USES OF 4,5,6,7-TETRAHYDROBENZO[*b*]THIOPHENE IN THE SYNTHESIS OF PYRIDAZINE, PYRAZOLE, THIAZOLE AND PYRIMIDINE DERIVATIVES TOGETHER WITH THEIR CYTOTOXICITY

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ABSTRACT

The reaction of N-phenylbutanamide derivative **1** with bromine afforded compound **2** which it was directed to reacts with activated methylene groups, malononitrile **(3a)** and ethylcyanoacetae **(3b)** to produce compounds **4a** and **4b** respectively, on the other hand the reaction of compound **2** with either hydrazine hydrate **(8a)** or phenylhydrazine **(8b)** afforded pyridazine derivatives **10a,b** respectively, Moreover the reaction of compound **2** with either potassium cyanide **(11a)** or potassium thiocyanate **(11b)** produced compounds **12a,b** respectively. Finally the reaction of compound **2** with thiourea **(13a)** afforded thiazole derivative **14.** Compound **4b** reacted with benzenediazonium chloride **(5)** afforded pyridazine derivative **7.** The reactivity of compound **12a** was introduced through the reaction with either hydrazine derivatives **8a,b** or aromatic aldehydes **16, 18** then compounds **15a,b, 17, 19** were produced respectively. As extension of compound **1** reactions, malononitrile **(3a)** reacted with compound **1** afforded two isomeric compounds **20** and **21,** the latter product **20** was reacted with either hydrazine derivatives **8a,b** or thiourea and urea **(13a,b)** to produce pyrazole derivatives **22a,b** and pyrimidine derivatives **23a,b** respectively.Their cytotoxic activities were tested using three different cell lines.

INTRODUCTION

Thiophene derivatives represent a class of important and well-studied heterocycles **(Eicher *et al.*, 2003 and Gronowitz Salo, 1991).** The interest in this kind of heterocycles has spread in drug design **(Wu *et al.*, 2004).** Pyridazine derivatives exhibit an interesting numbers of biological properties such as kinase inhibitors **(Kate *et al.*, 2004)** and antibacterial agents **(Rahul *et al.*, 2006),** also pyrazolo-pyridine derivatives it has antibacterial activity **(Focks *et al.*, 2005)**, on the other hand thiazole derivatives has many biological properties such as antiprotozoal agents **(Tapia *et al.*, 2003)** and potent anti-inflammatory agents **(Pawan *et al.*, 1997),** finally pyrimidine derivatives has a wide spectrum of biological and pharmacological activities. Thus many synthetic pyrimidines are considered as antiepileptic phenobarbital **(Kwan *et al.*, 2004),** dihydro-pyrimidinone unit like batzelladine alkaloids have been found to be potent HIV gp120-Human CD4 binding inhibitors **(Patil *et al.*, 1995 and Snider *et al.*, 1996).**

In this article we have synthesized some new heterocyclic compounds containing tetrahydrobenzo[*b*]thiophene moiety to try to improve their biological evaluations as well as their cytotoxic activity.

EXPERMENTAL

Synthetic methods, analytical and spectral data

All melting points were determined in open capillaries and are uncorrected. Elemental analyses were performed on a Yanaco CHNS Corder elemental analyzer (Japan). IR spectra were measured using KBr discs on a Pye Unicam SP-1000 spectrophotometer. ^{1}H NMR spectra were measured on a Varian EM 390-200 M*Hz* instrument in CD_3SOCD_3 as solvent using TMS as internal standard and chemical shifts are expressed as δ ppm. Mass spectra were recorded on Kratos (75 eV) MS equipment (Germany).

4-Bromo-2-[(3-cyano-4,5,6,7-tetrahydro-benzo[*b*]thiophen-2-yl)-hydrazono]-3-oxo-N-phenyl-butanamide (2)

To a solution of compound **1** (3.66 g, 0.01 mol) in glacial acetic acid (40 mL) at 60 ^{o}C, bromine (0.50 ml) in acetic acid solution (10 mL) was added drop wise. The reaction mixture, after addition of all bromine solution, was kept at room temperature for 1 h with continuous stirring. The solid product, formed upon pouring onto ice/water was collected by filtration.

Compound **2**: Pale brown crystals from ethanol, yield: 88 % (3.920g); mp: 124 ºC. IR (KBr): υ/cm^{-1} = 3479-3331 (2 NH), 3053 (CH-aromatic), 2888 (CH_2), 2225 (CN), 1705, 1689 (2 CO), 1633 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.74-1.79 (m, 4H, 2CH_2), 2.11-2.18 (m, 4H, 2CH_2), 3.88 (s, 2H, CH_2), 7.27-7.38 (m, 5H, C_6H_5), 8.32, 9.44 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 446, 444. Analysis for $C_{19}H_{17}BrN_4O_2S$ Calcd: C, 51.24; H, 3.85; N, 12.58; S, 7.20. Found: C, 51.48 H, 4.02; N, 12.39; S, 7.48 %.

2-(3-Cyano-2-hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene)-3(bromomethyl) -4,4-dicyano-N-phenylbut-3-enamide (4a) and Ethyl 4-(phenylcarbamoyl)-4-(3-cyano-2-hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]-thiophene)-3-(bromomethyl)-2-cyanobut-2-enoate (4b)

To a solution of compound **2** (2.22 g, 0.005 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL), either malononitrile (**3a**, 0.33 g, 0.005 mol) or ethyl cyanoacetate (**3b**, 0.57 g, 0.005 mol) was added. The reaction mixture was heated under reflux for 3h then left to cool and the formed solid product, so formed was collected by filtration.

Compound **4a**: Yellow crystals from ethanol, yield: 82 % (2.03 g); mp: 288 ºC. IR (KBr): υ/cm^{-1} = 3465-3328 (2 NH), 3056 (CH-aromatic), 2885 (CH_2), 2227- 2220 (3 CN), 1687 (CO), 1633 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.71-1.76 (m, 4H, 2CH_2), 2.15-2.19 (m, 4H, 2CH_2), 3.93 (s, 2H, CH_2), 7.28-7.39 (m, 5H, C_6H_5), 8.39, 9.42 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 494, 492. Analysis for $C_{22}H_{17}BrN_6OS$ Calcd: C, 53.56; H, 3.47; N, 17.03; S, 6.50. Found: C, 53.72; H, 3.55; N, 16.82; S, 6.36 %.

Compound **4b**: Yellow crystals from ethanol, yield: 77 % (2.08 g); mp: 193 ºC. IR (KBr): υ/cm^{-1} = 3471-3347 (2 NH), 3058 (CH-aromatic), 2882 (CH_2), 2227, 2222 (CN), 1785, 1689 (2CO), 1634 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.13 (t, 3H, J = 7.55 Hz, CH_3), 1.70-1.77 (m, 4H, 2CH_2), 1.97-2.05 (m, 4H, 2CH_2), 3.81 (s, 2H, CH_2), 4.22 (q, 2H, J = 7.55 Hz, CH_2), 7.29-7.41 (m, 5H, C_6H_5), 8.33, 9.40 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 541, 539. Analysis for $C_{24}H_{22}BrN_5O_3S$ Calcd: C, 53.34; H, 4.10; N, 12.96; S, 5.93. Found: C, 53.51; H, 4.27; N, 13.07 S, 6.22 %.

2-(3-Cyano-2-hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene)-2-(3-bromo-5-cyano-1,6-dihydro-6-oxo-1-phenylpyridazin-4-yl)-N-phenylacetamide (7)

To a solution of compound **4b** (2.7 g, 0.005 mol) in ethanol (40 mL) containing sodium hydroxide (10 mL, 10 %), a cold solution of benzenediazonium chloride **(5)** [prepared by the addition of sodium nitrite (0.35 g, 0.005 mol) solution (in 10 mL water) to a cold solution of aniline (0.47 g, 0.005 mol) in concentrated acetic/hydrochloric acid (10:3) with continuous stirring] was added with continuous stirring. The reaction mixture was stirred for an addition 1 h at room temperature and the formed solid product was collected by filtration.

Compound **7**: Pale yellow crystals from 1,4 dioxane, yield: 64 % (1.92 g); mp: 170-172 ºC. IR (KBr): υ/cm^{-1} = 3477-3320 (2 NH), 3054 (CH-aromatic), 2880 (CH_2), 2224, 2221 (2 CN), 1688, 1684 (2 CO), 1636 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.73-1.79 (m, 4H, 2CH_2), 1.89-1.99 (m, 4H, 2CH_2), 7.24-7.43 (m, 10H, 2C_6H_5), 8.32, 9.40 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 599, 597. Analysis for $C_{28}H_{20}BrN_7O_2S$ Calcd: C, 56.19; H, 3.37; N, 16.38; S, 5.36. Found: C, 56.05; H, 3.66; N, 16.52; S, 5.57 %.

3-Cyano-2-azo-(6-phenylamino-4-hydroxypyridazine-5-yl)-4,5,6,7-tetrahydro-benzo[*b*]thiophene (10a) and 3-Cyano-2-azo-(6-phenylamino-1-phenyl-4-hydroxy -pyridazine-5-yl)-4,5,6,7-tetrahydrobenzo-[*b*]thiophene (10b)

To a solution of compound **2** (2.22 g, 0.005 mol) in ethanol (40 mL), either hydrazine hydrate (**8a**, 0.30 mL, 0.005 mol) or phenylhydrazine (**8b**, 0.60 g, 0.005 mol) was added. The reaction mixture in each case was heated under reflux for 4 h then left to cool. The solid product, formed upon pouring onto ice/water containing few drops of hydrochloric acid (till pH 6) was collected by filtration.

Compound **10a**: Yellow crystals from ethanol, yield: 68 % (1.28 g); mp: 200-202 ºC. IR (KBr): υ/cm^{-1} = 3522-3348 (OH, NH), 3053 (CH-aromatic), 2886 (CH_2), 2224 (CN), 1638 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.73-1.78 (m, 4H, 2CH_2), 2.21-2.26 (m, 4H, 2CH_2), 6.83 (s, 1H, pyridazine H-3), 7.29-7.42 (m, 5H, C_6H_5), 8.28 (s, 1H, D_2O-exchangeable, NH), 9.38 (s, 1H, D_2O-exchangeable, OH). MS (relative intensity) m/z: 376. Analysis for $C_{19}H_{16}N_6OS$ Calcd: C, 60.62; H, 4.28; N, 23.33; S, 8.52. Found: C, 60.93; H, 4.09; N, 23.59; S, 8.35 %.

Compound **10b**: Yellow crystals from ethanol, yield: 81 % (1.84 g); mp: 162-163 ºC. IR (KBr): υ/cm^{-1} = 3555-3337 (OH, 2NH), 3056 (CH-aromatic), 2898 (CH_2), 2223 (CN), 1632 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.64-1.72 (m, 4H, 2CH_2), 2.06-2.14 (m, 4H, 2CH_2), 6.94 (s, 1H, pyridazine H-3), 7.28- 7.42 (m, 10H, 2C_6H_5), 8.32, 8.53 (2s, 2H, D_2O-exchangeable, 2NH), 9.18 (s, 1H, D_2O-exchangeable, OH). MS (relative intensity) m/z: 454. Analysis for $C_{25}H_{22}N_6OS$ Calcd: C, 66.06; H, 4.88; N, 18.49; S, 7.05. Found: C, 65.81; H, 4.92; N, 18.57; S, 7.32 %.

2-(3-Cyano-2-hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene)-4-cyano-3-oxo-N-phenylbutanamide (12a) and 2-(3-Cyano-2-hydrazinyl-4,5,6,7-tetrahydrobenzo [*b*] thiophene)- 3-oxo-N-phenyl-4-thiocyanatobutanamide (12b)

The solution of compound **2** (4.44 g, 0.01 mol) in ethanol (50 mL) either potassium cyanide (**11a**, 1.30 g, 0.02 mol) or potassium thiocyanate (**11b**, 1.94 g, 0.02 mol) solution in water (10 mL) was added drop wise and the reaction mixture was heated in water bath at 60 ^{o}C for 1h. The whole reaction mixture was stirred at room temperature for an additional 3 h then poured onto ice/water and few drops of hydrochloric acid were added, the formed solid product was collected by filtration.

Compound **12a**: White crystals from ethanol, yield: 58 % (2.270 g); mp: 178-179 ºC. IR (KBr): υ/cm^{-1} = 3444-3328 (2 NH), 3055 (CH-aromatic), 2918 (CH_2), 2226, 2220 (2 CN), 1688, 1685 (2 CO), 1633 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.71-1.75 (m, 4H, 2CH_2), 2.19-2.27 (m, 4H, 2CH_2), 3.93 (s, 2H, CH_2), 7.27- 7.36 (m, 5H, C_6H_5), 8.26, 8.45 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 391. Analysis for $C_{20}H_{17}N_5O_2S$ Calcd: C, 61.37; H, 4.38; N, 17.89; S, 8.19. Found: C, 61.58; H, 4.59; N, 17.92; S, 8.28 %.

Compound **12b**: Yellow crystals from ethanol, yield: 74 % (3.133 g); mp: 184-186 ºC. IR (KBr): υ/cm^{-1} = 3476-3332 (2NH), 3058 (CH-aromatic), 2916 (CH_2), 2222, 2220 (2 CN), 1636 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.62-1.70 (m, 4H, 2CH_2), 1.98-2.09 (m, 4H, 2CH_2), 3.89 (s, 2H, CH_2), 7.28- 7.42 (m, 5H, C_6H_5), 8.34, 8.58 (2s, 2H, D_2O-exchangeable, 2NH).

MS (relative intensity) m/z: 423. Analysis for $C_{20}H_{17}N_5O_2S_2$ Calcd: C, 56.72; H, 4.05; N, 16.54; S, 15.14. Found: C, 56.82; H, 3.89; N, 16.83; S, 15.08 %.

2-(3-Cyano-2-hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene)-2-(2-amino-thiazol-4-yl)-N-phenylacetamide (14)

To a solution of compound **2** (4.44 g, 0.01 mol) in ethanol (50 mL), thiourea (**13a**, 0.76 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then left to cool and the formed solid product upon pouring onto ice/water containing few drops of sodium hydroxide (5 %) was collected by filtration.

Compound **14**: Yellow crystals from DMF, yield: 65 % (2.746 g); mp: 252-253 ºC. IR (KBr): υ/cm^{-1} = 3483-3343 (NH_2, 2 NH), 3057 (CH-aromatic), 2883 (CH_2), 2224 (CN), 1687 (CO), 1638 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.67-1.74 (m, 4H, $2CH_2$), 2.02-2.08 (m, 4H, $2CH_2$), 4.22 (s, 2H, D_2O exchangeable, NH_2), 6.83 (s, 1H, thiazole H-5), 7.29- 7.43 (m, 5H, C_6H_5), 8.24, 8.83 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 422. Analysis for $C_{20}H_{18}N_6OS_2$ Calcd: C, 56.85; H, 4.29; N, 19.89; S, 15.18. Found: C, 56.59; H, 4.42; N, 19.58; S, 15.42 %.

2-(3-Cyano-2-hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene)-2-(3-amino-1*H*-pyrazol-5-yl)-N-phenylacetamide (15a) and 2-(3-Cyano-2-hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene)-2-(3-amino-1-phenyl-1*H*-pyrazol-5-yl)-N-phenyl-acetamide (15b)

To a solution of compound **12a** (3.91 g, 0.01 mol) in ethanol (40 mL) either hydrazine hydrate (**8a**, 0.50 g, 0.01 mol) or phenyl hydrazine (**8b**, 1.08 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

Compound **15a**: Yellow crystals from ethanol, yield: 76 % (3.081 g); mp: 285-287 ºC. IR (KBr): υ/cm^{-1} = 3485-3321 (NH_2, 3 NH), 3058 (CH-aromatic), 2884 (CH_2), 2224 (CN), 1689 (CO), 1636 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.70-1.77 (m, 4H, $2CH_2$), 2.19-2.28 (m, 4H, $2CH_2$), 4.66 (s, 2H, D_2O exchangeable, NH_2), 6.82 (s, 1H, pyrazole H-4), 7.29- 7.43 (m, 5H, C_6H_5), 8.34, 8.46, 9.11 (3s, 3H, D_2O-exchangeable, 3NH). MS (relative intensity) m/z: 405. Analysis for $C_{20}H_{19}N_7OS$ Calcd: C, 59.24; H, 4.72; N, 24.18; S, 7.91. Found: C, 58.98; H, 4.61; N, 24.42; S, 8.20 %.

Compound **15b**: Yellow crystals from ethanol, yield: 73 % (3.515 g); mp: 189-191 ºC. IR (KBr): υ/cm^{-1} = 3475-3331 (NH_2, 2 NH), 3054 (CH-aromatic), 2897 (CH_2), 2221 (CN), 1687 (CO), 1638 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.72-1.77 (m, 4H, $2CH_2$), 2.15-2.22 (m, 4H, $2CH_2$), 4.68 (s, 2H, D_2O exchangeable, NH_2), 6.80 (s, 1H, pyrazole H-4), 7.28- 7.46 (m, 10H, $2C_6H_5$), 8.32, 8.44 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 481. Analysis for $C_{26}H_{23}N_7OS$ Calcd: C, 64.85; H, 4.81; N, 20.36; S, 6.66. Found: C, 64.62; H, 4.58; N, 20.41; S, 6.48 %.

2-(3-Cyano-2-hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene)-4-cyano-3-oxo- 5-phenyl- N-phenylpent-4-enamide (17)

To a solution of compound **12a** (3.91 g, 0.01 mol) in 1,4-dioxane (40 mL) containing piperidine (0.50 mL), benzaldehyde (**16**, 1.06 g, 0.01 mol) was added. The 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 **17**: Yellow crystals from 1,4 dioxane, yield: 80 % (3.836g); mp: 183-185 ºC. IR (KBr): υ/cm^{-1} = 3523-3322 (2 NH), 3053 (CH-aromatic), 2877 (CH_2), 2227, 2220 (2 CN), 1718, 1684 (2 CO), 1636 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.67-1.74 (m, 4H, $2CH_2$), 2.13-

2.18 (m, 4H, $2CH_2$), 5.28 (s, 1H, CH=C), 7.30- 7.38 (m, 10H, $2C_6H_5$), 8.31, 8.42 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 479. Analysis for $C_{27}H_{21}N_5O_2S$ Calcd: C, 67.62; H, 4.41; N, 14.60; S, 6.69. Found: C, 67.48; H, 4.53; N, 14.88; S, 6.43 %.

2-(3-Cyano-2-hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene)-3-oxo-3-(2-oxo-2*H*-chromen-3-yl)-N-phenylpropanamide (19)

To a solution of compound **12a** (3.91 g, 0.01 mol) in 1,4-dioxane (40 mL) containing piperidine (0.50 mL), salicyladehyde (1.22 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 5 h then evaporated under vacuum. The remaining product was triturated with ethanol and the formed solid product was collected by filtration.

Compound **19**: Yellow crystals from 1,4 dioxane, yield: 80 % (3.972 g); mp: 215-217 ºC. IR (KBr): υ/cm^{-1} = 3477-3342 (2NH), 3055 (CH-aromatic), 2889 (CH_2), 2225 (CN), 1776, 1734, 1667 (3 CO), 1638 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.69-1.73 (m, 4H, $2CH_2$), 2.21-2.26 (m, 4H, $2CH_2$), 6.88 (s, 1H, coumarin H-4), 7.24- 7.47 (m, 9H, C_6H_5, C_6H_4), 8.33, 8.44 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 496. Analysis for $C_{27}H_{20}N_4O_4S$ Calcd: C, 65.31; H, 4.06; N, 11.28; S, 6.46. Found: C, 65.62; H, 4.27; N, 10.99; S, 6.69 %.

2-(3-Cyano-2-hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene)-4,4-dicyano-3-methyl-N-phenylbut-3-enamide (20) and 5-(2-Diazenyl-3cyano-4,5,6,7-tetrahydro benzo[*b*]thiophene)-2-amino-1,6-dihydro-4-methyl-6-oxo-1-phenylpyridine-3-carbonitrile (21)

To a solution of compound **1** (3.66 g, 0.01 mol) in DMF (40 mL) containing piperidine (0.50 mL), malononitrile (**3a**, 0.66 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then left to cool and the formed solid product, upon pouring onto ice/water containing few drops of hydrochloric acid was collected by filtration. The solid showed two spots through TLC, the ethanol soluble product was identified to show product **20** while the ethanol insoluble was identified to give product **21**.

Compound **20**: Pale yellow crystals from ethanol, yield: 68 % (2.818 g); mp: >290 ºC. IR (KBr): υ/cm^{-1} = 3449-3323 (2 NH), 3055 (CH-aromatic), 2955(CH_3), 2227- 2220 (3 CN), 1693 (CO), 1633 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.66-1.69 (m, 4H, $2CH_2$), 2.25-2.31 (m, 4H, $2CH_2$), 2.40 (s, 3H, CH_3), 7.29-7.40 (m, 5H, C_6H_5), 8.42, 9.29 (2s, 2H, D_2O-exchangeable, 2NH,). MS (relative intensity) m/z: 414. Analysis for $C_{22}H_{18}N_6OS$ Calcd: C, 63.75; H, 4.38; N, 20.28; S, 7.74. Found: C, 63.92; H, 4.66; N, 20.32; S, 7.49 %.

Compound **21**: Pale yellow crystals from DMF, yield: 79 % (3.274 g); mp: > 300 ºC. IR (KBr): υ/cm^{-1} = 3453-3343 (NH_2), 3053 (CH-aromatic), 2988 (CH_3), 2226, 2220 (2 CN), 1690 (CO), 1633 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.64-1.69 (m, 4H, $2CH_2$), 2.18-2.26 (m, 4H, $2CH_2$), 2.73 (s, 3H, CH_3), 4.82 (s, 2H, D_2O exchangeable, NH_2), 7.26-7.38 (m, 5H, C_6H_5), MS (relative intensity) m/z: 414. Analysis for $C_{22}H_{18}N_6OS$ Calcd: C, 63.75; H, 4.38; N, 20.28; S, 7.74. Found: C, 64.02; H, 4.49; N, 20.11; S, 7.49 %

2-[2-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)hydrazono]-3-(3,5-di- amino-4*H*-pyrazol-4-ylidene)-N-phenylbutanamide (22a) and 3-(3-Amino-5-imino-1-phenyl-1*H*-pyrazol-4(5*H*)-ylidene)-2-[2-(3-cyano-4,5,6,7-tetrahydro-benzo[*b*]thiophen-2-yl)hydrazono]-N-phenylbutanamide (22b)

To a solution of compound **20** (2.07 g, 0.005 mol) in ethanol (40 mL) either hydrazine hydrate (**8a**, 0.30 g, 0.005 mol) or phenyl hydrazine (**8b**, 0.59 g, 0.005 mol) was added. The reaction mixture was heated under reflux for 3h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

Compound **22a**: Pale yellow crystals from ethanol, yield: 68 % (1.52 g); mp: 290 ºC. IR (KBr): υ/cm^{-1} = 3462-3338 ($2NH_2$, 2NH), 3058 (CH-aromatic), 2976 (CH_3), 2224 (CN), 1688 (CO), 1638 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.62-1.65 (m, 4H, $2CH_2$), 2.25-2.32 (m, 4H, $2CH_2$), 2.84 (s, 3H, CH_3), 4.66, 5.21 (2s, 4H, $2NH_2$), 7.26-7.38 (m, 5H, C_6H_5), 8.38, 9.31 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 446. Analysis for $C_{22}H_{22}N_8OS$ Calcd: C, 59.18; H, 4.97; N, 25.09; S, 7.18. Found: C, 59.37; H, 4.73; N, 24.82; S, 7.28 %.

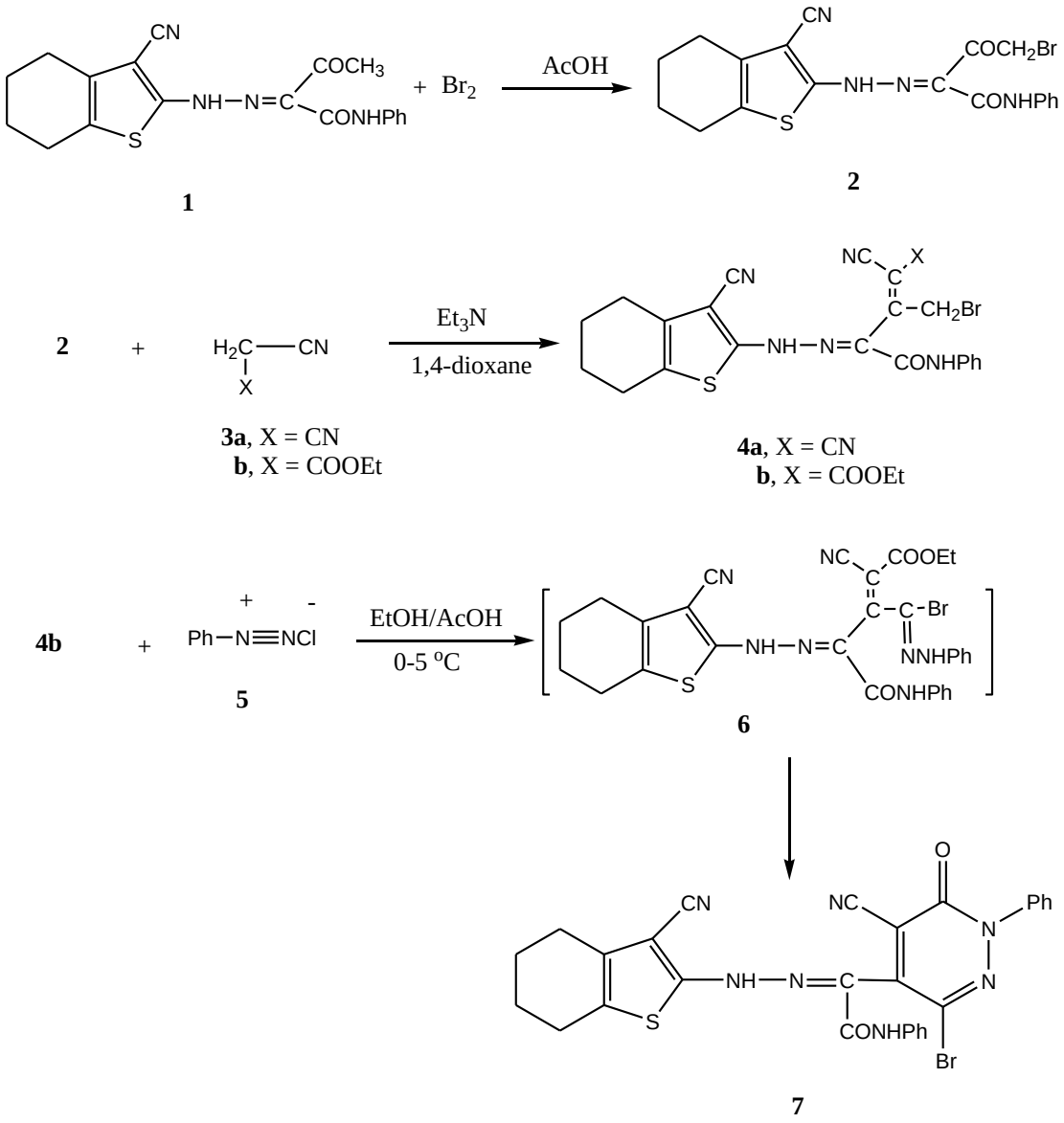
Compound **22b**: Orange crystals from ethanol yield: 62 % (1.62 g); mp: >290 ºC. IR (KBr): υ/cm^{-1} = 3460-3328 (NH_2, 3NH), 3054 (CH-aromatic), 2984 (CH_3), 2224 (CN), 1688 (CO), 1636 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.60- 1.67 (m, 4H, $2CH_2$), 1.94-1.98 (m, 4H, $2CH_2$), 2.84 (s, 3H, CH_3), 4.68 (s, 2H, D_2O exchangeable, NH_2), 7.23-7.36 (m, 10H, $2C_6H_5$), 8.36, 8.62, 9.33 (3s, 3H, D_2O-exchangeable, 3NH). MS (relative intensity) m/z: 522. Analysis for $C_{28}H_{26}N_8OS$ Calcd: C, 64.35; H, 5.01; N, 21.44; S, 6.14. Found: C, 64.29; H, 5.21; N, 21.73; S, 6.28 %.

2-(3-Cyano-2-hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene)-3-(4,6-diamino-2 -thioxopyrimidin-5(2*H*)-ylidene)-N-phenylbutanamide (23a) and 2-(3-Cyano-2-hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene)-3-(4,6-diamino-2-oxo pyrimidin-5(2*H*)-ylidene)-N-phenylbutanamide (23b)

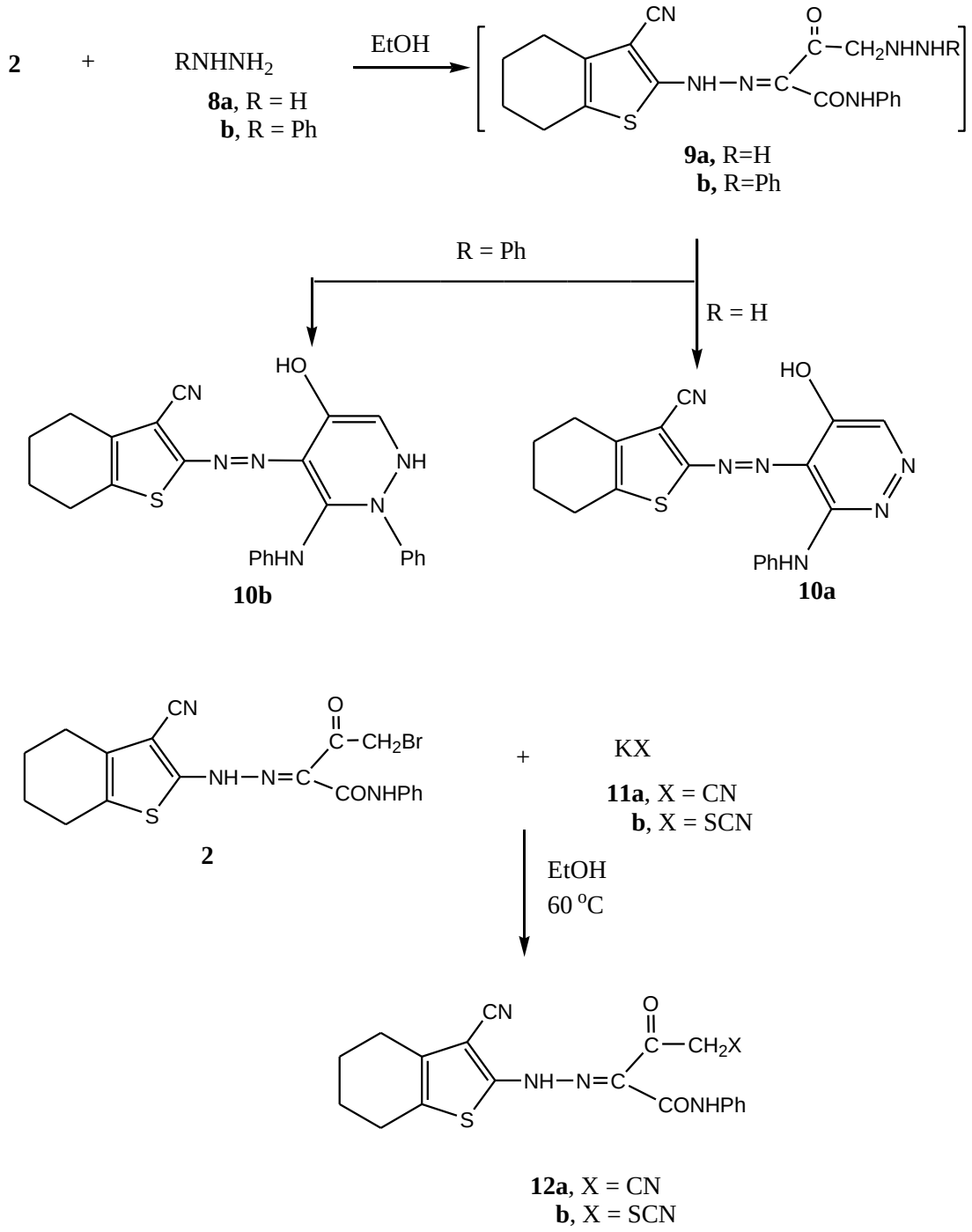
To a suspension of compound **20** (2.07 g, 0.005 mol) in sodium ethoxide {prepared by dissolving sodium metal (0.46 g, 0.02 mol) in absolute ethanol (40 mL)] either thiourea (**13a**, 0.38 g, 0.005 mol) or urea (**13b**, 0.30 g, 0.005 mol). The whole reaction mixture was heated in a boiling water bath for 3 h then poured onto ice/water containing few drops of hydrochloric acid (till pH 6) and the formed solid product was collected by filtration.

Compound **23a**: Yellow crystals from ethanol, yield: 58 % (1.42 g); mp: >290ºC. IR (KBr): υ/cm^{-1} = 3469-3312 ($2NH_2$, 2NH), 3058 (CH-aromatic), 2978 (CH_3), 2222 (CN), 1684 (CO), 1632 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.58-1.66 (m, 4H, $2CH_2$), 1.88-1.93 (m, 4H, $2CH_2$), 2.89 (s, 3H, CH_3), 4.69, 5.44 (2s, 4H, $2NH_2$), 7.23-7.38 (m, 5H, C_6H_5), 8.22, 9.33 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 490. Analysis for $C_{23}H_{22}N_8OS_2$ Calcd: C, 56.31; H, 4.52; N, 22.84; S, 13.07. Found: C, 56.07; H, 4.72; N, 22.63; S, 12.78 %.

Compound **23b**: White crystals from ethanol, yield: 73 % (1.73 g); mp:>290ºC. IR (KBr): υ/cm^{-1} = 3473-3348 (2 NH_2, 2 NH), 3056 (CH-aromatic), 2966 (CH_3), 2220 (CN), 1690, 1686 (2CO), 1635 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.60-1.67 (m, 4H, $2CH_2$), 1.91-1.97 (m, 4H, $2CH_2$), 2.86 (s, 3H, CH_3), 4.68, 5.40 (2s, 4H, $2NH_2$), 7.25-7.40 (m, 5H, C_6H_5), 8.28, 9.38 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 474. Analysis for $C_{23}H_{22}N_8O_2S$ Calcd: C, 58.21; H, 4.67; N, 23.61; S, 6.76. Found: C, 57.92; H, 4.81; N, 23.88; S, 7.03 %.


CN
COCH3
NH–N=C
CONHPh
+ Br2
AcOH
COCH2Br
1
2
Et3N
1,4-dioxane
NC
X
CH2Br
3a, X = CN
b, X = COOEt
4a, X = CN
b, X = COOEt
4b
Ph–N≡NCl
5
EtOH/AcOH
0-5 °C
COOEt
C–C–Br
NNHPh
6
O
Ph
N
Br
7

Scheme (1)


2
+
RNHNH2
8a, R = H
b, R = Ph
EtOH
CN
O
C
CH2NHNHR
NH
N
C
CONHPh
S
9a, R=H
b, R=Ph
R = Ph
R = H
HO
CN
N=N
NH
N
PhHN
Ph
10b
HO
CN
N=N
N
N
PhHN
10a
CN
O
C
CH2Br
NH
N
C
CONHPh
S
2
+
KX
11a, X = CN
b, X = SCN
EtOH
60 °C
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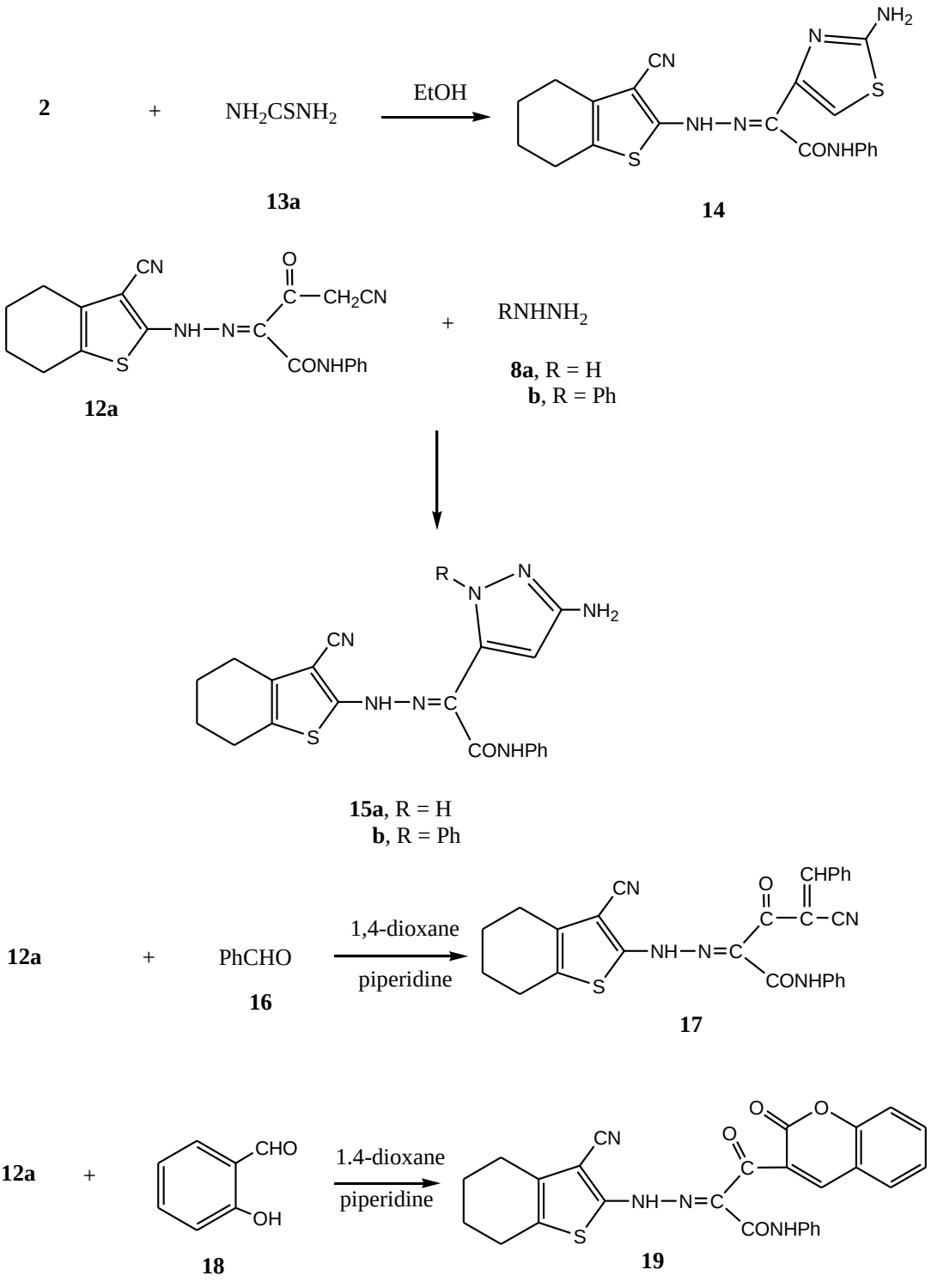
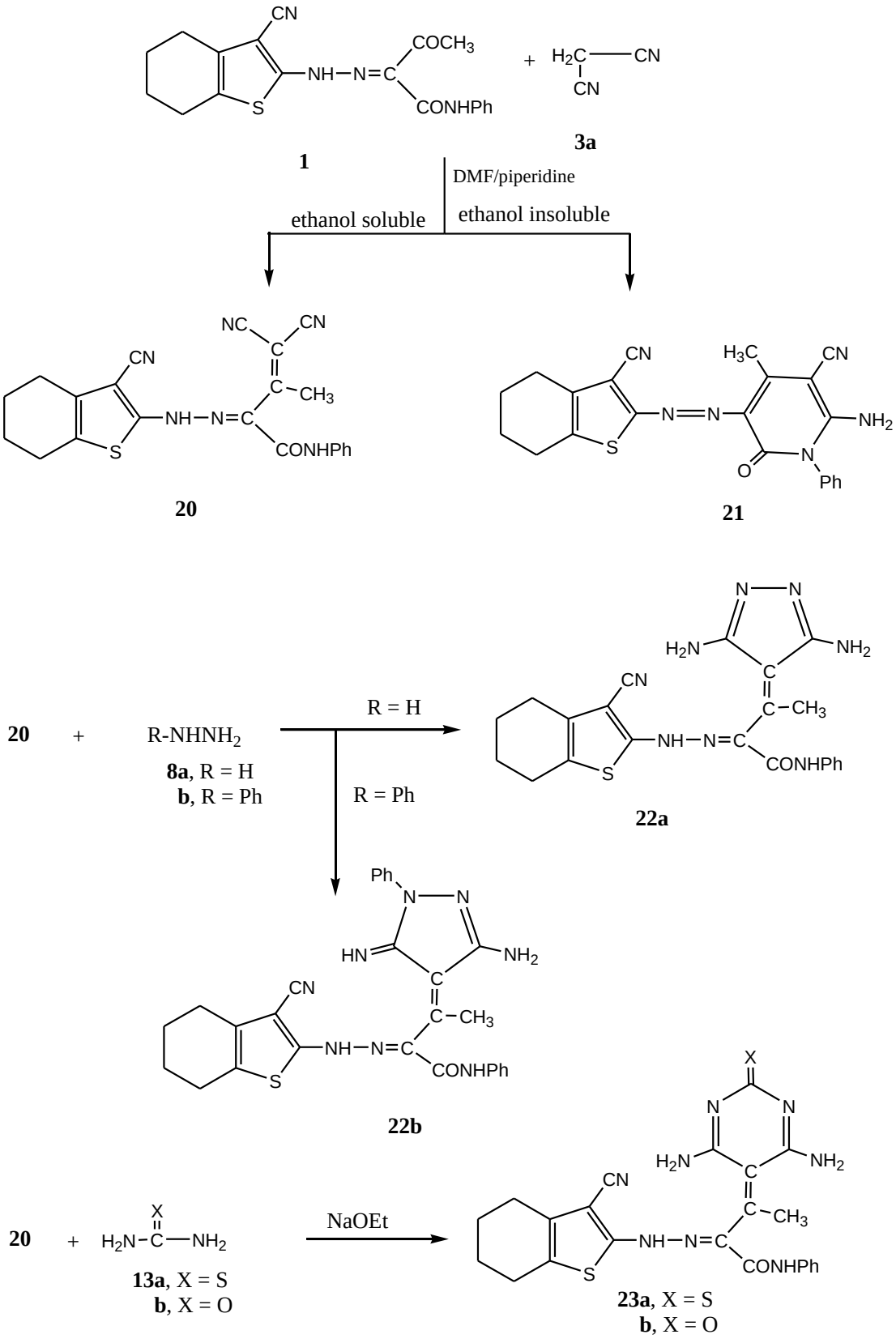
O
C
CH2X
NH
N
C
CONHPh
S
12a, X = CN
b, X = SCN

Scheme (2)


2
+
NH2CSNH2
EtOH
13a
14
12a
+
RNHNH2
8a, R = H
b, R = Ph
15a, R = H
b, R = Ph
12a
+
PhCHO
16
1,4-dioxane
piperidine
17
12a
+
18
1.4-dioxane
piperidine
19

Scheme (3)


1
3a
DMF/piperidine
ethanol soluble
ethanol insoluble
20
21
20 + R-NHNH2
8a, R = H
b, R = Ph
R = H
R = Ph
22a
22b
20 + H2N-C(=X)-NH2
13a, X = S
b, X = O
NaOEt
23a, X = S
b, X = O

Scheme (4)

RESULTS AND DISCUSSION

Recently, we were involved through comprehensive program involving the uses of 4,5,6,7-tetrahydrobenzo[*b*]thiophene derivatives **(Mohareb *et al.*, 2009)** together with their further reactions with chemical reagents to give heterocyclic and fused heterocyclic derivatives with antitumor activities, In continuation of this program, we report here the reactivity of the 2-(3-Cyano-2-hydrazinyl-4,5,6,7-tetrahydrobenzo- [*b*]thiophene)-3-oxo-N-phenylbutanamide **(1)** with some chemical reagents. Thus, reaction of **1** with bromine in acetic acid solution to give the α-bromocarbonyl compound **2**. The structure of compound **2** was based on analytical and spectral data. Thus, the ^{1}H NMR spectrum showed two multiplets at δ 1.74-1.79 & 2.11-2.18 ppm indicating to the four CH_2 groups, a singlet at δ 3.88 ppm corresponding to the CH_2 group, multiplet at δ 7.27-7.38 ppm for the C_6H_5 group and two singlets, D_2O-exchangeable, at δ 8.32 & 9.44 ppm for the two NH groups. The reaction of compound **2** with either malononitrile **(3a)** or ethyl cyanoacetate **(3b)** in refluxing 1,4-dioxane containing a catalytic amount of triethylamine gave the condensate products **4a** and **4b** respectively. The structures of the latter products were based on analytical and spectral data. Thus, the ^{1}H NMR spectrum of **4a** showed two multiplets at δ 1.71-1.76 & 2.15-2.19 ppm indicating the four CH_2 groups, a singlet at δ 3.93 ppm corresponding to CH_2 group, a multiplet at δ 7.28-7.39 ppm for C_6H_5 group and two singlets, D_2O-exchangeable, at δ 8.39 & 9.42 ppm for the two NH groups. Moreover, the reaction of compound **4b** with benzenediazonium chloride at 0-5 ^oC gave the pyridazine derivative **7**, its formation is explained in terms of the intermediate formation of the arylhydrazo derivative **6 (Scheme1)**.

The reaction of α-oxobromo derivative **2** with either hydrazine hydrate **(8a)** or phenylhydrazine **(8b)** gave the pyridazine derivatives **10a** and **10b** respectively. Formation of the latter products was based on the intermediate formation α-hydrazinoxo derivatives **9a, b** followed by water elimination. The structural elucidations were based on the obtained analytical and spectral data. Thus, the ^{1}HNMR spectrum of **10a** showed two multiplets at δ 1.73-1.78 & 2.21-2.26 ppm indicating to the four CH_2 groups, a singlet at δ 6.83 ppm corresponding to the pyridazine H-3 group, a multiplet at δ 7.29-7.42 ppm for the C_6H_5 group, a singlet, D_2O-exchangeable at δ 8.28 ppm for the NH group and a singlet at δ 9.38 ppm for the OH group.

The reaction of compound **2** with either potassium cyanide **(11a)** or potassium thiocyanate **(11b)** gave either α-oxonitrile derivative **12a** or the α-oxothiocyanate derivative **12b** respectively **(Scheme2)**.

On the other hand, the reaction of compound **2** with thiourea **(13a)** in refluxing ethanol gave the thiazole derivative **14**. The analytical and spectral data were in agreement with the assigned structure. Next, we moved towards studying the reactivity of the α-oxonitrile derivative **12a** in order to form new heterocyclic compounds derivatives with potential biological activities. Thus, the reaction of **12a** with either hydrazine hydrate **(8a)** or phenylhydrazine **(8b)** gave pyrazole derivatives **15a,b**. On the other hand, the reaction of **12a** with benzaldehyde **(16)** in refluxing 1,4-dioxane containing a catalytic amount of piperidine gave the benzal derivative **17**. Moreover, the reaction of **12a** with salicylaldehyde **(18)** gave the coumarin derivative **19 (Scheme3)**. The analytical and spectral data of the latter product were in agreement with the assigned structure.

The reaction of compound **1** with malononitrile **(3a)** in DMF/piperidine solution gave two isomeric products with the same molecular formula $C_{22}H_{18}N_6OS$, the ethanol soluble product assigned the acyclic structure **20** while the ethanol insoluble product is the pyridine derivative **21**. The structures of compounds **20** and **21** were based on analytical and spectral

data. The dicyanomethino group present in compound **20** showed interesting reactivity towards the reaction with diamino reagents. Thus, the reaction of compound **20** reacted with either hydrazine hydrate **(8a)** or phenylhydrazine **(8b)** gave the pyrazole derivatives **22a** and **22b** respectively. On the other hand the reaction of compound **20** with either thiourea **(13a)** or urea **(13b)** in sodium ethoxide solution to give the pyrimidine derivatives **23a** and **23b** respectively **(Scheme4)**.

Antitumor activity tests

Reagents: Fetal bovine serum (FBS) and L-glutamine from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium 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 together with the normal cell lines the normal fibroblast cells (WI 38). MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and NCI-H460 and SF-268 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^{o}C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5 X 10^5 cells/mL for MCF-7 and SF-268 and 0.75 X 10^4 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 **2–23a,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-wellplates were then exposed for 48 h to five serial concentrations of each compound **(Skehan *et al*., 1990),** 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 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 growth inhibition of 50% (GI_{50}), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth, was calculated as described elsewhere **(Monks *et al*., 1991).** Doxorubicin was used as a positive control and tested in the same manner.

Table 1. Effect of compounds 2–23a,b on the growth of human tumor cell lines and a normal cell line

Compound	GI_{50} (μ mol l^{-1})			
	MCF-7	NCI-H460	SF-268	WI 38
2	42.6 ± 12.2	36.6 ± 8.6	62.4 ± 14.8	22.3 ± 6.0
4a	32.4 ± 10.6	26.1 ± 2.7	28.9 ± 6.8	40.1 ± 6.0
4b	22.2 ± 1.2	32.6 ± 1.4	36.4 ± 0.6	32.1 ± 4.8
7	14.6 ± 2.4	12.9 ± 0.8	11.8 ± 0.6	44.2 ± 10.2
10a	20.6 ± 0.4	24.3 ± 0.8	32 ± 0.8	4.2 ± 1.8
10b	38.4 ± 1.8	42.0 ± 0.8	22.5 ± 1.1	64.2 ± 12.6
12a	33.1 ± 0.6	27.3 ± 1.4	24.3 ±1.5	62.5 ± 22.6
12b	0.6 ± 0.2	0.2 ± 0.02	0.2 ± 0.05	22.6 ± 8.0
14	22.0 ± 0.6	28.0 ± 0.4	30.5 ± 8.0	56.2 ± 12.9
15a	33.9 ± 17.5	40.2 ± 12.8	52.0 ± 9.0	46.5 ± 8.0
15b	34.0 ± 1.8	46.0 ± 0.8	22.5 ± 1.1	12.3 ± 2.6
17	0.01 ± 0.004	0.02 ± 0.002	0.01 ± 0.001	66.5 ± 14.7
19	0.03 ± 0.007	0.02 ± 0.008	0.01 ± 0.004	> 100
20	26.7 ± 17.8	24.2 ± 12.6	36.0 ± 6.0	72.1 ± 22.3
21	28.7 ± 11.5	22.2 ± 10.	22.0 ± 8.0	20.7 ± 8.3
22a	22.4 ± 0.2	22.6 ± 1.4	33.4 ± 0.6	40.3 ± 10.6
22b	10.2 ± 0.4	12.1 ± 0.6	18.3 ± 0.5	66.4 ± 16.7
23a	2.0 ± 1.0	3.6 ± 1.4	2.4 ± 0.8	70.4 ± 22.6
23b	20.0 ± 0.6	22.0 ± 0.4	31.5 ± 8.0	80.3 ± 18.4
Doxorubicin	0.04 ± 0.008	0.09±0.007	0.09±0.007	> 100

GI_{50} mean value ± standard error of mean of 3 independent experiments performed in duplicate.

Effect on the Growth of Human Tumor Cell Lines

The effect of compounds **2-23a,b** 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) and the normal fibroblast cells (WI 38) after a continuous exposure for 48h.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. The results indicated through **Table 1** revealed that "compounds **17** and **19** showed the highest inhibitory effect against all the three tumor cell lines", such activity is higher than the reference doxorubicin.

While compounds **12b** and **23a** showed high inhibitory effects against non-small cell lung cancer (NCI-H460) and breast adenocarcinoma (MCF-7) and CNS cancer (SF-268) respectively, which are less than the reference doxorubicin. Compounds **2, 4a, 10b, 12a, 15a** and **15b** showed the lowest inhibitory effect. The rest of the compounds showed a moderate growth inhibitory effect. Comparing of **12a** with **12b** it is obvious that the presence of SCN group in **12b** is responsible for the greater inhibitory effect towards the three cell lines than

that of **12a**. Similarly comparing compound **23a** and **23b**, it is obvious that the presence of the sulpher atom in compound **23a** is responsible for their reactivity over **23b**.

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تطبيقات على 4,5,6,7 رباعى هيدروبنزو-ب-ثيوفين فى تخليق مشتقات البيريدازين والبيرازول والثيازول والبيريميدين مع تقييمها كمضادات للاورام

كرم احمد الشرقاوى[1] و فاتن اسماعيل حامد[2]

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يتضمن البحث تحضير مشتق مركب برومو البيوتان اميد **2** الذى تم توجيهه للتفاعل مع المالونونيتريل والايثيل سيانو اسيتات ليكون المركب المقابل **4ا** و **4ب** ثم التفاعل مع مركب الهيدرازين المائى وفينيل الهيدرازين ليعطى مركبات **10ا و 10ب** اضافة لتفاعل مركب **2** ايضا مع البوتاسيوم سيانيد والبوتاسيوم ثيوسيانيد ليعطى مركبات **12ا و 12ب** على التوالى. اخيرا مركب **2** تفاعل مع الثيويوريا لينتج مركب **14.** على الجانب الاخر تفاعل مركب **4ب** مع مركب ملح الدايزونيم **5** لينتج البيريدازين **7** . نشاط مركب **12ا** تم تاكيده بتفاعله مع كلا من الهيدرازين ومشتقاته و بعض الالدهيدات الاروماتيه ليكون المركبات المقابله **15ا** و **ب و17** و **19** على التتابع. امتدادا لتفاعل مركب **1** تم تفاعله مع المالونونيتريل **3ا** و قد اعطى مركبين متماثلين فى الشكل الجزيئى **20** و **21** تم تفاعل مركب **20** مع مشتقات الهيدرازين واليوريا ليكون مركبات **22ا** و **ب** اضافة لمركبى **23ا** و **ب.**

وقد تم فصل جميع المركبات السابقة ايضا تم اثبات تركيبها بوا سطةالتحليل العنصرى الدقيق والاشعة تحت الحمراء والرنين النووى المغناطيسى ومطياف الكتله .

وبدراسة النشاط البيولوجى تبين ان لبعض هذه المركبات نشاطا بيولوجيا تجاه بعض انواع مختلفه من اورام بعض الخلايا المستخدمة والتى يمكن الاستفاده منها فى المجالات الطبيه المختلفه .