Synthesis of Pyrazole and Thiophene Derivatives Together with their Antitumor Evaluations

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Abstract: The reaction of cyclohexanone, cyanoacetylhydrazine and elemental sulfur gave the 4,5,6,7-tetrahydrobenzo[*b*]thiophene derivative **3**. The latter compound was used as the key starting material for the synthesis of pyrazoles, thiazole derivatives. The newly synthesized products were screening against the three cancer cell lines namely the human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) and the human normal cell line the normal fibroblast WI 38. The results showed that compounds **7a** and **19c** were the most potent compounds among the tested compounds.

Keywords: Pyrazole, thiophene, tetrahydrobenzo[*b*]thiophene, cytotoxicity.

INTRODUCTION

2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-carboxylate its 3-carboxylate regioisomer and derivatives were recently found to be important intermediates in the synthesis of pharmaceutically active compounds. Their structural framework can be found in products with antimicrobial, antimycobacterial, anti-inflamatory [1-3] and antitumor properties [4]. Especially interesting are those molecules with a fused pyrimidine ring as they can be considered as potential nucleic acid antimetabolites. Moreover, Substituted 2-aminothiophenes are a kind of important structures which usually show diverse pharmacological profiles with antimicrobial, anticonvulsant and anti-inflammatory activities [5], and thus are continually used as a scaffold to construct a series of natural products, dyes and agrochemicals [6]. They can also serve as potential JNK2 and JNK3 kinase inhibitor and adenosine agonists [7]. Many other commercial medicines and drug candidates have been increasingly evolved from such scaffold as well [8]. The preparation of 2-aminothiophene derivatives is always achieved by a Gewald reaction (Scheme **1**), which is a multi-component condensation of a ketone with an activated hydrogen, a cyanomethylene containing an electron-withdrawing group, e.g., cyanoacetate, and elemental sulfur in the presence of an organic base [9] such as morpholine [10], diethylamine [11], etc. Alternative catalysts like lproline [12] have also been developed for Gewald reaction. Yet, existing chemical procedures are always associated with all kinds of weaknesses, like high catalyst loading [13], complicated procedures [14], and hazardous solvents [15], which hinder the further development of the Gewald reaction.

RESULTS AND DISCUSSION

In the present work, the reaction of cyclohexanone with elemental sulfur and cyanoacetylhydrazine in ethanol containing triethylamine gave the 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbohydrazide (**3**). The structure of compound **3** was based on its analytical and spectral data. Thus, ^{1}H NMR spectrum showed two multiplets at δ 1.66-1.77 and 2.31-2.38 ppm corresponding to the four CH_2 groups, two singlets at δ 4.85, 5.22 ppm (D_2O exchangeable) indicating the two NH_2 groups, a singlet at δ 8.30 (D_2O exchangeable) corresponding to the NH group. Compound **3** reacted with either acetophenone (**4a**) or 4-chloro acetophenone (**4b**) to give the corresponding hydrazide-hydrazone derivatives **5a** and **5b** respectively. Analytical and spectral data were the tools of their structural elucidation (see experimental section). The reaction of compound **3** with either malononitrile (**6a**) or ethyl cyanoacetate (**6b**) gave the pyrazole derivatives **7a** and **7b**, respectively. Similarly, the reaction of compound **3** with either acetylacetone (**8a**) or ethyl acetoacetate (**8b**) gave the pyrazole derivatives **9a** and **9b**, respectively. On the other hand, the reaction of compound **3** with formaldehyde **10** gave the Schiff base **11**. Compound **11** reacted with malononitrile (**6a**) in 1,4-dioxane containing triethylamine to give the tetrhydropyrazole derivative **12** (Scheme **1**).

The hydrazido moiety present in compound **3** showed interesting activity towards ethyl orthoformate **13** gave the N-ethoxymethino derivative **14**. On the other hand, the reaction of compound **3** with ethyl

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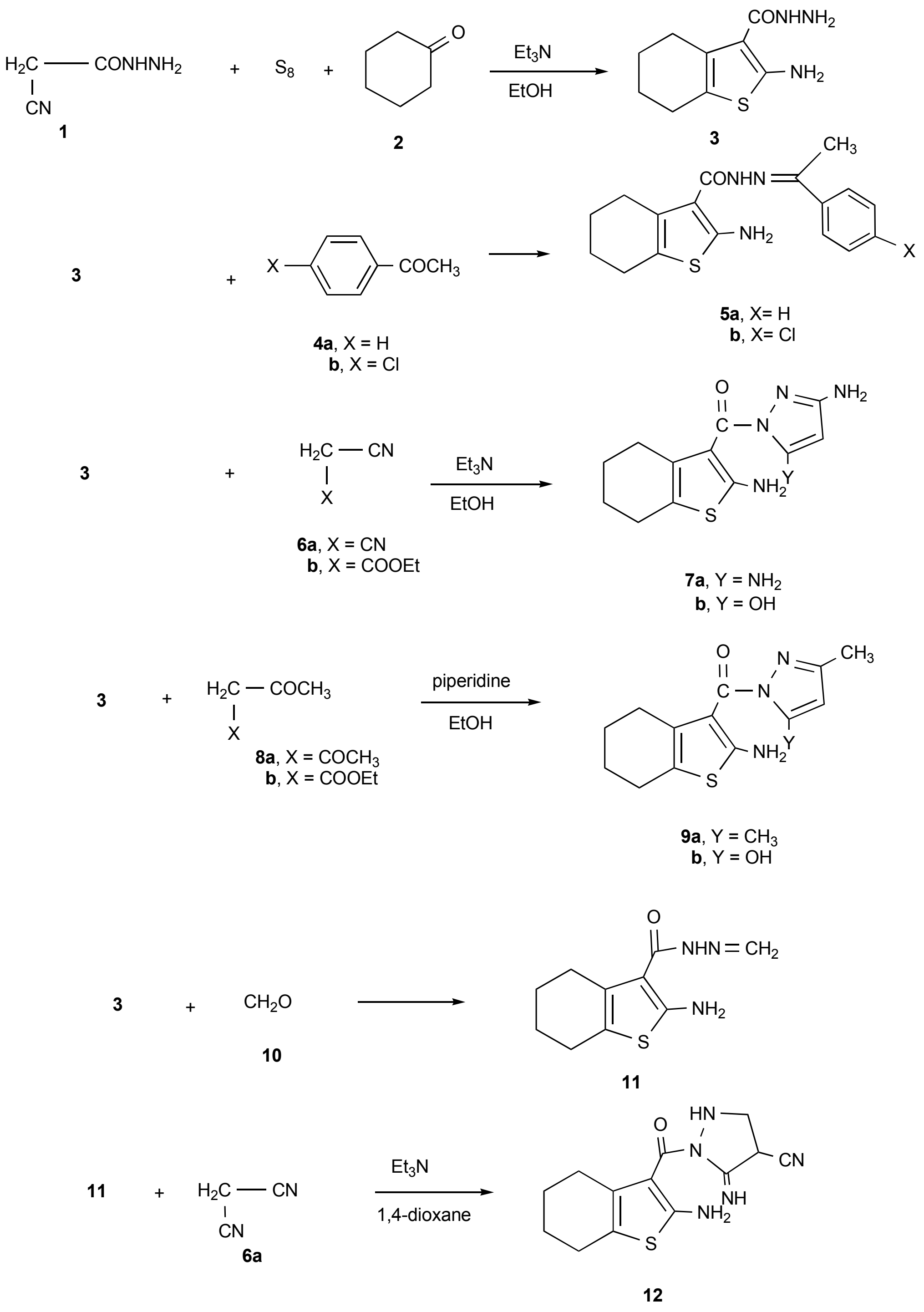
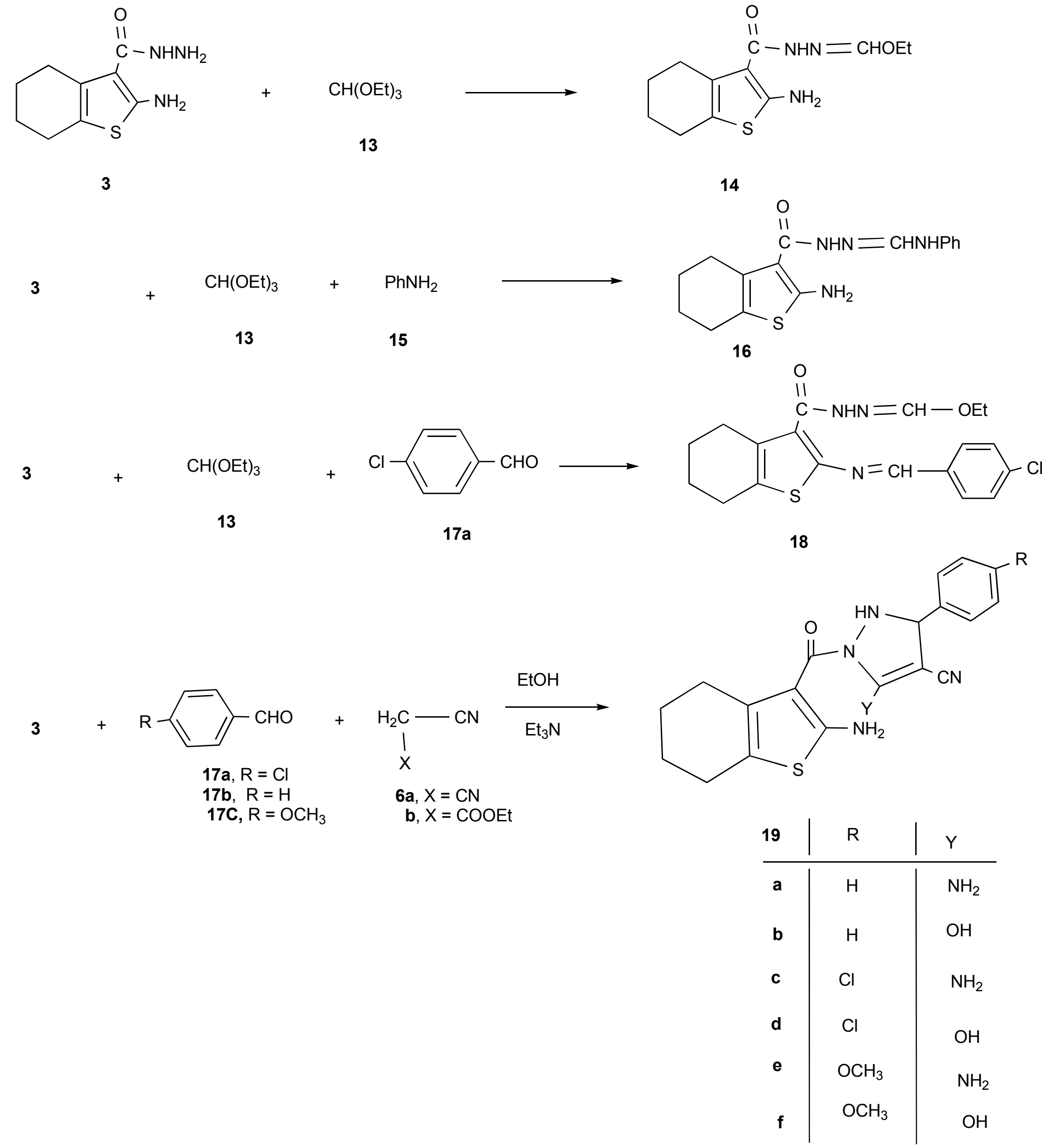


Scheme 1:

orthoformate and aniline gave the N-phenylmethino derivative **16**. The analytical and spectral data of the latter product are in agreement with its structure. On the other hand the reaction of compound **3** with ethyl orthoformate (**13**) and 4-chlorobenzaldehyde (**17a**) gave the 2-N-benzal-3-hydrazino-N-ethoxymethino derivative **18**. The analytical and spectral data of compounds **16** and **18** were the basis of their structural elucidations.

The multi-component reaction of compound **3** with either of 4-chlorobenzaldehyde (**17a**), benzaldehyde (**17b**) or 4-methoxybenzaldehyde (**17c**) and either of malononitrile (**6a**) or ethyl cyanoacetate (**6b**) gave the pyrazole derivatives **19a-f**, respectively (Scheme **2**). The ^{1}H NMR spectra were the tools of confirming of their structures. Thus, ^{1}H NMR spectrum of compound **19a** showed two multiplets at δ at 1.60-1.77 and 2.29-2.37 ppm for the four CH_2 groups, two singlts at δ 4.80,



Scheme 2:

4.97 ppm (D_2O exchngeable) corresponding to the two NH_2 groups. A singlet at δ 5.93 ppm for the pyrazole H-3, a multiplet at δ 7.28-7.39 ppm equivalent to the C_6H_5 group and a singlet at δ 8.25 (D_2O exchangeable) for the NH group.

The hydrazido group present in compound **3** underwent ready hydrolysis to the carboxylic group when compound **3** was heated in ethanol containing sodium hydroxide. Compound **3** reacted with phenylisothiocyanate in 1,4-dioxane gave 4-substituted-2-thiosemicarbazide derivative **22**. The latter compound reacted with either α- chloroacetone (**23a**) or ethyl chloroacetate (**23b**) to give the thiazole derivatives **24a** and **24b**, respectively (Scheme **3**).

ANTITUMOR EVALUATIONS

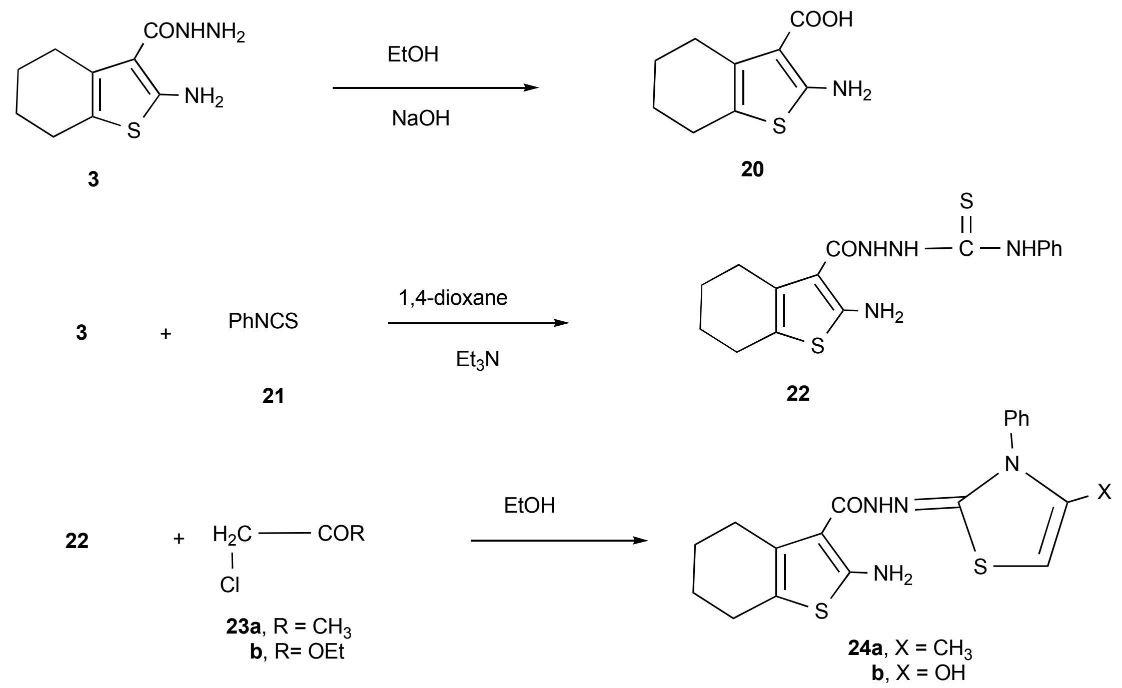
Antitumor and Normal Cell Line Activity Tests

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



Scheme 3:

cancer), and SF-268 (CNS cancer) were used. MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK), NCI-H460, SF-268 and normal fibroblast cells (WI 38) 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 μM 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 x 105 cells/mL for MCF-7 and SF-268 and 0.75 x 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 **3-24a,b** 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. Briefly, exponentially, cells growing in 96-well plates were then exposed for 48 hr 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 solubilised and the absorbance was measured at 492 nm in a plate reader (Bio-Tek Instruments Inc., Power wave XS, Wincoski, USA). For each test compound and cell line, a dose–response curve was obtained and the minimum inhibitory concentration of 50% (IC_{50}), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth was calculated as described elsewhere. Doxorubicin was used as a positive control and tested in the same manner.

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

STRUCTURE ACTIVITY RELATIONSHIP

It is clear from Table **1** that the 2-amino-3-hydrazide-4,5,6,7-tetrahydrobenzo[*b*]thiophene derivative **3** showed high cytotoxicity. On the other hand, the 4-chloroaryl derivative **4b** is more cytotoxic than the unsubstituted aryl derivative. The reaction of compound **3** with either malononitrile of ethyl cyanoacetate gave the pyrazole derivatives **7a** and **7b**, respectively. The cytoyoxicity of compound **7a** with the 2-amino moiety is higher than compound **7b** with the 2-hydroxy moiety. Similarly the reaction of compound **3** with either acetylacetone or ethyl acetoacetate gave the pyrazole derivatives **9a** and **9b**, respectively. It is clear from

Table 1: Effect of Compounds 3-24a,b on the Growth of Three Human Tumor Cell Lines

Compound	**IC$_{50}$ (μ mol L^{-1})**			
	MCF-7	**NCI-H460**	**SF-268**	**WI 38**
3	0.30 ± 0.02	1.80 ± 0.09	4.20 ± 1.04	na
4a	37.0 ± 7.3	16.7 ± 2.3	38.4 ± 2.6	30.6 ± 6.2
4b	20.4 ± 2.8	16.2 ± 3.2	18.6 ±2.6	16.2 ± 8.6
7 a	0.02 ± 0.002	0.01 ± 0.002	0.06 ± 0.008	na
7b	14.0 ± 1.4	22.8 ± 0.3	22.3 ± 0.8	32.4 ± 0.6
9a	28.4 ± 8.8	20.7 ± 6.2	34.4 ± 2.4	30.6 ±3.0
9b	40.8 ± 8.8	33.3 ± 4.7	25.6 ± 5.2	18.2 ± 3.4
11	4.8 ± 1.0	6.8 ± 0.3	12.8 ± 4.2	26.8 ± 4.0
14	30.2 ± 3.6	38.3 ± 12.5	42.6 ± 5.8	58.7 ± 8.6
16	22.1 ± 10.4	30.8 ± 10.8	26.1 ± 2.8	28.2 ± 0.8
18	6.8 ± 1.4	4.5 ± 0.4	3.8 ± 0.6	4.0 ± 2.3
19a	0.02 ± 0.008	0.03 ± 0.006	0.05 ± 0.001	na
19b	26.6 ± 6.2	8.0 ± 3.6	10.6 ± 1.8	8.2 ±2.0
19c	0.01 ± 0.002	0.02 ± 0.001	0.02 ± 0.01	na
19d	33.6 ± 8.5	40.3 ± 12.3	30.4 ± 2.8	62.2 ± 2.0
19e	60.2 ± 2.4	44.6 ± 2.8	46.8 ± 8.5	42.2 ±4.6
19f	22.6 ± 8.0	22.6 ± 8.6	12.4 ± 3.6	40.4 ± 11.3
20	16.2 ± 3.4	23.8 ± 2.6	26.3 ± 2.6	36.4 ±1.4
22	22.3 ± 0.2	1.80 ± 0.09	4.20 ± 1.04	na
24a	42.6 ± 2.6	26.6 ± 2.6	35.2 ± 12.8	10.5 ± 5.1
24b	40.6 ± 3.8	22.6 ± 4.6	38.2 ± 10.6	10.8± 5.1
Doxorubicin	0.04 ± 0.008	0.09±0.008	0.09±0.007	> 100

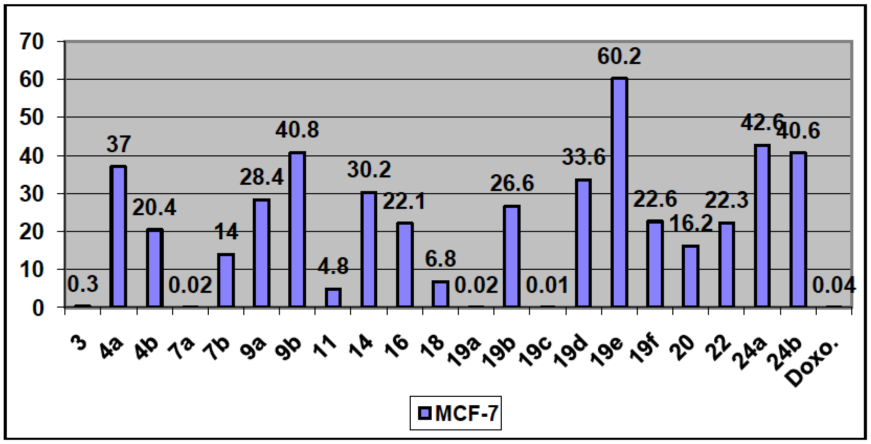


Figure 1: Antitumor effect of synthesized compounds against MCF-7.

Table **1** that both of them with low cytotoxicity, however, compound **9a** with the 2-methyl moiety is more cytotoxic than compound **9b**. The formation of the Schiff's base **11** showed remarkable cytotoxicity with the IC$_{50}$ 4.5 μmol L^{-1} towards MCF-7 cell lines and IC$_{50}$ 6.80 μmol L^{-1} against NCI-H460. Such cytotoxicitys are higher than that of the Schiff bases **14** and **18**. The pyrazole derivatives **19a-f** showed different varieties of IC$_{50}$ where compounds **19a** with the amino group and compound **19c** with the NH$_2$ and Cl moieties showed

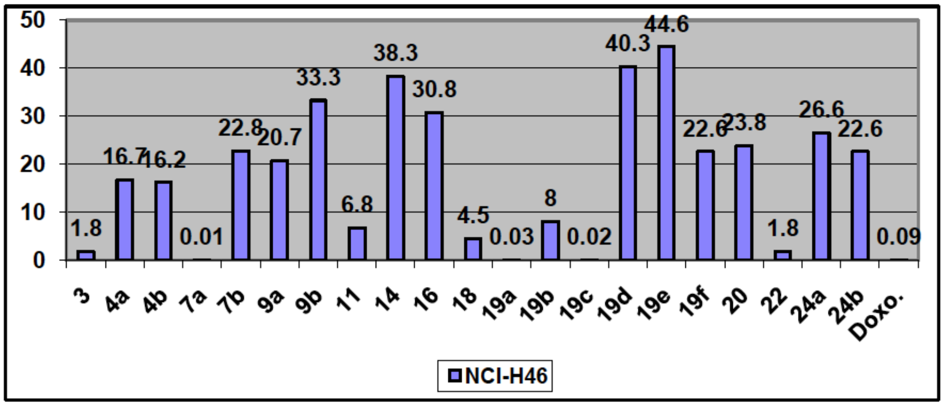


Figure 2: Antitumor effect of synthesized compounds against NCI-H46.

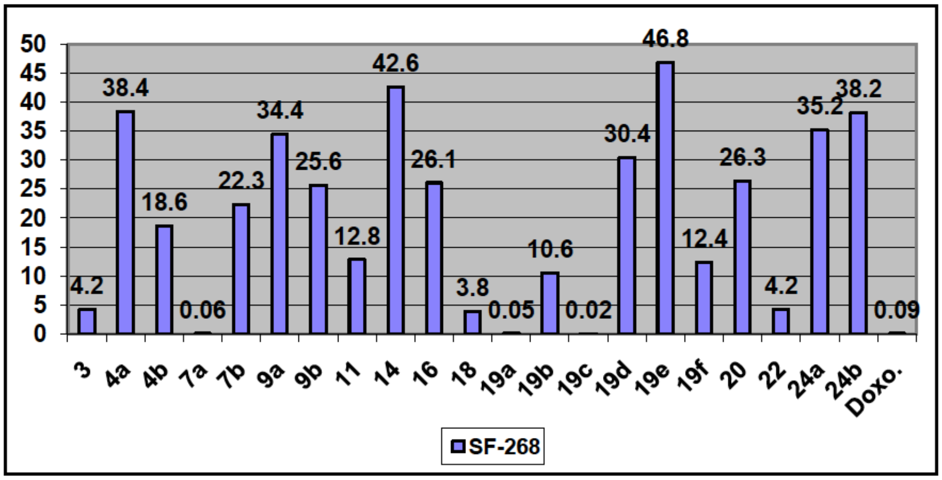


Figure 3: Antitumor effect of synthesized compounds against SF-268.

the highest cytotoxicity among such series of compounds. The reaction of compound **3** with phenylisothiocyanate produced the thiosemicarbazide derivative **22**. The latter showed high cytotoxicity against the two cell lines NCI-H460 and SF-268 with IC_{50} 1.80 and 4.20 (μmol L^{-1}). On the other hand, the reaction of compound **22** reacted with the α-haloketones to produce the thiazole derivatives **24a** and **24b** showed low cytotoxicity.

EXPERIMENTAL

All melting points were determined on an Electrothermal digital melting point apparatus and are uncorrected. IR spectra (KBr discs) were recorded on a FTIR plus 460 or PyeUnicam SP-1000 spectrophotometer. 1H NMR spectra were recorded with Varian Gemini-200 (200 MHz) and Jeol AS 500 MHz instruments spectra were performed in DMSO-d6 as solvent using TMS as internal standard and chemical shifts are expressed as δ ppm. MS (EI) spectra were recorded with Hewlett Packard 5988 A GC/MS system and GCMS-QP 1000 Ex Shimadzu instruments. Analytical data were obtained from the Micro-analytical Data Unit at Cairo University and were performed on Vario EL III Elemental analyzer.

Synthesis of amino-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid hydrazide (3)

To a solution of compound (**1**) (0.99, 0.01 mol) in ethanol (40 mL), containing triethylamine(0.50 ml, 0.01mol) elemental sulphur (0.32g, 0.01mol), cyclopentanone (**2**) (0.98, 0.01mol) was added. The reaction mixture was heated under reflux for 1 h. The separated solid was filtered off to give compound **3**.

Compound **3**: Yellow crystals from acetic acid, yield 95% (2.00g), m.p150°C. *Anal.* calculated for $C_9H_{13}N_3OS$ (211.28): C, 51.16; H, 6.20; N, 19.89; S, 15.18. Found: C, 51.08; H, 5.88; N; 20.05; S, 15.32. IR, υ: 3463-3348 ($2NH_2$, NH), 3056 (CH, aromatic), 1688 (CO), 1634 (C=C). ^{1}H-NMR, δ: 1.66-1.77 (m, 4H, $2CH_2$), 2.31-2.38 (m, 4H, $2CH_2$), 4.85, 5.22 (2s, 4H,

D_2O exchangeable, $2NH_2$), 8.30 (s, 1H, D_2O exchangeable, NH).

Synthesis of 2-amino-4,5,6,7-tetrahydrobenzo [b]thiophene-3-carboxylic acid [1-(4-chloro-phenyl)-ethylidene]-hydrazide (5a) and Amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylic acid [1-(4-chloro-phenyl)-ethylidene]hydrazid (5b)

General Procedure

To a solution of compound **3** (2.11g, 0.01 mol) in absolute ethanol (40 mL) and dimethyl formamide (10 mL) either of acetophenone (1.20 g, 0.01mol) or 4-chloroacetophenone (1.54 g, 0.01mol). The whole reaction mixture, in each case, was heated under reflux for 4h and the formed solid product, obtained upon cooling, was filtered off and dried to give compounds **5a,b**.

Compound **5a**: Brown crystals from acetic acid, yield 87% (2.72g), m.p140°C. *Anal*. calculated for $C_{17}H_{19}N_3OS$ (313.42): C, 65.15; H, 6.11; N, 13.41; S, 10.23. Found: C, 65.08; H, 5.98; N; 13.23; S, 10.41. IR, υ: 3473-3326 (NH_2, NH), 3054 (CH, aromatic), 1689 (CO), 1637 (C=C). ^{1}H-NMR, δ: 1.64-1.77 (m, 4H, $2CH_2$), 2.30-2.39 (m, 4H, $2CH_2$), 2.67 (s, 3H, CH_3), 4.87 (s, 2H, D_2O exchangeable, NH_2), 7.29-7.40 (m, 5H, C_6H_5), 8.27 (s, 1H, D_2O exchangeable, NH).

Compound **5b**: Pale brown crystals from acetic acid, yield 84 % (2.91g), m.p120°C. *Anal*. calculated for $C_{17}H_{18}ClN_3OS$ (347.86): C, 58.70; H, 5.22; N, 12.08; S, 9.22. Found: C, 58.99; H, 5.31; N; 11.89; S, 9.30. IR, υ: 3459-3316 (NH_2, NH), 3056 (CH, aromatic), 1686 (CO), 1632 (C=C). ^{1}H-NMR, δ: 1.62-1.76 (m, 4H, $2CH_2$), 2.31-2.37 (m, 4H, $2CH_2$), 2.68 (s, 3H, CH_3), 4.89 (s, 2H, D_2O exchangeable, NH_2), 7.27-7.42 (m, 4H, C_6H_4), 8.28 (s, 1H, D_2O exchangeable, NH).

Synthesis of (2-amino-4,5,6,7-tetrahydro-benzo[b] thiophen-3-yl)-(3,5-diamino-pyrazol-1-yl)-methanone (7a) and (3-Amino-5-hydroxy-pyrazol-1-yl)-(2-amino-4,5,6,7-tetrahydro-benzo[b]thiophen-3-yl)-methanone (7b)

General Procedure

To a solution of compound **3** (2.11g, 0.01mol) in absolute ethanol (40 mL) and dimethyl formamide (10 mL) containing triethylamine (0.50 g, 0.01mol), either malononitrile (0.66 g, 0.01mol) or ethyl cyanoacetate (1.07g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 3h and the formed solid product, obtained in each case, upon cooling, was collected by filtration to give compounds **7a,b**.

Compound **7a**: Deep brown crystals from 1,4-dioxane, yield 90% (2.49g), m.p190°C. *Anal*. calculated for $C_{12}H_{15}N_5OS$ (277.35): C, 51.97; H, 5.45; N, 25.25; S, 11.56. Found: C, 52.38; H, 5.75; N; 25.27; S, 11.80. IR, υ: 3486-3319 ($3NH_2$), 3057 (CH, aromatic), 1687 (CO), 1634 (C=C). ^{1}H-NMR, δ: 1.62-1.77 (m, 4H, $2CH_2$), 2.31-2.37 (m, 4H, $2CH_2$), 4.84, 4.92, 5.21 (3s, 6H, D_2O exchangeable, $3NH_2$), 6.22 (s, 1H, pyrazole H-4).

Compound **7b**: Brown crystals from 1,4-dioxane, yield 82 % (2.27 g), m.p100°C. *Anal*. calculated for $C_{12}H_{14}N_4O_2S$ (278.33): C, 51.78; H, 5.07; N, 20.13; S, 11.52. Found: C, 52.08; H, 5.36; N; 20.37; S, 11.66. IR, υ: 3520-3327 (OH, $2NH_2$), 1693 (CO), 1658 (C=N), 1631 (C=C). ^{1}H-NMR, δ: 1.60-1.79 (m, 4H, $2CH_2$), 2.32-2.39 (m, 4H, $2CH_2$), 4.82, 4.93 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.24 (s, 1H, pyrazole H-4), 10.30 (s, 1H, D_2O exchangeable, OH).

Synthesis of (2-amino-4,5,6,7-tetrahydro-benzo[b] thiophen-3-yl)-(3,5-dimethyl-pyrazol-1-yl)-methanone (9a) and (2-amino-4,5,6,7-tetrahydro-benzo[b] thiophen-3-yl)-(5-hydroxy-3-methyl-pyrazol-1-yl)-methanone (9b)

General Procedure

To a solution of compound **3** (2.11g, 0.01 mol) in absolute ethanol (40 mL) and dimethylformamide (15 mL) containing piperidine (0.85 g, 0.01mol), either acetylacetone (1.00 g. 0.01mol) or ethyl acetoacetate (1.30 g, 0.01 mol). The reaction mixture, in each case, was heated under reflux for 2h. The formed solid product was filtered off to afford **9a,b**.

Compound **9a**: Orange crystals from 1,4-dioxane, yield 88% (2.42g), m.p 110°C. *Anal*. calculated for $C_{14}H_{17}N_3OS$ (275.37): C, 61.06; H, 6.22; N, 15.26; S, 11.64. Found: C, 60.93; H, 6.09; N; 15.42; S, 11.92. IR, υ: 3459-3326 (NH_2), 3056 (CH, aromatic), 2899 (CH_3), 1689 (CO), 1659 (C=N), 1632 (C=C). ^{1}H-NMR, δ: 1.63-1.75 (m, 4H, $2CH_2$), 2.33-2.39 (m, 4H, $2CH_2$), 2.68, 2.81 (2s, 6H, $2CH_3$), 4.82 (s, 2H, D_2O exchangeable, NH_2), 6.18 (s, 1H, pyrazole H-4).

Compound **9b**: Brown crystals from 1,4-dioxane, yield 90% (2.49 g), m.p120°C. *Anal*. calculated for $C_{13}H_{15}N_3O_2S$ (277.34): C, 56.30; H, 5.45; N, 15.15; S, 11.56. Found: C, 56.42; H, 5.62; N; 15.25; S, 11.63. IR, υ: 3533-3320 (OH, NH_2), 3053 (CH, aromatic), 2889 (CH_3), 1687 (CO), 1653 (C=N), 1630 (C=C). ^{1}H-NMR, δ: 1.61-1.76 (m, 4H, $2CH_2$), 2.32-2.38 (m, 4H, $2CH_2$), 2.69 (s, 3H, CH_3), 4.80 (s, 2H, D_2O exchangeable,

NH_2), 6.19 (s, 1H, pyrazole H-4), 10.30 (s, 1H, D_2O exchangeable, OH).

Synthesis of 2-amino-4,5,6,7-tetrahydro-benzo[b] thiophene-3-carboxylic acid methylene-hydrazide (11)

To a solution of **3** (2.11g, 0.01 mol) in 1,4-dioxane (40 mL) formaldehyde (0.60 g, 0.02 mol) was added. The whole reaction mixture was heated under reflux for 3 h then evaporated under vacuum. The remaining product was triturated with ethanol and the formed solid product was collected by filtration.

Compound **11**: Pale brown crystals from 1,4-dioxane, yield 89 % (1.98 g), m.p 120°C. *Anal*. calculated for $C_{10}H_{13}N_3OS$ (223.29): C, 53.79*;* H, 5.87; N, 18.82; S, 14.36. Found: C, 53.82; H, 5.66; N; 18.91; S, 14.23. IR, υ: 3466-3313 (NH_2, NH), 3053 (CH, aromatic), 2893 (CH_3), 1687 (CO), 1653 (C=N), 1630 (C=C). ^{1}H-NMR, δ: 1.61-1.76 (m, 4H, $2CH_2$), 2.32-2.38 (m, 4H, $2CH_2$), 3.81 (s, 2H, $N=CH_2$), 4.84 (s, 2H, D_2O exchangeable, NH_2), 8.18 (s, 1H, D_2O exchangeable, NH).

Synthesis of 1-(2-amino-4,5,6,7-tetrahydro-benzo[b] thiophene-3-carbonyl)-5-imino-pyrazolidine-4-carbonitrile (12)

To a solution of compound **1** (2.23 g, 0.01mol) in 1,4-dioxane containing triethylamine (0.50 mL), malononitrile (0.66 g, 0.01 mol) was added. The whole reaction mixture was heated under reflux for 3h then left to cool. The solid product, so formed, was collected by filtration. The reaction mixture was evaporated under vacuum and the remaining product was triturated with ethanol and the separated solid was collected by filtration.

Compound **12**: Brown crystals from 1,4-dioxane, yield 78 % (2.23g), m.p120°C. *Anal*. calculated for $C_{13}H_{15}N_5OS$ (289.36): C, 53.96*;* H, 5.23; N, 24.20; S, 11.08. Found: C, 54.12; H, 4.91; N; 24.53; S, 11.40. IR, υ: 3466-3313 (NH_2, NH), 3053 (CH, aromatic), 2893 (CH_3), 2220 (CN), 1689 (CO), 1652 (C=N), 1634 (C=C). ^{1}H-NMR, δ: 1.60-1.78 (m, 4H, $2CH_2$), 2.31-2.38 (m, 4H, $2CH_2$), 4.51 (2s, 2H, D_2O exchangeable, NH_2), 6.23-6.26 (m, 3H, pyrazole CH_2, H-4), 8.22, 8.31 (2s, 2H, D_2O exchangeable, 2NH).

Synthesis of 2-amino-4,5,6,7-tetrahydro-benzo[b] thiophene-3-carboxylic acid ethoxymethylene-hydrazide (14)

To a solution of compound (**3**) (2.11g, 0.01 mol) in 1,4-dioxane (50 mL) triethylorthoformate (1.48 g, 0.01mol) was added. The reaction mixture was heated under reflux for 2h then left to cool and the separated solid was filtered off to afford (**14**).

Compound **14**: Deep brown crystals from 1,4-dioxane, yield 75 % (2.00 g), m.p 100°C. *Anal*. calculated for $C_{12}H_{17}N_3O_2S$ (267.35): C, 53.91*;* H, 6.41; N, 15.72; S, 11.99. Found: C, 53.77; H, 6.29; N; 15.88; S, 11.84. IR, υ: 3494-3340 (NH_2, NH), 2943, 2870 (CH_3, CH_2), 1687 (CO), 1650 (C=N), 1634 (C=C). ^{1}H-NMR, δ: 1.14 (t, 3H, J = 7.22 Hz, CH_3), 1.62-1.79 (m, 4H, $2CH_2$), 2.32-2.39 (m, 4H, $2CH_2$), 4.26 (q, 2H, J = 7.22 Hz, CH_2), 4.73 (s, 2H, D_2O exchangeable, NH_2), 6.21 (s, 1H, N=CH), 8.28 (s, 1H, D_2O exchangeable NH).

Synthesis of 2-amino-4,5,6,7-tetrahydro-benzo[b] thiophene-3-carboxylic acid phenylaminomethylene-hydrazide (16)

To a solution of compound **3** (2.11 g, 0.01 mol) in 1,4-dioxane (40 mL) each of triethylorthoformate (1.48 g, 0.01 mol) and aniline oil (0.93 g, 0.01 mol) were added. The whole reaction mixture was heated under reflux for 4 h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

Compound **16**: Brown crystals from 1,4-dioxane, yield 96% (3.01 g), m.p110°C. *Anal*. calculated for $C_{16}H_{18}N_4OS$ (314.41): C, 61.12*;* H, 5.77; N, 17.82; S, 10.20. Found: C, 61.07; H, 6.01; N; 17.67; S, 10.41. IR, υ: 3483-3327 (NH_2, 2NH), 2893 (CH_2), 1692 (CO), 1651 (C=N), 1632 (C=C). ^{1}H-NMR, δ: 1.61-1.78 (m, 4H, $2CH_2$), 2.31-2.38 (m, 4H, $2CH_2$), 4.69 (s, 2H, D_2O exchangeable, NH_2), 6.25 (s, 1H, N=CH), 7.29-7.39 (m, 5H, C_6H_5), 8.27, 8.37 (2s, 2H, D_2O exchangeable, 2NH).

Synthesis of 2-(4-Chloro-benzylidene)-amino]-4,5, 6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethoxymethylene-hydrazide (18)

A solution of compound **3** (2.11 g, 0.01 mol) in dimethylformamide (20 mL) each of triethylorthoformate (1.48 g, 0.01 mol) and p-chloro benzaldehyde (140 g, 0.01 mol) were added. The whole reaction mixture was heated under reflux for 3h then left to cool and the separated solid was filtered off to afford **18**.

Compound **18**: Deep yellow crystals from 1,4-dioxane, yield 95% (3.69g), m.p115°C. *Anal*. calculated for $C_{19}H_{20}ClN_3O_2S$ (389.90): C, 58.53*;* H, 5.17; N, 10.78; S, 8.22. Found: C, 58.71; H, 5.09; N; 10.53; S, 8.40. IR, υ: 3469-3347 (NH), 2890 (CH_2), 1690 (CO), 1641 (C=N), 1632 (C=C). ^{1}H-NMR, δ: 1.13 (t, 3H, J =

6.94 Hz, CH_3), 1.60-1.79 (m, 4H, 2CH_2), 2.31-2.40 (m, 4H, 2CH_2), 4.21 (q, 2H, J = 6.94 Hz, CH_2), 6.28, 6.32 (2s, 2H, two N=CH), 7.25-7.42 (2d, 4H, C_6H_4), 8.25 (s, 1H, D_2O exchangeable NH).

Synthesis of 5-amino-1-(2-amino-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carbonyl)-3-phenyl-2,3-dihydro-1H-pyrazole-4-carbonitrile (19a), 5-amino-1-(2-amino-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carbonyl)-3-(4-hydroxy-phenyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (19b), 5-amino-1-(2-amino-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carbonyl)-3-(4-chloro-phenyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (19c), 1-(2-amino-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carbonyl)-3-(4-chloro-phenyl)-5-hydroxy-2,3-dihydro-1H-pyrazole-4-carbonitrile (19d), 5-amino-1-(2-amino-4,5,6,7-tetrahydro-benzo [b]thiophene-3-carbonyl)-3-(4-methoxy-phenyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (19e), 1-(2-amino-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carbonyl)-5-hydroxy-3-(4-methoxy-phenyl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (19f)

General Procedure

To a solution of compound **3a** (2.11 g, 0.01 mol), in ethanol (40 mL) containing triethylamine (0.50 ml, 0.01 mol), either benzaldehyde (1.06 g, 0.01mol), or p-chlorobenzaldehyde (1.40 g, 0.01 mol) or p-methoxybenzaldehyde (1.36 g, 0.01 mol) and either of malononitrile (0.66 g, 0.01mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) were added. The whole reaction mixture, in each case, was heated under reflux for 6h and the produced solid product, upon cooling, was collected by filtration.

Compound **19a**: Brown crystals from acetic acid, yield 87 % (g), m.p 90°C. *Anal.* calculated for $C_{19}H_{19}N_5OS$ (365.45): C, 62.44; H, 5.24; N, 19.16; S, 8.77. Found: C, 62.39; H, 5.39; N; 19.26; S, 8.93. IR, υ: 3469-3347 (2NH_2, NH), 2893 (CH_2), 2220 (CN), 1688 (CO), 1648 (C=N), 1630 (C=C). ^{1}H-NMR, δ: 1.60-1.77 (m, 4H, 2CH_2), 2.29-2.37 (m, 4H, 2CH_2), 4.80, 4.97 (2s, 4H, D_2O exchangeable, 2NH_2), 5.93 (s, 1H, pyrazole H-3), 7.28-7.39 (m, 5H, C_6H_5), 8.25 (s, 1H, D_2O exchangeable NH).

Compound **19b**: Brown crystals from acetic acid, yield 82 % (3.00 g), m.p120°C. *Anal.* calculated for $C_{19}H_{18}N_4O_2S$ (366.44): C, 62.28; H, 4.95; N, 15.29; S, 8.75. Found: C, 62.18; H, 5.19; N; 15.41; S, 8.69. IR, υ: 3552-3341 (OH, NH_2, NH), 2893 (CH_2), 2222 (CN), 1689 (CO), 1643 (C=N), 1635 (C=C). ^{1}H-NMR, δ: 1.62-1.77 (m, 4H, 2CH_2), 2.31-2.39 (m, 4H, 2CH_2), 4.82 (s, 2H, D_2O exchangeable, NH_2), 5.91 (s, 1H, pyrazole H-3), 7.29-7.37 (m, 5H, C_6H_5), 8.28 (s, 1H, D_2O exchangeable, NH), 10.04 (s, 1H, D_2O exchangeable, OH).

Compound **19c**: Brown crystals from 1,4-dioxane, yield 89 % (3.55 g), m.p100°C. *Anal.* calculated for $C_{19}H_{18}ClN_5OS$ (399.90): C, 57.07; H, 4.54; N, 17.51; S, 8.02. Found: C, 56.95; H, 4.63; N; 17.26; S, 7.93. IR, υ: 3452-3331 (2NH_2, NH), 2890 (CH_2), 2223 (CN), 1686 (CO), 1643 (C=N), 1635 (C=C). ^{1}H-NMR, δ: 1.62-1.77 (m, 4H, 2CH_2), 2.31-2.38 (m, 4H, 2CH_2), 4.82, 4.86 (2s, 4H, D_2O exchangeable, 2NH_2), 5.91 (s, 1H, pyrazole H-3), 7.25-7.37 (2d, 4H, C_6H_4), 8.27 (s, 1H, D_2O exchangeable NH).

Compound **19d**: Brown crystals from 1,4-dioxane, yield 88 % (3.52g), m.p100°C. *Anal.* calculated for $C_{19}H_{17}ClN_4O_2S$ (400.88): C, 56.93; H, 4.27; N, 13.98; S, 8.00. Found: C, 56.82; H, 4.23; N; 13.77; S, 7.89. IR, υ: 3531-3320 (OH, NH_2, NH), 2893 (CH_2), 2220 (CN), 1689 (CO), 1643 (C=N), 1635 (C=C). ^{1}H-NMR, δ: 1.61-1.77 (m, 4H, 2CH_2), 2.34-2.39 (m, 4H, 2CH_2), 4.83 (s, 2H, D_2O exchangeable, NH_2), 5.82 (s, 1H, pyrazole H-3), 7.23-7.39 (2d, 4H, C_6H_4), 8.24 (s, 1H, D_2O exchangeable NH), 10.16 (s, 1H, D_2O exchangeable, OH).

Compound **19e**: Brown crystals from 1,4-dioxane, yield 85 % (3.35 g), m.p 105 °C. *Anal.* calculated for $C_{20}H_{21}N_5O_2S$ (395.48): C, 60.74; H, 5.35; N, 17.71; S, 8.11. Found: C, 60.83; H, 5.11; N; 17.94; S, 8.03. IR, υ: 3485-3334 (2 NH_2, NH), 2893 (CH_2), 2223 (CN), 1687 (CO), 1623 (C=N), 1632 (C=C). ^{1}H-NMR, δ: 1.59-1.78 (m, 4H, 2CH_2), 2.30-2.39 (m, 4H, 2CH_2), 3.13 (s, 3H, CH_3), 4.85, 8.93 (4H, D_2O exchangeable, 2NH_2), 5.89 (s, 1H, pyrazole H-3), 7.26-7.41 (2d, 4H, C_6H_4), 8.26 (s, 1H, D_2O exchangeable NH).

Compound **19f**: Brown crystals from 1,4-dioxane, yield 80 % (3.16g), m.p120°C. *Anal.* calculated for $C_{20}H_{20}N_4O_3S$ (396.46): C, 60.59; H, 5.08; N, 14.13; S, 8.09. Found: C, 60.79; H, 5.22; N; 14.21; S, 8.27. IR, υ: 3540-3312 (OH, NH_2, NH), 2896 (CH_2), 2224 (CN), 1688 (CO), 1626 (C=N), 1634 (C=C). ^{1}H-NMR, δ: 1.63-1.79 (m, 4H, 2CH_2), 2.32-2.41 (m, 4H, 2CH_2), 3.14 (s, 3H, CH_3), 4.82 (2H, D_2O exchangeable, NH_2), 5.79 (s, 1H, pyrazole H-3), 7.28-7.38 (2d, 4H, C_6H_4), 8.31 (s, 1H, D_2O exchangeable NH), 10.25 (s, 1H, D_2O exchangeable, OH).

Synthesis of 2-amino-4,5,6,7-tetrahydro-benzo[b] thiophene-3-carboxylic acid (20)

To a solution of compound **3** (2.11 g, 0.01mol) in absolute ethanol (40 mL), sodium hydroxide (0.40 g, 0.01 mol) was added. The whole reaction mixture was heated under reflux for 6h the poured onto ice/water mixture containing few drops of concentrated hydrochloric acid (till pH 6). The formed solid product was collected by filtration.

Compound **20**: crystals from 1,4-dioxane, yield % (g), m.p 220-223°C. *Anal*. Calculated for $C_9H_{11}NO_2S$ (197.25): C, 54.80*;* H, 5.62; N, 7.10; S, 16.26. Found: C, 55.13; H, 5.49; N; 7.28; S, 16.19. IR, υ: 3523-3338 (OH, NH_2), 2898 (CH_2), 1702 (CO), 1628 (C=N), 1638 (C=C). ^{1}H-NMR, δ: 1.61-1.79 (m, 4H, $2CH_2$), 2.30-2.39 (m, 4H, $2CH_2$), 4.80 (s, 2H, D_2O exchangeable, NH_2), 10.28 (s, 1H, D_2O exchangeable, OH).

Synthesis of 2-(2-amino-4,5,6,7-tetrahydrobenzo [b]thiophene-3-carbonyl)-N-phenylhydrazinecarbothioamide (22)

To a solution of compound **3** (2.11g, 0.01mol), in 1,4dioxane (25 mL) and dimethylformamide (10 mL) containing triethylamine (0.5 mL,0.01mol), phenylisothiocyanate (1.35 g, 0.01mol) was added. The whole mixture was heated under reflux for 4h then left to cool and the formed solid product upon pouring onto ice/water containing few drops of hydrochloric acid.

Compound **22**: Orange crystals from acetic acid, yield 95 % (3.28g), m.p110°C. *Anal*. Calculated for $C_{16}H_{18}N_4OS_2$ (346.47): C, 55.47*;* H, 5.24; N, 16.17; S, 18.51. Found: C, 55.63; H, 5.41; N; 15.94; S, 18.72. IR, υ: 3483-3322 (NH_2, 3NH), 2885 (CH_2), 1687 (CO), 1632 (C=C), 1205 (C=S). ^{1}H-NMR, δ: 1.59-1.78 (m, 4H, $2CH_2$), 2.34-2.41 (m, 4H, $2CH_2$), 4.87 (s, 2H, D_2O exchangeable, NH_2), 7.26-7.42 (m, 5H, C_6H_5), 8.26, 8.29, 8.31 (3s, 3H, D_2O exchangeable, 3NH).

Synthesis of 2-amino-4,5,6,7-tetrahydro-benzo[b] thiophene-3-carboxylic acid (4-methyl-3-phenyl-3H-thiazol-2-ylidene)-hydrazide (24a) and 2-Amino-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid (4-hydroxy-3-phenyl-3H-thiazol-2-ylidene)-hydrazide (24b)

General Procedure

To a solution of compound **22** (3.46 g, 0.01 mol) in ethanol (40 mL) either α-chloroacetone (0.92 g, 0.01 mol) or ethyl chloroacetate (1.22g, 0.01 mol). The whole reaction mixture, in each case, was heated under reflux for 4 h then left to cool and the separated crystals were collected by filtration.

Compound **24a**: Brown crystals from 1,4-dioxane, yield 75 % (2.88 g), m.p105°C. *Anal*. calculated for $C_{19}H_{20}N_4OS_2$ (384.52): C, 59.35*;* H, 5.24; N, 14.57; S, 16.68. Found: C, 59.48; H, 5.09; N; 14.82; S, 16.82. IR, υ: 3474-3320 (NH_2, NH), 3055 (H aromatic), 2890 (CH_2), 1690 (CO), 1653 (C=N), 1630 (C=C). ^{1}H-NMR, δ: 1.60-1.75 (m, 4H, $2CH_2$), 2.34-2.41 (m, 4H, $2CH_2$), 2.69 (s, 3H, CH_3), 4.80 (s, 2H, D_2O exchangeable, NH_2), 6.16 (s, 1H, thiazole H-5), 7.31-7.42 (m, 5H, C_6H_5), 8.24 (s, 1H, D_2O exchangeable, NH).

Compound **24b**: Brown crystals from 1,4-dioxane, yield 76 % (2.93g), m.p110 °C. *Anal*. calculated for $C_{18}H_{18}N_4O_2S_2$ (386.49): C, 55.94*;* H, 4.69; N, 14.50; S, 16.59. Found: C, 56.27; H, 4.81; N; 14.68; S, 16.69. IR, υ: 3523-3331 (OH, NH_2, NH), 3059 (CH aromatic), 2893 (CH_2), 1688 (CO), 1653 (C=N), 1632 (C=C). ^{1}H-NMR, δ: 1.62-1.75 (m, 4H, $2CH_2$), 2.33-2.39 (m, 4H, $2CH_2$), 4.82 (s, 2H, D_2O exchangeable, NH_2), 6.19 (s, 1H, thiazole H-5), 7.26-7.40 (m, 5H, C_6H_5), 8.27 (s, 1H, D_2O exchangeable, NH), 8.32 (s, 1H, D_2O exchangeable, OH).

REFERENCES

[1] Sultana SS, Antony SA, Balachandran C. Thiophene and benzodioxole appended thiazolyl-pyrazoline compounds: Microwave assisted synthesis, antimicrobial and molecular docking studies. Bioorg Med Chem Lett 2015; 25: 2753-7. http://dx.doi.org/10.1016/j.bmcl.2015.05.033

[2] Magd-El-Din AA, Atta SMS, Abd-El-All AS Galal SA, Abdalah MM. New synthesis of tetrahydrobenzo[4,5]thieno[2,3d] pyrimidine derivatives and Schiff bases derived from 2-aminotetrahydro benzothiophenes and hetarylcarboxaldehydes studies on their antitumor and antimicrobial activities. World J Chem 2009; 4: 112-7.

[3] Soliman R, Habib NS, El-Tombary AA, El-Hawash SAM, Shaaban OG. Synthesis of tetrahydrobenzothieno[2,3-d]pyrimidine and tetrahydrobenzothieno[3,2-e][1,2,4]triazolo [4,3-*c*]pyrimidine derivatives as potential antimicrobial agents. Sci Pharm 2009; 77: 755-73. http://dx.doi.org/10.3797/scipharm.0904-17

[4] Sayyedeh Shadfar Pourpanah SS, Sayyed Mostafa Habibi-Khorassani SM, Mehdi Shahraki M. Fructose-catalyzed synthesis of tetrahydrobenzo[*b*]pyran derivatives: Investigation of kinetics and mechanism. Chinese J of Catalysis 2015; 36: 757-63. http://dx.doi.org/10.1016/S1872-2067(14)60302-8

[5] Elmegeed GA, Wardakhan WW, Younis M, Louca NA. Synthesis and antimicrobial evaluation of some novel cholestane heterocyclic derivatives. Arch Pharm 2004; 337: 140-7. http://dx.doi.org/10.1002/ardp.200300825

[6] Behbehani H, Ibrahim HM, Makhseed S, Elnagdi MH, Mahmoud H. 2-Aminothiophenes as building blocks in heterocyclic synthesis: Synthesis and antimicrobial evaluation of a new class of pyrido[1,2-*a*]thieno[3,2-*e*]pyrimidine, quinoline and pyridin-2-one derivatives. Eur J Med Chem 2012; 52: 51-65. http://dx.doi.org/10.1016/j.ejmech.2012.03.004

[7] Angell RM, Atkinson FL, Brown MJ, *et al.* N-(3-Cyano-4,5,6,7-tetrahydro-1-benzothien-2-yl)amides as potent, selective, inhibitors of JNK2 and JNK3. Bioorg Med Chem Lett 2007; 17: 1296-1301. http://dx.doi.org/10.1016/j.bmcl.2006.12.003

[8] Cohen SM, Duffy JL, Miller C, *et al*. Direct observation (NMR) of the efficacy of glucagon receptor antagonists in murine liver expressing the human glucagon receptor. Bioorg Med Chem 2006; 14: 1506-17.
http://dx.doi.org/10.1016/j.bmc.2005.10.008

[9] Gouda MA, Berghot MA, El-Ghani GF, Elattar KM, Khalil AE. Chemistry of 2-aminothiophene-3-carboxamide and related compounds. Turk J Chem 2011; 35: 815 - 37.

[10] Silva WL, De Lima CA, Galdino SL, Pitta IR, De Simone CA. 2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile, Acta Crystallogr 2011; 67: O3161-U3836.

[11] Abu-Hashem AA, Abu-Zied KM, El-Shehry, MF. Synthetic utility of bifunctional thiophene derivatives and antimicrobial evaluation of the newly synthesized agents. Monatsh Chem 2011; 142: 539-45.
http://dx.doi.org/10.1007/s00706-011-0456-z

[12] Wang T, Huang XG, Liu J, *et al*. An Efficient One-Pot Synthesis of Substituted 2-aminothiophenes *via* three-component Gewald reaction catalyzed by L-Proline. Synlett 2010; 1351-4.
http://dx.doi.org/10.1002/chin.201040097

[13] Fujita M, Seki T, Ikeda N. Synthesis and bioactivities of novel bicyclic thiophenes and 4,5,6,7-tetrahydrothieno[2,3-c]pyridines as inhibitors of tumor necrosis factor-α (TNF-α) production. Bioorg Med Chem Lett 2002; 12: 1897-1990.
http://dx.doi.org/10.1016/S0960-894X(02)00332-3

[14] Feroci M, Chiarotto I, Rossi L, Inesi A. Activation of elemental sulfur by electrogenerated cyanomethyl anion: synthesis of substituted 2-aminothiophenes by the Gewald reaction. Adv Synth Catal 2008; 350: 2740-6.
http://dx.doi.org/10.1002/adsc.200800503

[15] Castanedo GM, Sutherlin DP. Synthesis of tetrasubstituted thiophenes on solid-support using the Gewald reaction. Tetrahedron Lett 2001; 42: 7181-4.
http://dx.doi.org/10.1016/S0040-4039(01)01470-8

[16] Xian-Gui H, Jia L, Jiangmeng R, Tao W, Weidong C, Bu-Bing Z. A facile and practical one-pot synthesis of multisubstituted 2-aminothiophenes *via* imidazole-catalyzed Gewald reaction. Tetrahedron 2011; 67: 6202-5.
http://dx.doi.org/10.1016/j.tet.2011.06.061

[17] William CS, Maxwell ZS, James M. A Solvent-Free, One-step, one-pot Gewald reaction for alkyl-aryl ketones *via* mechanochemistry. Appl Sci 2014; 4: 171-9.
http://dx.doi.org/10.3390/app4020171

[18] Tormyshev VM, Trukhin DV, Rogozhnikova OY, Mikhalina TV, Troitskaya TI, Flinn A. Aryl alkyl ketones in a one-pot Gewald synthesis of 2-aminothiophenes. Synlett 2006; 2559-64.
http://dx.doi.org/10.1055/s-2006-951484

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