-1})[a] HePG2	MCF-7
3	85.57 ± 4.5	79.22 ± 4.4
4	61.83 ± 3.4	79.87 ± 4.3
5	16.62 ± 1.3	11.22 ± 1.2
6	5.95 ± 0.6	6.49 ± 0.7
7	74.04 ± 3.8	41.45 ± 2.5
8	>100	93.85 ± 5.2
9	9.49 ± 0.9	7.47 ± 0.8
10	63.85 ± 3.6	75.60 ± 3.9
11	40.35 ± 2.6	32.74 ± 2.2
13	57.13 ± 3.1	76.47 ± 4.1
14	92.87 ± 4.9	83.87 ± 4.6
15	40.56 ± 2.7	19.67 ± 1.6
17a	48.19 ± 2.8	29.95 ± 2.0
17b	24.05 ± 1.8	17.10 ± 1.5
18	34.94 ± 2.4	57.87 ± 3.3
DOX	4.50 ± 0.2	4.17 ± 0.2

[a]IC_{50} (μg mL^{-1}): 1–10 (very strong), 11–20 (strong), 21–50 (moderate), 51–100 (weak), above 100 (non-cytotoxic).
DOX, doxorubicin.

0.8 μg mL^{-1} for MCF-7 cell line, respectively. Compounds **5** showed strong activity toward HePG-2 and MCF-7 cell lines with IC_{50} 16.62 ± 1.3, 11.22 ± 1.2 μg mL^{-1}, respectively. Moderate activity toward HePG-2 and MCF-7 cell lines was observed with compounds **7, 11, 15**, 17**a, b**, and **18**.

Structure–activity relationship

DNA is made of chemical building blocks called nucleotides. The four types of nitrogen bases found in nucleotides are: adenine (A), thymine (T), guanine (G), and cytosine (C). The base adenine always pairs with thymine, while guanine always pairs with cytosine through hydrogen bond. The cytotoxic activity of the tested compounds toward different cell lines depends on two factors[29,30]: (i) The formation of intermolecular hydrogen bond with DNA bases. (ii) The positive charge on the tested compounds attracted to the negative charge on the cell wall. By comparing the experimental cytotoxicity of the compounds reported in this study to their structures, the following structure–activity relationship was postulated.

- Compound **6** showed very strong activity, this is due to the presence of 3 NH and 2 NH_2 groups available to form hydrogen bond with either one of the nucleobases of DNA and causes damage.
- Compound **9** showed very strong activity, this is due to the presence of SH group which may be added to any unsaturated moiety in DNA or forming hydrogen bond with either one of the nucleobases of the DNA and causes damage.

Conclusion

The objectives of the present study were to synthesize some new compounds with thienopyrimidoisoquinoline moiety to assess the pharmacological profile of this class of compounds. The tested compounds showed very strong to weak cytotoxic activity against two anticancer cell lines. The best results were observed for compounds **6** and **9** (very strong activity). Compound **6** showed approximately equal activity to doxorubicin as a standard against HePG2 and MCF-7 cell lines.

Experimental

All melting points were taken on a Griffin and Geory melting point apparatus and are uncorrected. IR spectra were recorded on Pye Unicam SP1200 spectrophotometer using the KBr wafer technique. ^{1}H-NMR and ^{13}C NMR spectra were determined on a Varian Gemini 300 MHz using tetramethylsilane as an internal standard (chemical shifts in δ scale). EI-MS were measured on a Schimadzu-GC-MS operating at 70 eV. Elemental analyses were performed at the Microanalytical Unit, Faculty of Science, Ain Shams University, using a Perkin-Elmer 2400 CHN elemental analyzer, and satisfactory analytical data (±0.4) were obtained for all compounds. Microwave experiments were performed using a CEM Discover Labmate microwave apparatus (300 W with CHEMDRIVER software; Matthews, NC). The homogeneity of the synthesized compounds was controlled by TLC using aluminum sheet silica gel F_{254} (Merck). The pharmacological activity assays were performed at Pharmacology Department, Faculty of Pharmacy, El-Mansoura University, El-Mansoura, Egypt.

Synthesis of 2-(2-((3-(ethoxycarbonyl)-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl) amino-2-oxoethyl)benzoic acid 3

A mixture of 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzothiophene (2.25 g, 10 mmol) and homophthalic anhydride (1.62 g, 10 mmol) was exposed to microwave at 900 W for 3 min. After cooling, the reaction mixture was treated with MeOH and the solid formed was filtered off, dried, and recrystallized from benzene to give **3** as white crystals; mp: 176–180 °C, yield: 51.67%. Anal. Calcd. for $C_{20}H_{21}NO_5S$ (387.11): C, 62.00; H, 5.46; N, 3.62; S, 8.27. Found: C, 61.89; H, 5.33; N, 3.51; S, 8.18. IR (υ/cm^{-1}): 3237 (NH), 1747 ($C{=}O_{ester}$), 1693 ($C{=}O_{acid}$), 1664 ($C{=}O_{amide}$). MS *m/z* (%): 387 (63.5), 373 (51.3), 323 (22.5), 262 (33.5), 225 (99.9), 211 (99.9), 179 (99.9), 151 (76.2), 135 (79.8), 89 (34.2), 77 (31.5). ^{1}H-NMR (DMSO-d_6) δ (ppm): 13.0 (s, 1H, COOH, exchangeable with D_2O), 10.89 (s, 1H, NH, exchangeable with D_2O), 7.97–7.45 (m, $4H_{arom.}$), 4.21–4.16 (q, 2H, $COO\underline{CH_2}CH_3$, $J = 7.2$ Hz), 3.69 (s, 2H, $COCH_2$), 2.67–2.56 (m, 4H, $2CH_2$), 1.69 (m, 4H, $2CH_2$), 1.26–1.22 (t, 3H, $COOCH_2\underline{CH_3}$, $J = 7.2$ Hz). ^{13}C-NMR (DMSO-d_6) (ppm) δ: 13.9, 22.1, 22.1, 22.3, 23.5, 25.5, 25.6, 41.6, 59.9, 110.8, 125.6, 127.4, 130.2, 130.3, 130.6, 132.1, 132.1, 132.5, 135.2, 146.1, 164.5, 167.7, 167.9.

Pharmacological activity

Cytotoxicity assay

The cytotoxic activity of 15 compounds was tested against two human tumor cell lines, namely, hepatocellular carcinoma (liver) HePG-2, and mammary gland (breast) MCF-7. The cell lines were obtained from the ATCC through the Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). Doxorubicin was used as a standard anticancer drug for comparison. The reagents used were RPMI-1640 medium, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), DMSO, and 5-fluorouracil (Sigma Co., St. Louis, MO, USA), and fetal bovine serum (GIBCO, Paisley, UK).

The different cell lines[31,32] mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of yellow tetrazolium bromide (MTT) to a purple formazan derivative by

mitochondrial succinate dehydrogenase in viable cells. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units mL^{-1} penicillin and 100 µg mL^{-1} streptomycin at 37 °C in a 5% CO_2 incubator. The cell lines were seeded [33] in a 96-well plate at a density of 1.0×10^4 cells/well at 37 °C for 48 h under 5% CO_2 incubator. After incubation, the cells were treated with different concentrations of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µL of MTT solution at 5 mg mL^{-1} was added and incubated for 4 h. DMSO in volume of 100 µL was added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, BioTech, Winoosky, VT, USA).

The relative cell viability in percentage was calculated as (A_{570} of treated samples/A_{570} of untreated sample) × 100.

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