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The Reaction of 2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophenes with Benzoyl-Isothiocyanate: Synthesis of Annulated Thiophene Derivatives and Their Antitumor Evaluations

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ABSTRACT

The reaction of the tetrahydrobenzo[*b*]thiophene derivatives **1a**,**b** with benzoylisothiocyanate **(2)** afforded the thiourea derivatives **3a**,**b**. Cyclization of the latter products gave the annulated products **4a**,**b**. Compounds **3a**,**b** reacted with either hydrazine hydrate **(5a)** or phenyl hydrazine **(5b)** afforded compounds **7a-d** which underwent cyclization for compounds **7a**,**c** afforded compound **9**. On the other hand compounds **4a**,**b** reacted with either hydrazine hydrate **(5a)** or phenyl hydrazine **(5b)** afforded compounds **6a-d** a second pathway was applied to synthesize compound **6c** which underwent cyclization afforded compound **9**. Also compounds **4a**,**b** reacted with either phenacyl bromide **(10)** afforded compounds **11a**,**b** or ethylchloroacetae **(12)** compounds **13a**,**b** were produced. The latter products reacted with each hydrazine hydrate **(5a)** and phenyl hydrazine **(5b)** afforded compounds **14a-d**. Their antitumor activities were tested using three different cell lines.

Keywords: Tetrahydrobenzo[*b*]thiophene; Pyrimidine; Thiourea; Antitumor Activity

1. Introduction

Over the past few years some research groups and our group were interested to introduce a comprehensive study program towards the synthesis of thiophenes and their fused derivatives [1-4]. The importance of such compounds based on their uses as anti-inflammatory [5-7] thiophene derivatives was evaluated as antiprotozoal [8,9], on the other hand thiophene derivatives was applied as a new antitumor agents [10,11], in addition to fused thiophene derivatives was tested as templates for serine protease inhibition [12] and alternate substrate inhibitors of cholesterol esterase [13]. In this article we are using the 2-amino-4,5,6,7-tetrahydrobenzo[*b*] thiophene derivatives [14] **1a**,**b** in the synthesis of fused derivatives of pharmaceutical interest.

2. Results and Discussion

Thus, the reaction of **1a**,**b** with benzoylisothiocyanate **(2)** in 1,4-dioxane at room temperature gave the N-benzoylthiourea derivatives **3a** and **3b**, respectively. The structures of compounds **3a**,**b** were based on analytical and spectral data. Thus, 1H NMR spectrum of **3a** (as an example) showed beside the expected regular data for the cyclohexenyl moiety, two multiplet at δ 2.14 - 2.16 (4H, $2CH_2$), δ 2.23 - 2.26 (4H, $2CH_2$), a multiplet at δ 7.30 - 7.41 (5H), corresponding to aromatic protons. Two singlet at δ 8.26, 8.30 (2H) corresponding to 2NH. The reactions of isothiocyanates with NH_2 compounds was reported earlier [15]. Compounds **3a**,**b** underwent ready cyclization when heated in sodium ethoxide/ethanol solution in a boiling water bath to give the tetrahydro bezo[*b*]thieno [2,3-*d*]pyrimidine derivatives **4a** and **4b**, respectively. The ^{1}HNMR spectrum of each compound which revealed, in case of **4a** showed three multiplet at δ 1.64 - 1.72 (4H, 2CH2), δ 2.21 - 2.23 (4H, $2CH_2$) and multiplet at δ 7.32 - 7.44 (5H) corresponding to aromatic protons. Two singlet at δ 8.26, 8.29 (2H) corresponding to 2NH. Further confirmations for the structures of **4a** and **4b** were obtained through studying their reactivity towards some chemical reagents. Thus, the reaction of either **4a** or **4b** with either

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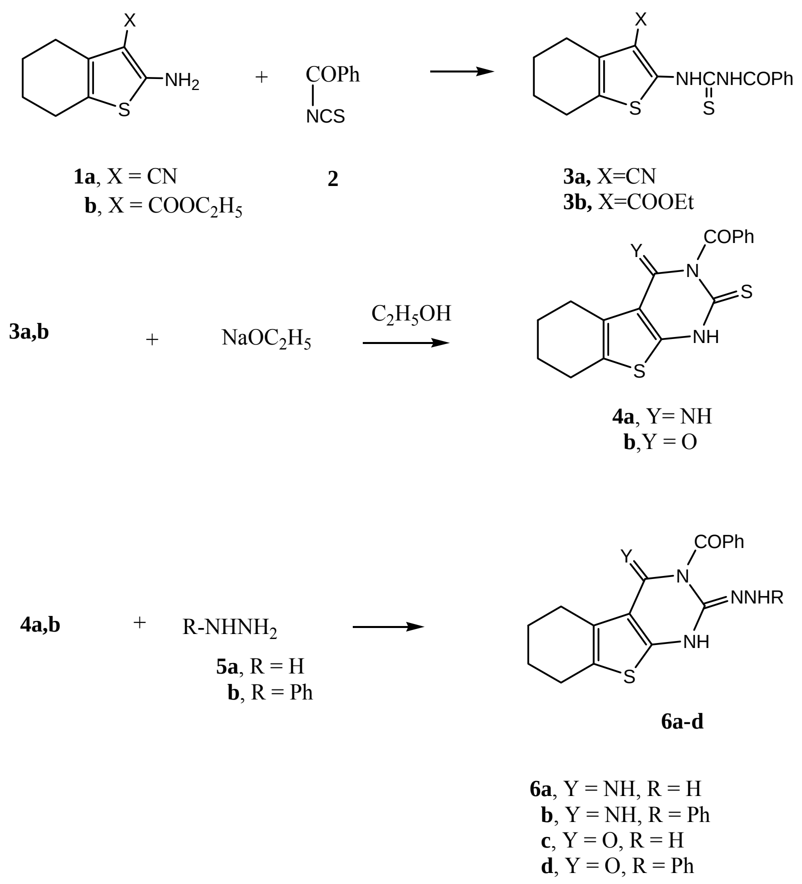
hydrazine hydrate **(5a)** or phenylhydrazine **(5b)** gave the corresponding compounds **6a-d** (**Scheme 1**). On the other hand the reaction of either **3a** or **3b** with either hydrazine hydrate or phenylhydrazine gave the hydrazone derivatives **7a-d**. The latter products **7a**,**c** were cyclized when heated in DMF/piperdine solution to give the annulated derivative **9**. Formation of the latter product took place through the intermediate triazole derivatives **8a**,**b**. The structure of compound **9** was based on analytical and spectral data. Formation of such compound **9** from either **7a** or **7c** is explained in terms of the first addition of NH group to CN group followed by hydrolysis of the C=NH group [16,17], however the addition of NH on COOEt group was followed by loss of ethanol took place. Confirmation structure of compound **9** was carried out by applying the second pathway to synthesize such compound **9** through reaction of compound **4b** with hydrazine hydrate **(5a)** in 1,4 dioxan at room temperature where the hydrazide derivative **6c** was separated which underwent ready cyclization when heated in dimethylformamide solution to give the same product **9** (m.p. and mixed m.p.) (**Scheme 2**). The reaction of either compound 4a or 4b with ω-bromoacetophenone **(10)** in ethanol solution gave the thioether derivatives **11a** and **11b**, respectively. Structures of the latter products were based on analytical and spectral data. On the other hand, the reaction of either **4a** or **4b** with ethyl chloroacetate **(12)** gave the thioether derivatives **13a** and **13b** respectively. The spectral data of the latter are in consistent with the proposed structures.

The reaction of either compounds **13a** or **13b** with either hydrazine hydrate or phenylhydrazine afforded the corresponding 2-hydrazino-4,5,6,7-tetrahydrobenzo[*b*] thieno-[2,3-*d*]pyrimidine derivatives **14a-d** (**Scheme 3**). The analytical and spectral data of the latter products are in agreement with the proposed structures.

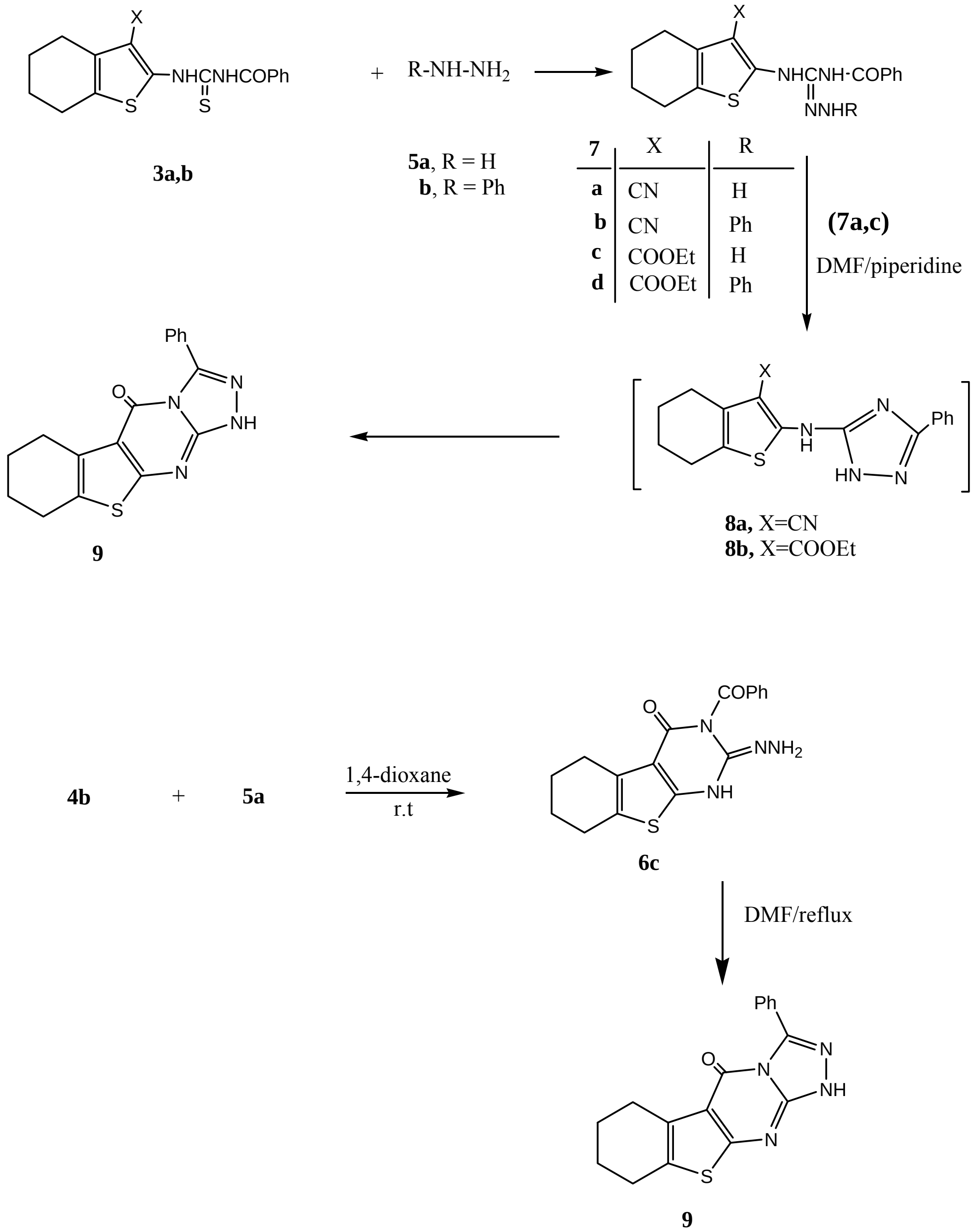
2.1. Antitumor Activity Tests

Reagents: Fetal bovine serum (FBS) and L-glutamine, were from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were from Sigma Chemical Co. (Saint Louis, USA).

Cell cultures: Three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) were used. MCF-7 was obtained from the European Collection of Cell Cultures



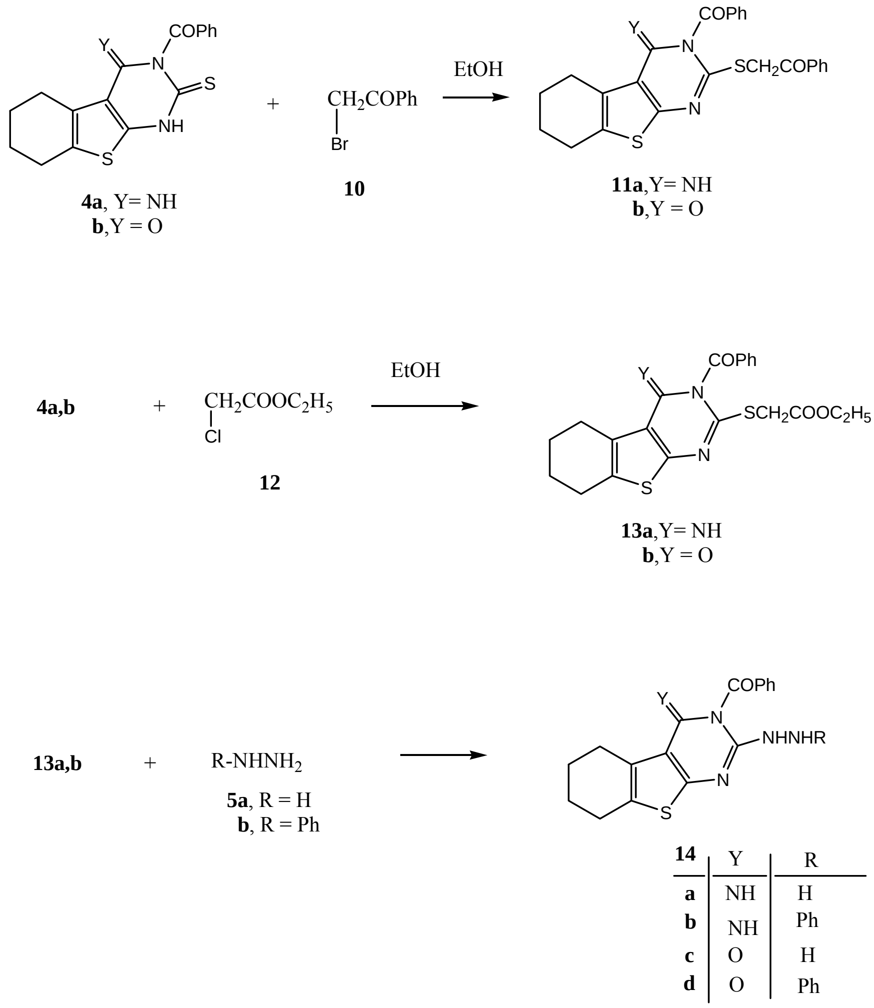
Scheme 1. Synthesis of compounds 3a,b-6a-d.



Scheme 2. Synthesis of compounds 7a-d-9.

(ECACC, Salisbury, UK) and NCI-H460 and SF268 were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 µg/mL), at 37˚C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5 × 105 cells/mL for MCF-7 and SF-268 and 0.75 × 104 cells/mL for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

Tumor cell growth assay: The effects of **3a-14d** on the in vitro growth of human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the "*In vitro* Anticancer Drug Discovery Screen" that uses the protein-binding dye sulforhodamine B to assess cell growth [18]. Briefly, exponentially, cells growing in 96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 µM. Following this exposure period adherent cells were fixed, washed, and stained. The bound stain was solubilized and the absorbance was measured at 492 nm



Scheme 3. Synthesis of compounds 11a,b-14a-d.

in a plate reader (Bio-Tek Instruments Inc., Powerwave XS, Wincoski, USA). For each test compound and cell line, a dose-response curve was obtained and the growth inhibition of 50% (GI50), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth, was calculated as described elsewhere [19]. Doxorubicin was used as a positive control and tested in the same manner.

2.2. Effect on the Growth of Human Tumor Cell Lines

The effect of compounds **3a-14d** was evaluated on the *in vitro* growth of 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) after a continuous exposure for 48 h. The results are summarized in **Table 1**. All of the tested compounds were able to inhibit the growth of the tested human tumor cell lines in a dose-dependent manner (data not shown). The results indicated through **Table 1** revealed that "compounds **11a** and **14c** showed the highest inhibitory effect against all the three tumor cell lines corresponding to reference standard material (Doxorubicin) for compounds **6a**, **7d**, and **14d** showed the highest inhibitory effect against all the three tumor cell lines with respect to the remaining synthesized compounds". While compounds **6d**, **7b**, **7c** and **9** showed moderate inhibitory effects against the three cancer cell lines. The rest of the compounds showed a low growth inhibitory effect. Comparing compound **6a**, **6b**, **6c** and **6d** it is obvious that the presence of the NH group and R =H in compound **6a** increased the inhibitory effect higher than that of the corresponding compounds **6b**, **6c** and **6d** where as compound **6d** with Y=O and R=Ph showed the lowest inhibitory effect. Comparing the tetrahydrobenzo [*b*]thiophene derivatives **7a**, **7b**, **7c** and **7d** it is obvious that compound **7d** with the X=COOEt and the R=Ph showed the highest inhibitory effect among the fours compounds but compound **7c** with X=COOEt, R=H showed moderate inhibitory effect among the four compounds.

Table 1. Effect of compounds 3a-14d on the growth of three human tumor cell lines.

Compound	GI_{50} (μ mol·L^{-1})		
	MCF-7	NCI-H460	SF-268
3a	24.0 ± 4.6	22.0 ± 2.4	23.5 ± 6.0
3b	32.0 ± 1.8	12.0 ± 0.8	14.5 ± 4.1
4a	30.1 ± 0.6	17.3 ± 1.4	22.3 ±1.5
4b	33.7 ± 17.5	42.2 ± 12.8	14 ± 0.8
6a	0.2 ± 0.04	0.1 ± 0.06	0.3 ± 0.05
6b	50.1 ± 0.7	23.2 ± 4.8	18.4 ± 1.8
6c	38.0 ± 1.8	44.0 ± 0.8	20.5 ± 1.1
6d	11.8 ± 0.6	14.5 ± 0.8	16.7 ± 1.6
7a	20.0 ± 0.2	22.6 ± 1.4	32.4 ± 0.6
7b	8.2 ± 0.4	6.1 ± 0.6	4.3 ± 0.5
7c	6.0 ± 0.6	4.0 ± 0.4	2.5 ± 8.0
7d	0.9 ± 0.2	0.1 ± 0.02	0.3 ± 0.05
9	6.6 ± 22.2	46 ± 2.6	2.4 ± 1.8
11a	0.01 ± 0.003	0.02 ± 0.001	0.01 ± 0.001
11b	25.0 ± 0.6	22.0 ± 0.4	31.5 ± 8.0
13a	22.7 ± 17.5	20.2 ± 12.8	33.0 ± 9.0
13b	20.0 ± 0.2	30.6 ± 1.4	38.4 ± 0.6
14a	34.0 ± 1.8	44.0 ± 0.8	20.5 ± 1.1
14b	24.2 ± 11.5	20.2 ± 10.8	24.0 ± 8.0
14c	0.03 ± 0.007	0.02 ± 0.008	0.01 ± 0.004
14d	1.0 ± 0.2	3.6 ± 0.4	1.4 ± 0.8
Doxorubicin	0.04 ± 0.008	0.09 ± 0.008	0.09 ± 0.007

Results are given in concentrations that were able to cause 50% of cell growth inhibition (GI_{50}) after a continuous exposure of 48 h and show means ± SEM of three-independent experiments performed in duplicate.

Finally for Hydrazopyrimidine derivatives **14a**, **14b**, **14c**, **14d** it is found that compounds **14c** and **14d** with the Y =O and the R=H or Ph respectively showed the highest inhibitory effect among the fours compounds but compounds **14a** and **14b** with the Y=NH and the R=H or Ph respectively showed the lowest inhibitory effect.

3. Experimental Section

3.1. General

All melting points were uncorrected; the IR spectra expressed in cm^{-1} and recorded using KBr pellets and a Pa-9721 IR spectrometer. ^{1}H NMR spectra were obtained on a Varian EM-390 90 Hz spectrometer in DMSO-d_6 as solvent and TMS as internal reference. Chemical shifts (δ) are expressed in ppm. Elemental analyses were carried out by the Microanalytical Data Unit at the National Research Center, Giza, Egypt and the Microanalytical data Unit at Cairo University.

3.2. General Procedure for the Synthesis of: 3-Cyano-2-(N-benzoylthiouryl)-4,5,6,7-tetrahydro-benzo[*b*]-thiophene (3a) and Ethyl 2-(N-benzoylthiouryl)-4,5,6,7-tetrahydro-benzo[*b*]thiophen-3-carboxy-late (3b)

General procedure: Equimolar amounts of either **1a** (5.34 g, 0.03 mol) or **1b** (6.75 g, 0.03 mol) in 1,4-dioxane (50 mL), benzoylisothiocyanate (3.93 g, 0.03 mol) [prepared by adding ammonium isothiocyanate (0.03 mol) to a solution of benzoyl chloride (0.03 mol) in 1,4-dioxan (50 mL) and heat for 1/2 h followed by isolation of the by-product, ammonium chloride] was added. The whole reaction mixture, in each case, was stirred at room temperature overnight and the formed solid product upon pouring onto ice/water was collected by filtration.

Compound **3a**: Yellow crystals from acetic acid, yield 70% (7.17 g), m.p. 200˚C - 203˚C. *Anal.* Calculated for $C_{17}H_{15}N_3OS_2$ (341.45): C, 59.80; H, 4.43; N, 12.31; S, 18.78. Found: C, 60.07; H, 4.42; N, 12.58; S, 19.01. IR (υ/cm^{-1}): 3456 - 3334 (2NH), 3030 (CH aromatic), 2888 (CH_2), 2227 (CN), 1690 (CO), 1660 - 1649 (C=S), 1639 (C=C). ^{1}H NMR (δ ppm): 2.14 - 2.16 (m, 4H, 2CH_2); 2.23 - 2.26 (m, 4H, 2CH_2), 7.30 - 7.41 (m, 5H, C_6H_5), 8.26, 8.30 (2s, 2H, 2NH).

Compound **3b**: Pale yellow crystals from acetic acid, yield 60% (6.99 g), m.p. 160˚C. *Anal.* Calculated for $C_{19}H_{20}N_2O_3S_2$ (388.50): C, 58.74; H, 5.19; N, 7.21; S, 16.51. Found: C, 58.89; H, 5.24; N, 7.31; S, 16.44. IR (υ/cm^{-1}): 3466 - 3339 (2NH), 3042 (CH aromatic), 2986, 2893 (CH_3, CH_2), 1692, 1685 (2 CO), 1665 - 1652 (C=S), 1636 (C=C). ^{1}H NMR (δ ppm): 1.32 (t, 3H, CH_3), 2.18 - 2.19 (m, 4H, 2CH_2); 2.27 - 2.29 (m, 4H, 2CH_2), 4.23 (q, 2H, CH_2), 7.18 - 7.35 (m, 5H, C_6H_5) 8.27, 8.30 (2s, 2H, 2NH).

3-Benzoyl-4-imino-2-thioxo-4,5,6,7-tetrahydro-benzo[*b*]thieno[2,3-*d*]pyrimidin (4a), 3-Benzoyl-4-oxo-2-thioxo-4,5,6,7-tetrahydro-benzo[*b*]thieno [2,3-*d*]-pyrimidin (4b)

General procedure: A suspension of either **3a** (3.41 g, 0.01 mol), **3b** (3.88 g, 0.01 mol), in sodium ethoxide (0.01 mol) [prepared by dissolving sodium metal (0.23 g, 0.01 mol) in absolute ethanol (40 mL)] was heated in a boiling water bath for 6 hr then left to cool. The solid product was formed upon pouring onto ice/water containing few drops from hydrochloric acid (till pH = 6) was collected by filtration.

Compound **4a**: Colorless crystals from 1,4-dioxan, yield 70% (2.39 g), m.p. 233˚C - 235˚C. *Anal.* Calculated for $C_{17}H_{15}N_3OS_2$ (341.45): C, 59.80; H, 4.43; N, 12.31; S, 18.78. Found: C, 59.57; H, 4.35; N, 12.48; S, 18.99. IR (υ/cm^{-1}): 3446 - 3336 (2NH), 3038 (CH aromatic), 2887 (CH_2), 1687 (CO), 1661 - 1652 (C=S), 1639 (C=C). ^{1}H NMR (δ ppm): 1.64 - 1.72 (m, 4H, 2CH_2); 2.21 - 2.23 (m, 4H, 2CH_2), 7.32 - 7.44 (m, 5H, C_6H_5), 8.26, 8.29 (2s, 2H, 2NH).

Compound **4b**: Pale yellow crystals from ethanol, yield 88% (3.01 g), m.p. 185˚C - 187˚C. *Anal.* Calculated for $C_{17}H_{14}N_2O_2S_2$ (342.44): C, 59.63; H, 4.12; N, 8.18; S, 18.73. Found: C, 59.87; H, 4.23; N, 8.38; S, 18.58. IR (υ/cm^{-1}): 3456 - 3336 (NH), 3042 (CH aromatic), 2890 (CH_2), 1692, 1687 (2 CO), 1638 (C=C), 1668 - 1651 (C=S). ^{1}H NMR (δ ppm): 1.63 - 1.70 (m, 4H, 2CH_2); 2.20 - 2.26 (m, 4H, 2CH_2), 7.32 - 7.39 (m, 5H, C_6H_5), 8.22 (s, 1H, NH).

3-Benzoyl-2-hydrazono-4-imino-4,5,6,7-tetrahydro benzo[*b*]thieno[2,3-*d*]pyrimidine (6a), 3-benzoyl-2-phenylhydrazono-4-imino-4,5,6,7-tetrahy-drobenzo[*b*] thieno[2,3-*d*]pyrimidine (6b), 3-benzoyl-2-hydrazono-4-oxo-4,5,6,7-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyramidine (6c) and 3-benzoyl-2-phenyl hydrazono-4-oxo-4,5,6,7-tetrahydrobenzo[*b*]thieno-[2,3-*d*]pyrimidine (6d)

Method (A): To a solution of either **4a** (0.68 g, 0.002 mol) or **4b** (0.68 g, 0.002 mol) in DMF (40 mL) either hydrazine hydrate (0.1 g, 0.002 mol) or phenylhydrazine (0.22 g, 0.002 mol) was added. The reaction mixture, in each case was heated under reflux for 2 hr till evolution of hydrogen sulphide ceased. The reaction mixture, in each case, was left to cool then poured onto ice/water containing few drops of hydrochloric acid (till pH 6) and the formed solid product was collected by filtration.

Method (B): 3-Benzoyl-2-hydrazono-4-oxo-4,5,6,7-tetrahydro-benzo[*b*]-thieno-[2,3-*d*]pyrimidine (6c)

General procedure: To a solution of **4b** (1.03 g, 0.003 mol) in 1,4-dioxane (40 mL) hydrazine hydrate (0.15 g, 0.003 mol) was added. The reaction mixture was stirred at room temperature for 24 hr and the formed crystals was collected by filtration.

Compound **6a**: Yellow crystals from 1,4-dioxane, yield 75% (0.51 g), m.p. 188˚C - 191˚C. *Anal.* Calculated for $C_{17}H_{17}N_5OS$ (339.41): C, 60.16; H, 5.05; N, 20.63; S, 9.45. Found: C, 60.23; H, 5.11; N, 20.88; S, 9.77. IR (υ/cm^{-1}): 3504 - 3332 (NH_2, 2NH), 3043 (CH aromatic), 1690 (CO), 1678, 1665 (exocyclic 2C=N), 1643 (C=C). ^{1}H NMR (δ ppm): 1.62 - 1.70 (m, 4H, 2CH_2); 2.21 - 2.27 (m, 4H, 2CH_2), 4.22 (s, 2H, NH_2), 7.29 - 7.38 (m, 5H, C_6H_5), 8.12 - 8.28 (2s, 2H, 2NH).

Compound **6b**: Yellowish white crystals from 1,4-dioxan, yield 62% (0.52 g), m.p. 233˚C - 236˚C. *Anal.* Calculated for $C_{23}H_{21}N_5OS$ (415.51): C, 66.48; H, 5.09; N, 16.85; S, 7.72. Found: C, 66.53; H, 4.93; N, 16.90; S, 8.02. IR (υ/cm^{-1}): 3449 - 3324 (3NH), 3052 (CH aromatic), 1692 (CO), 1669, 1660 (exocyclic 2C=N), 1642 (C=C). ^{1}H NMR (δ ppm): 1.63 - 1.66 (m, 4H, 2CH_2); 2.22 - 2.28 (m, 4H, 2CH_2), 7.26 - 7.53 (m, 10H, 2C_6H_5), 7.88, 8.03, 8.25 (3s, 3H, 3NH).

Compound **6c**: Yellow crystals from DMF, yield 86% (0.59 g), m.p. 180˚C - 183˚C. *Anal.* Calculated for $C_{17}H_{16}N_4O_2S$ (340.4): C, 59.98; H, 4.74; N, 16.46; S, 9.42. Found: C, 60.21; H, 4.82; N, 16.68; S, 9.61. IR (υ/cm^{-1}): 3555 - 3312 (NH_2, NH), 3050 (CH aromatic), 1698, 1690 (2CO), 1670 (exocyclic C=N), 1643 (C=C). ^{1}H NMR (δ ppm): 1.64 - 1.72 (m, 4H, 2CH_2); 2.25 - 2.29 (m, 4H, 2CH_2), 4.48 (s, 2H, NH_2), 7.32 - 7.43 (m, 5H, C_6H_5), 8.22 (s, 1H, NH).

Compound **6d**: Yellow crystals from DMF, yield 70 % (0.58 g), m.p. 266˚C - 269˚C. *Anal.* Calculated for $C_{23}H_{20}N_4O_2S$ (416.50): C, 66.33; H, 4.84; N, 13.45; S, 7.70. Found: C, 66.06; H, 4.76; N, 13.74; S, 7.98. IR (υ/cm^{-1}): 3555 - 3312 (2NH), 3058 (CH aromatic), 1692, 1680 (2CO), 1662 (C=N), 1643 (C=C). ^{1}H NMR (δ ppm): 1.68 - 1.77 (m, 4H, 2CH_2); 2.23 - 2.28 (m, 4H, 2CH_2), 7.29 - 7.35 (m, 10H, 2C_6H_5), 8.24 - 8.26 (2s, 2H, 2NH).

1-(3-Cyano-4,5,6,7-tatrayhdrobenzo[*b*]thiophene-2-yl)-2-hydrazono-3-benzoyl urea (7a), 1-(3-Cyano-4,5,6,7-tatrayhdrobenzo[*b*]thiophene-2-yl)-2-phenyl hydrazono-3-benzoyl urea (7b), 1-(Ethyl-4,5,6,7-tatrayhdrobenzo[*b*]thiophene-3-carboxylate-2-yl)-2-hydrazono-3-benzoyl urea (7c), 1-(Ethyl-4,5,6,7-tatrayhdrobenzo[*b*]thio-phene-3-carboxylate-2-yl)-2-phenyl hydra-zono-3-benzoyl urea (7d)

To a solution of either **3a** (1.7 g, 0.005 mol), 3b (1.94 g, 0.005 mol) in ethanol (50 mL), either hydrazine hydrate (0.25 g, 0.005 mol) or phenyl hydrazine (0.59 g, 0.005 mol) was added. The reaction mixture, in each case, was heated under reflux for 6 hr then poured onto ice/water containing few drops of hydrochloric acid (till pH 6) and the formed solid product was collected by filtration.

Compound **7a**: Yellowish white crystals from acetic acid, yield 78% (1.32 g), m.p. 167˚C - 170˚C. *Anal.* Calculated for $C_{17}H_{17}N_5OS$ (339.41): C, 60.16; H, 5.05; N, 20.63; S, 9.45. Found: C, 59.93; H, 4.86; N, 20.41; S, 9.62. IR (υ/cm^{-1}): 3578 - 3332 (NH_2, NH), 3045(CH aromatic), 2227 (CN), 1688 (CO), 1660 (C=N), 1636 (C=C). ^{1}H NMR (δ ppm): 1.68 - 1.74 (m, 4H, 2CH_2); 2.22 - 2.28 (m, 4H, 2CH_2), 4.56 (s, 2H, NH_2), 7.31 - 7.45 (m, 5H, C_6H_5), 8.20, 8.34 (2s, 2H, 2NH).

Compound **7b**: Yellow crystals from acetic acid, yield 66 % (1.37 g), m.p. 268˚C - 271˚C. *Anal.* Calculated for $C_{23}H_{21}N_5OS$ (415.51): C, 66.48; H, 5.09; N, 16.85; S, 7.72. Found: C, 66.32; H, 4.89; N, 16.95; S, 7.69. IR (υ/cm^{-1}): 3566 - 3332 (3 NH), 3055 (CH aromatic), 2223 (CN), 1688 (CO), 1662 (C=N), 1633 (C=C). ^{1}H NMR (δ ppm): 1.64 - 1.70 (m, 4H, 2CH_2), 2.24 - 2.37 (m, 4H, 2CH_2), 7.08 - 7.38 (m, 10H, 2C_6H_5), 8.02, 8.25 - 8.28 (3s, 3H, 3NH).

Compound **7c**: Orange crystals from acetic acid, yield 69% (1.33 g), m.p. 190˚C - 192˚C. *Anal.* Calculated for $C_{19}H_{22}N_4O_3S$ (386.47): C, 59.05; H, 5.74; N, 14.50; S, 8.30. Found: C, 59.28; H, 5.88; N, 14.79; S, 8.48. IR (υ/cm^{-1}): 3569 - 3322 (NH_2, 2NH), 3057 (CH aromatic), 2983, 2856 (CH_3, CH_2), 1692, 1987 (2CO), 1670 (C=N), 1636 (C=C). ^{1}H NMR (δ ppm): 1.33 (t, 3H, J = 7.43 Hz, CH_3), 1.64 - 1.72 (m, 4H, 2CH_2); 2.26 - 2.29 (m, 4H, 2CH_2), 4.23 (q, 2H, J = 7.43 Hz, CH_2), 4.62(s, 2H, NH_2), 7.30 - 7.38 (m, 5H, C_6H_5), 8.02, 8.24 (2s, 2H, 2NH).

Compound **7d**: Buff crystals from DMF, yield 60% (1.39 g), m.p. 173˚C - 175˚C. *Anal.* Calculated for $C_{25}H_{26}N_4O_3S$ (462.56): C, 64.91; H, 5.67; N, 12.11; S, 6.93. Found: C, 65.12; H, 5.92; N, 12.19; S, 7.02. IR (υ/cm^{-1}): 3484 - 3329 (3NH), 3051 (CH aromatic), 2992, 2890 (CH_3, CH_2), 1692, 1687 (2CO), 1663 (C=N), 1639 (C=C). ^{1}H NMR (δ ppm): 1.36 (t, 3H, J = 7.03 Hz, CH_3), 1.68 - 1.73 (m, 4H, 2CH_2), 2.24 - 2.28 (m, 4H, 2CH_2), 4.26 (q, 2H, J = 7.03 Hz, CH_2), 7.28 - 7.39 (m, 10H, 2C_6H_5), 8.02, 8.28, 8.34 (3s, 3H, 3NH).

3-Phenyl-5,6,7,8-tetrahydro-1*H*-9-thia-1,2,3a,10-te traaza cyclopenta[*b*]fluoren-4-one (9):

Method (A): A solution of either **7a** (0.68 g, 0.002 mol), **7c** (0.77 g, 0.002 mol), in dimethylformamide (40 mL) containing piperidine (0.5 mL) was heated under reflux for 6 hr then left to cool. The solid product, formed upon pouring onto ice/water containing hydrochloric acid (till pH = 6) was collected by filtration.

Method (B): A solution of **6c** (0.34 g, 0.001 mol) in dimethylformamide (30 mL) was heated under reflux for 4 hr. The reaction mixture was poured onto ice/water and the formed solid product was filtered off, crystallized from 1,4-dioxane and identified as compound **9** (m.p., mixed m.p.), yield 70% (0.23 g).

Compound **9**: Pale yellow crystals from 1,4 dioxan, yield 70% (0.45 g), m.p. 210˚C - 212˚C. *Anal.* Calculated for $C_{17}H_{14}N_4OS$ (322.38): C, 63.33; H, 4.38; N, 17.38; S, 9.95. Found: C, 63.52; H, 4.55; N, 17.60; S, 10.05, IR (υ/cm^{-1}): 3380 - 3335 (NH), 3052 (CH aromatic), 1691 (CO), 1657 (C=N), 1644 (C=C). ^{1}H NMR (δ ppm): 1.60 - 1.66 (m, 4H, 2CH_2); 2.20 - 2.32 (m, 4H, 2CH_2), 7.32 - 7.37 (m, 5H, C_6H_5), 8.20 (s, 1H, NH).

3-Benzoyl-4-imino-2-phenylthioacetyl-4,5,6,7-tetrahydrobenzo[*b*]-thieno[2,3-*d*]pyrim-idine (11a) and 3-Benzoyl-2-phenylthioacetyl-4-oxo-4,5,6,7-tetrahydro benzo[*b*]-thieno[2,3-*d*]pyrimidine (11b)

To dry solid of either **4a** (1.02 g, 0.003 mol) or **4b** (1.03 g, 0.003 mol) in ethanol (40 mL), phenacylbromide (0.6 g, 0.003 mol) was added. The reaction mixture was heated under reflux for 2 hr. then left to cool and the remaining product was triturated with diethyl ether and the formed solid product was collected by filtration.

Compound **11a**: Yellowish white crystals from acetic acid, yield 84% (1.16 g), m.p. 220˚C - 223˚C. *Anal.* Calculated for $C_{25}H_{21}N_3O_2S_2$ (459.58): C, 65.33; H, 4.61; N, 9.14; S, 13.95. Found: C, 65.52; H, 4.43; N, 8.92; S, 14.01. IR (υ/cm^{-1}): 3489(NH), 3053 (CH aromatic), 2880 (CH_2), 1710, 1682 (2CO), 1669 (C=N), 1637 (C=C), 1202 - 1191 (C-S). ^{1}H NMR (δ ppm): 1.64 - 1.68 (m, 4H, 2CH_2); 2.25 - 2.28 (m, 4H, 2CH_2), 2.86 (s, 2H, CH_2), 7.03 - 7.38 (m, 10H, 2C_6H_5), 8.21 (s, 1H, NH),

Compound **11b**: Yellow crystals from acetic acid, yield 73% (1.0 g), m.p. 229˚C - 231˚C. *Anal.* Calculated for $C_{25}H_{20}N_2O_3S_2$ (460.57): C, 65.20; H, 4.38; N, 6.08; S, 13.92. Found: C, 65.41; H, 4.41; N, 5.99; S, 13.89. IR (υ/cm^{-1}): 3056 (CH aromatic), 2890 (CH_2), 1715 - 1688 (3CO), 1664 (C=N), 1637 (C=C), 1198 - 1190 (C-S). ^{1}H NMR (δ ppm): 1.68 - 1.72 (m, 4H, 2CH_2); 2.23 - 2.24 (m, 4H, 2CH_2), 2.74 (s, 2H, CH_2), 7.33 - 7.42 (m, 10H, 2C_6H_5).

3-Benzoyl-2-ethyl-thioglycolato-4-imino-4,5,6,7-tetrahydrobenzo[*b*]-thieno[2,3-*d*]pyrimidine(13a) and 3-Benzoyl-2-ethyl-thioglycolato-4-oxo-4,5,6,7-tetrahydrobenzo[*b*]-thieno[2,3-*d*]pyrimidine (13b)

A suspension of either **4a** (1.02 g, 0.003 mol) **4b** (1.03 g, 0.003 mol) in ethanol (50 mL), ethyl chloroacetate (0.37 g, 0.003 mol) was added. The reaction mixture was heated under reflux for 4 hr. then left to cool and the remaining product was triturated with diethyl ether and the formed solid product was collected by filtration.

Compo und **13a**: Orange crystals from 1,4-dioxan, yield 61% (0.78 g), m.p. > 300˚C. *Anal.* Calculated for $C_{21}H_{21}N_3O_3S_2$ (427.54): C, 58.99; H, 4.95; N, 9.83; S, 15.00. Found: C, 58.79; H, 4.72; N, 10.02; S, 14.83. IR (υ/cm^{-1}): 3378 (NH), 3059 (CH aromatic), 2971, 2885 (CH_3, CH_2), 1699, 1689 (2CO), 1670 (exocyclic C=N), 1644 (C=C), 1205 - 1192 (C-S). ^{1}H NMR (δ ppm): 1.36 (t, 3H, J = 7.13 Hz, CH_3), 1.68 - 1.70 (m, 4H, 2CH_2); 2.25 - 2.29 (m, 4H, 2CH_2), 2.88(s, 2H, CH_2), 4.23 (q, 2H, J = 7.13 Hz, CH_2), 7.22 - 7.36 (m, 5H, C_6H_5), 8.26(s, 1H, NH).

Compound **13b**: Orange crystals from DMF, yield 62% (0.8 g), m.p. 246˚C - 248˚C. *Anal.* Calculated for $C_{21}H_{20}N_2O_4S_2$ (428.52): C, 58.86; H, 4.70; N, 6.54; S, 14.97. Found: C, 59.12; H, 4.53; N, 6.76; S, 14.94. IR (υ/cm^{-1}): 3049 (CH aromatic), 2986, 2876(CH_3, CH_2), 1698, 1693, 1685 (3CO), 1637 (C=C), 1203 - 1196 (C-S). ^{1}H NMR (δ ppm): 1.16 (t, 3H, J = 7.22 Hz, CH_3), 1.65 - 1.74 (m, 4H, 2CH_2); 2.22 - 2.27 (m, 4H, 2CH_2), 2.75(s, 2H, CH_2), 4.24 (q, 2H, J = 7.22 Hz, CH_2), 7.28 - 7.38 (m, 5H, C_6H_5).

3-Benzoyl-2-hydrazino-4-imino-4,5,6,7 tetrahydrobenzo[*b*]-thieno[2,3-*d*]pyrimidine (14a), 3-Benzoy-4-imino l-2-phenylhydrazino-4,5,6,7-tetrahydrobenzo[*b*] thieno[2,3-*d*]pyrimidine (14b), 3-Benzoyl-2-hydrazino-4-oxo-4,5,6,7-tetrahydrobenzo[*b*]-thieno-[2,3-*d*] pyrimidine (14c) and 3-Benzoyl-2-phenylhydrazino-4-oxo-4,5,6,7-tetrahydro benzo[*b*]-thieno[2,3-*d*]pyrimidine (14d)

To a solution of either **13a** (0.43 g, 0.001 mol) or **13b** (0.43 g, 0.001 mol) in 1,4-dioxane (40 mL) either hydrazine hydrate (0.05 g, 0.001) or phenylhydrazine (0.11 g, 0.001) was added. The reaction mixture, in each case, was heated under reflux for 2 hr then poured onto ice/

water containing few drops of hydrochloric acid (till pH 6) and the formed solid product was collected by filtration.

Compound **14a**: Pale brown crystals from 1,4-dioxan, yield 74% (0.25 g), m.p. 192˚C - 195˚C. *Anal.* Calculated for $C_{17}H_{17}N_5OS$ (339.41): C, 60.16; H, 5.05; N, 20.63; S, 9.45. Found: C, 59.93; H, 4.82; N, 20.67; S, 9.72. IR (υ/cm^{-1}): 3455 - 3330 (NH_2, 2NH), 3064 (CH aromatic), 1690 (CO), 1677 (exocyclic C=N), 1639 (C=C). ^{1}H NMR (δ ppm): 1.65 - 1.70 (m, 4H, $2CH_2$); 2.22 - 2.31 (m, 4H, $2CH_2$), 4.21 (s, 2H, NH_2), 7.33 - 7.39 (m, 5H, C_6H_5), 8.38, 8.94 (2s, 2H, 2NH).

Compound **14b**: Brown crystals from 1,4-dioxan, yield 62% (0.27 g), m.p. 171˚C - 173˚C. *Anal.* Calculated for $C_{23}H_{21}N_5OS$ (415.51): C, 66.48; H, 5.09; N, 16.85; S, 7.72. Found: C, 66.50; H, 5.02; N, 16.72; S, 7.48 IR (υ/cm^{-1}): 3442 - 3321 (3NH), 3052 (CH aromatic), 1693 (CO), 1636 (C=C). ^{1}H NMR (δ ppm): 1.66 - 1.73 (m, 4H, $2CH_2$); 2.24 - 2.28 (m, 4H, $2CH_2$), 7.31 - 7.40 (m, 10H, 2 C_6H_5), 8.18, 8.30, 8.39 (3s, 3H, 3NH).

Compound **14c**: Pale brown crystals from 1,4-dioxan, yield 78% (0.27 g), m.p. 215˚C - 217˚C. *Anal.* Calculated for $C_{17}H_{16}N_4O_2S$ (340.40): C, 59.98; H, 4.74; N, 16.46; S, 9.42. Found: C, 59.81; H, 4.88; N, 16.67; S, 9.70. IR (υ/cm^{-1}): 3455 - 3330 (NH_2, NH), 3064 (CH aromatic), 1690, 1687 (2 CO), 1639 (C=C). ^{1}H NMR (δ ppm): 1.65 - 1.77 (m, 4H, $2CH_2$); 2.20 - 2.31 (m, 4H, $2CH_2$), 4.46 (s, 2H, NH_2), 7.31 - 7.36 (m, 5H, C_6H_5), 8.36 (s, 1H, NH).

Compound **14d**: Yallow crystals from 1,4-dioxan, yield 65% (0.27 g), m.p. 103˚C - 105˚C. *Anal.* Calculated for $C_{23}H_{20}N_4O_2S$ (416.50): C, 66.33; H, 4.84; N, 13.45; S, 7.70. Found: C, 66.29; H, 4.62; N, 13.62; S, 7.60. IR (υ/cm^{-1}): 3442 - 3321 (2NH), 3052 (CH aromatic), 1693, 1684 (2CO), 1636 (C=C). ^{1}H NMR (δ ppm): 1.62 - 1.75 (m, 4H, $2CH_2$); 2.24 - 2.34 (m, 4H, $2CH_2$), 7.31 - 7.44 (m, 10H, $2C_6H_5$), 8.26 - 8.30 (2s, 2H, 2NH).

4. Conclusion

The work described in this article showed the synthesis of fused thieophene derivatives, most of the newly synthesized products showed high antitumor activities. The 4,5,6,7-tetrahydrobenzo[*b*]-thieno[2,3-*d*]pyrimidine derivatives **11a** and **14c** showed the maximum inhibitory effect among the newly synthesized products where such activity is higher than that of the reference doxorubicin.

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