SYNTHESIS AND ANTIMICROBIAL EVALUATIONS OF NEW BIOACTIVE DYES AND THEIR CYCLIZED DERIVATIVES SYNTHESIZED FROM 4,5,6,7-TETRAHYDROBENZO[*b*]-THIOPHENE

Rafat M. Mohareb[a], Amira E. M. Abdallah[b], Maher H. E. Helal[b], Heba Allah N. A. Mohammed[b]

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The reaction of 3-cyano-2-diazo-4,5,6,7-tetrahydrobenzo[*b*]thiophene **(1)** with active methylene reagents **2a-e** gave the respective hydrazono derivatives **3a-e**. The reactivity of the latter derivatives towards different chemical reagents was studied. The antimicrobial activity of the newly obtained products was studied and evaluated in terms of minimal inhibitory concentration (MIC) in μg mL^{-1}. The results showed that compounds **3b**, **7a** and **15a** are the most active compounds towards *E. coli* ECT 101; compounds **5f**, **13b**, **17a** and **23** are active towards *B. Cereus* CECT 148; while **10**, **19a** and **19b** towards *B. subtilis* CECT 498 and **3c**, **5c** and **13b** towards *C. albicans* CECT 1394.

Corresponding Authors
E-Mail: raafat_mohareb@yahoo.com
[a] Department of Chemistry, Faculty of Science, Cairo University, Giza, A. R. Egypt
[b] Department of Chemistry, Faculty of Science, Helwan University, Ain Helwan, Cairo A. R. Egypt

Introduction

2-Aminothiophene derivatives are an important class of heterocycles found in several biologically active and natural compounds. This class of compounds has demonstrated a broad spectrum of activities and applications as pharmaceuticals and agrochemicals, dyes, biodiagnostics, and electronic and optoelectronic devices.[1] They have been reported to exert antitubercular,[2] anti-inflammatory,[3] antimicrobial[4] and antianxiety[5] properties. A survey of the literature also reveals that substituted 2-aminothiophenes are potent and selective inhibitors of human leukocyte elastase,[6] kinesin spindle protein (KSP),[7] tubulin[8] and tyrosine kinases of the fibroblast growth factor receptors (FGRF),[9] as well as adenosine A1 receptor allosteric enhancers.[10] Antifungal[11] and antitumor[12] properties have also been extensively described, resulting in marketed antifungal agents such as sertaconazol.

The synthetic strategy of the investigated dyes and their cyclized products depended on the competition of the reaction pathways which followed nucleophilic displacement,[13,14] β-attack, Gewald type reaction,[15, 16] dinucleophilic bielectrophilic attack, dipolar cyclization and condensation reactions. This led to the diversity of the reaction products.

Within the scope of these diverse synthetic methods and the utility of thiophene-based systems and in continuation to our interest in the design of bioactive heterocycles,[17-21] we focused our efforts to synthesized a series of hydrazono dyes **3a-e** based on the key precursor 3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-diazonium chloride (**1**) which coupled with some active methylene reagents. The antimicrobial activity of the new systems was studied and evaluated.

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 Pye Unicam SP-1000 spectro-photometer. ^{1}H-NMR spectra were recorded with Varian Gemini-200 (200 MHz) (Cairo University) instrument in DMSO-d6 as solvent using TMS as internal standard and chemical shifts are expressed as δ ppm. The mass 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 CHNS analyzer.

Synthesis of 3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thio-phen-2-yl-hydrazono derivatives (3a-e)

General procedure: To a cold solution (0–5 °C) of 2-cyano-*N*-phenyl-acetamide **2a** (1.60 g, 0.01 mol), malonic acid diethyl ester **2b** (1.60 g, 0.01 mol), *N*-(4-chloro-phenyl)-2-cyano-acetamide **2c** (1.94 g, 0.01 mol), 2-cyano-*N*-(4-methoxy-phenyl)-acetamide **2d** (1.90 g, 0.01 mol) and 2-cyano-*N*-*p*-tolyl-acetamide **2e** (1.74 g, 0.01 mol) in ethanol (20 mL) containing sodium hydroxide (1.00 g) an equimolar amount of diazotized 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile **1** [which was prepared by adding $NaNO_2$ (0.7 g, 0.01 mol) solution to a cold solution of 2-amino-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carbonitrile[22] (1.78 g, 0.01 mol) in acetic acid (20 mL), HCl (6.0 mL)] was gradually added while stirring. The solid product formed upon cooling in an ice-bath was collected by filtration, washed with water and crystallized from acetic acid.

2-Cyano-2-[3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-hydrazono]-*N*-phenyl-acetamide (3a)

Reddish brown crystals, m.p. 138–140 °C, yield: 3.04 g (87%); Anal. for $C_{18}H_{15}N_5OS$ (349.41), (% Calcd./Found): 61.87/61.50 (C), 4.33/3.99 (H), 20.04/19.70 (N), 9.18/8.80 (S); IR (υ, cm^{-1}): 3431 (2NH), 3050 (CH aromatic), 2935 (CH_2), 2219, 2200 (2CN), 1687 (C=O), 1596, 1496 (C=C), 1531(=N-NH); ^{1}H-NMR (δ, ppm): 1.76-1.98 (m, 4H, cyclohexene $2CH_2$), 2.20-2.89 (m, 4H, cyclohexene $2CH_2$), 6.80-7.80 (m, 5H, C_6H_5), 8.40 (s, 1H, NH), 9.40 (s, 1H, NH); MS *m/z* (%): 349 [M^+] (63.41), 348 [M^+-1] (67.07), 328 (100.00), 76 (75.61).

2-[3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-hydrazono]-malonic acid diethyl ester (3b)

Pale brown crystals, m.p. 98–100 °C, yield: 2.51 g (72%); Anal. for $C_{16}H_{19}N_3O_4S$ (349.40), (% Calcd./Found): 55.00/55.38 (C), 5.48/5.10 (H), 12.03/12.40 (N), 9.18/9.50 (S); IR (υ, cm^{-1}): 3434 (2NH), 3100 (CH aromatic), 2935 (CH_2, CH_3), 2215 (CN), 1682, 1680 (2C=O), 1540, 1441 (C=C), 1530 (=N-NH); ^{1}H-NMR (δ, ppm): 1.14-1.21 (2t, 6H, $2CH_3$); 1.71-1.92 (m, 4H, cyclohexene $2CH_2$), 1.99-2.92 (m, 4H, cyclohexene $2CH_2$), 4.10-4.20 (2q, 4H, $4CH_2$), 6.91 (s, 1H, NH); MS *m/z* (%): 350 [M^++1] (3.54), 349 [M^+] (3.73), 178 (68.13), 177 (47.73).

***N*-(4-Chloro-penta-2,4-dienyl)-2-cyano-2-[3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-hydrazono]-acetamide (3c)**

Pale brown crystals, m.p. 108–110 °C, yield: 3.45 g (90%); Anal. for $C_{18}H_{14}N_5OSCl$ (383.85), (% Calcd./Found): 56.32/55.92 (C), 3.68/3.99 (H), 18.24/17.84 (N), 8.35/8.70 (S); IR (υ, cm^{-1}): 3433 (2NH), 3100 (CH aromatic), 2935 (CH_2), 2215, 2190 (2CN), 1687 (C=O), 1589, 1494 (C=C), 1533 (=N-NH); ^{1}H-NMR (δ, ppm): 1.76-1.98 (m, 4H, cyclohexene $2CH_2$), 2.40-2.90 (m, 4H, cyclohexene $2CH_2$), 7.45-7.83 (m, 4H, C_6H_4), 8.40 (s, 1H, NH), 10.50 (s, 1H, NH); MS *m/z* (%): 382 [M^+-1] (1.72), 381 [M^+-2] (3.00), 127 (100.00), 176 (2.30).

2-Cyano-2-[3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-hydrazono]-*N*-(4-methoxy-phenyl)-acetamide (3d)

Brown crystals, m.p. 101–103 °C, yield: 3.42 g (90%); Anal. for $C_{19}H_{17}N_5O_2S$ (379.44), (% Calcd./Found): 60.14/59.75 (C), 4.52/4.58 (H), 18.46/18.06 (N), 8.45/8.84 (S); IR (υ, cm^{-1}): 3420 (2NH), 3100 (CH aromatic), 2934 (CH_2), 2213-2191 (2CN), 1682 (C=O), 1601, 1445 (C=C), 1510 (=N-NH); ^{1}H-NMR (δ, ppm): 1.23 (s, 3H, CH_3), 1.71-1.98 (m, 4H, cyclohexene $2CH_2$), 2.13-2.82 (m, 4H, cyclohexene $2CH_2$), 6.89-7.64 (m, 4H, C_6H_4), 8.30 (s, 1H, NH), 10.10 (s, 1H, NH); MS *m/z* (%): 381 [M^+-2] (0.37), 380 [M^++1] (0.15), 379 [M^+] (0.14), 77 $[C_6H_5]^+$, 64 (100.00).

2-Cyano-2-[3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-hydrazono]-*N*-*p*-tolyl-acetamide (3e)

Pale brown crystals, m.p. 103–105 °C, yield: 3.38 g (93%); Anal. for $C_{19}H_{17}N_5OS$ (363.44), (% Calcd./Found): 62.79/62.44 (C), 4.71/5.10 (H), 19.27/18.88 (N), 8.82/9.20 (S); IR (υ, cm^{-1}): 3433 (2NH), 3090 (CH aromatic), 2934 (CH_2), 2214, 2192 (2CN), 1685 (C=O), 1591, 1410 (C=C), 1520 (=N-NH); ^{1}H-NMR (δ, ppm): 1.23 (s, 3H, CH_3), 1.77-1.98 (m, 4H, cyclohexene $2CH_2$), 2.19-2.89 (m, 4H, cyclohexene $2CH_2$), 6.98-7.48 (m, 4H, C_6H_4), 8.30 (s, 1H, NH), 10.30 (s, 1H, NH); MS *m/z* (%): 365 [M^++2] (0.18), 364 [M^++1] (0.11), 363 [M^+] (0.26), 107 (100.00), 77 $[C_6H_5]^+$ (47.77).

Synthesis of 3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl-functionalized pyridazine carboxylic acid derivatives (5a-f)

General procedure: To a solution of either compound **3a** (3.49 g, 0.01 mol), **3c** (3.83 g, 0.01 mol) or **3d** (3.79 g, 0.01 mol) in 1,4-dioxane (35 mL) containing triethylamine (1.00 mL), either malononitrile 4a (0.66 g, 0.01 mol) or ethyl cyanoacetate 4b (1.13 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 5 h, then cooled and neutralized by pouring onto ice/water mixture containing few drops of hydrochloric acid. The solid product formed in each case was collected by filtration and crystallized from 1,4-dioxane.

4-Amino-5-cyano-1-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-6-imino-1,6-dihydro-pyridazine-3-carboxylic acid phenylamide (5a)

Dark brown crystals, m.p. 198–200 °C, yield: 3.49 g (84%); Anal. for $C_{21}H_{17}N_7OS$ (415.47), (% Calcd./Found): 60.71/60.35 (C), 4.12/3.89 (H), 23.60/23.22 (N), 7.72/8.10 (S); IR (υ, cm^{-1}): 3429-3300 (2NH, NH_2), 3100 (CH aromatic), 2932 (CH_2), 2298, 2201 (2CN), 1685 (C=O), 1588, 1435 (C=C); ^{1}H-NMR (δ, ppm): 1.81-2.27 (m, 4H, cyclohexene $2CH_2$), 2.57-2.94 (m, 4H, cyclohexene $2CH_2$), 4.44 (s, 2H, NH_2), 6.91-7.40 (m, 5H, C_6H_5), 8.30 (s, 1H, NH), 8.40 (s, 1H, NH); MS *m/z* (%): 415 [M^+] (3.01), 77 $[C_6H_5]^+$ (22.35), 64 (100.00), 50 (6.40).

5-Amino-2-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-3-imino-6-phenylcarbamoyl-2,3-dihydro-pyridazine-4-carboxylic acid ethyl ester (5b)

Dark brown crystals, m.p. 143–145 °C, yield: 4.21 g (91%); Anal. for $C_{23}H_{22}N_6O_3S$ (462.52), (% Calcd./Found): 59.73/59.68 (C), 4.79/4.96 (H), 18.17/17.80 (N), 6.93/7.30 (S); IR (υ, cm^{-1}): 3431-3200 (2NH, NH_2), 3100 (CH aromatic), 2931 (CH_2, CH_3), 2203 (CN), 1734, 1689 (2C=O), 1523, 1440 (C=C); ^{1}H-NMR (δ, ppm): 1.06 (t, 3H. CH_3), 1.15-1.78 (m, 4H, cyclohexene $2CH_2$), 2.61-2.73 (m, 4H, cyclohexene $2CH_2$), 4.00 (s, 2H, NH_2), 4.20 (q, 2H, CH_2), 6.80-7.90 (m, 5H, C_6H_5), 8.29 (s, 1H, NH), 9.90 (s, 1H, NH); MS *m/z* (%): 463 [M^++1] (0.03), 462 [M^+] (0.03), 135 (100.00), 77 $[C_6H_5]^+$ (20.94).

4-Amino-5-cyano-1-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-6-imino-1,6-dihydro-pyridazine-3-carboxylic acid (4-chloro-phenyl)-amide (5c)

Pale brown crystals, m.p. 178–180 °C, yield: 3.82 g (85%); Anal. for $C_{21}H_{16}N_7OSCl$ (449.92), (% Calcd./Found): 56.06/56.19 (C), 3.58/3.63 (H), 21.79/21.40

(N), 7.13/6.78 (S); IR (υ, cm^{-1}): 3329-3209 (2NH, NH_2), 3090 (CH aromatic), 2922-2850 (CH_2), 2260, 2199 (2CN), 1685 (C=O), 1520, 1435 (C=C); ^{1}H-NMR (δ, ppm): 1.16-2.40 (m, 4H, cyclohexene $2CH_2$), 2.56-2.89 (m, 4H, cyclohexene $2CH_2$), 3.57 (s, 2H, NH_2), 7.15-7.76 (m, 4H, C_6H_4), 8.29 (s, 1H, NH), 9.40 (s, 1H, NH); MS *m/z* (%): 451 [M^++1] (12.50), 450 [M^+] (10.96), 76 $[C_6H_4]^+$ (35.77), 57 (100.00).

5-Amino-6-(4-chloro-phenylcarbamoyl)-2-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-3-imino-2,3-dihydro-pyridazine-4-carboxylic acid ethyl ester (5d)

Pale brown crystals, m.p. 248–250 °C, yield: 4.62 g (93%); Anal. for $C_{23}H_{21}N_6O_3SCl$ (496.97), (% Calcd./Found): 55.59/55.16 (C), 4.26/3.88 (H), 16.91/16.55 (N), 6.45/6.14 (S); IR (υ, cm^{-1}): 3433 (2NH, NH_2), 3100 (CH aromatic), 2927 (CH_2, CH_3), 2207 (CN), 1684, 1682 (2C=O), 1610, 1438 (C=C); ^{1}H-NMR (δ, ppm): 1.05 (t, 3H, CH_3), 1.21-2.33 (m, 4H, cyclohexene $2CH_2$), 2.63-2.95 (m, 4H, cyclohexene $2CH_2$), 3.57 (s, 2H, NH_2), 3.99 (q, 2H, CH_2), 6.00 (s, 1H, NH), 6.60 (s, 1H, NH), 6.90-7.60 (m, 4H, C_6H_4); MS *m/z* (%): 496 [M^+-1] (0.72), 495 [M^+-2] (0.96), 105 (100.00), 76 $[C_6H_4]^+$ (5.36).

4-Amino-5-cyano-1-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-6-imino-1,6-dihydro-pyridazine-3-carboxylic acid (4-methoxy-phenyl)-amide (5e)

Brown crystals, m.p. over 300 °C, yield: 3.43 g (77%); Anal. for $C_{22}H_{19}N_7O_2S$ (445.50), (% Calcd./Found): 59.31/58.99 (C), 4.30/3.90 (H), 22.01/21.70 (N), 7.20/6.80 (S); IR (υ, cm^{-1}): 3431 (2NH, NH_2), 3100 (CH aromatic), 2930 (CH_2), 2200, 2190 (2CN), 1687 (C=O), 1590, 1431 (C=C); ^{1}H-NMR (δ, ppm): 1.22 (s, 3H, CH_3), 1.82-2.40 (m, 4H, cyclohexene $2CH_2$), 2.56-2.90 (m, 4H, cyclohexene $2CH_2$), 3.99 (s, 2H, NH_2), 6.00 (s, 1H, NH), 6.89-7.23 (m, 4H, C_6H_4), 8.10 (s, 1H, NH); MS *m/z* (%): 445 [M^+] (10.75), 444 [M^+-1] (2.04), 76 $[C_6H_4]^+$ (12.24), 55 (100.00).

5-Amino-2-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-3-imino-6-(4-methoxy-phenylcarbamoyl)-2,3-di-hydro-pyrid-azine-4-carboxylic acid ethyl ester (5f)

Brown crystals, m.p. 248–250 °C, yield: 3.69 g (75%); Anal. for $C_{24}H_{24}N_6O_4S$ (492.55), (% Calcd./Found): 58.52/58.20 (C), 4.91/4.55 (H), 17.06/16.70 (N), 6.51/6.16 (S); IR (υ, cm^{-1}): 3434 (2NH, NH_2), 3090 (CH aromatic), 2930 (CH_2, CH_3), 2208 (CN), 1686, 1680 (2C=O), 1509, 1450 (C=C); ^{1}H-NMR (δ, ppm): 1.19 (t, 3H, CH_3), 1.77-2.44 (m, 4H, cyclohexene $2CH_2$), 2.55-2.97 (m, 4H, cyclohexene $2CH_2$), 4.00 (s, 2H, NH_2), 4.10 (q, 2H, CH_2), 6.91-7.70 (m, 4H, C_6H_4), 7.90 (s, 1H, NH), 8.30 (s, 1H, NH); MS *m/z* (%): 492 [M^+] (23.47), 491 [M^+-1] (20.58), 64 (100.00).

Synthesis of 3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl- functionalized 3-thioxo-2,3,4,5-tetrahydro-[1,2,4]triazine-6-carboxylic acid derivatives, 7a-c

General procedure: Equimolar amounts of **3a** (3.49 g, 0.01 mol), **3b** (3.49 g, 0.01 mol), **3d** (3.79 g, 0.01 mol) and phenylisothiocyanate **6** (1.35 g, 0.01 mol) in 1,4-dioxane (35 mL) containing triethylamine (1.0 mL) were heated under reflux for 5h. After cooling, the reaction mixture in each case was acidified by hydrochloric acid and the crude product was precipitated, collected by filtration and crystallized from 1,4-dioxane .

2-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-5-imino-4-phenyl-3-thioxo-2,3,4,5-tetrahydro-[1,2,4]triazine-6-carboxy-lic acid phenylamide (7a)

Reddish brown crystals, m.p. 203–205 °C, yield: 3.49 g (72%); Anal. for $C_{25}H_{20}N_6OS_2$ (484.60), (% Calcd./Found): 61.96/61.72 (C), 4.16/4.47 (H), 17.34/17.00 (N), 13.23/13.39 (S); IR (υ, cm^{-1}): 3355 (2NH), 3100 (CH aromatic), 2930 (CH_2, CH_3), 2206 (CN), 1680 (C=O), 1592, 1499 (C=C), 1317, 1239 (C=S); ^{1}H-NMR (δ, ppm): 1.18-2.43 (m, 4H, cyclohexene $2CH_2$), 2.56-2.89 (m, 4H, cyclohexene $2CH_2$), 7.12-7.50 (m, 10H, $2C_6H_5$), 8.29 (s, 1H, NH), 9.80 (s, 1H, NH); MS *m/z* (%): 486 [M^++2] (26.80), 485 [M^++1] (53.09), 484 [M^+] (44.85), 483 [M^+-1] (31.96), 82 (100.00).

2-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-5-oxo-4-phenyl-3-thioxo-2,3,4,5-tetrahydro-[1,2,4]triazine-6-carboxy-lic acid ethyl ester (7b)

Reddish brown crystals, m.p. 233–235 °C, yield: 3.46 g (79%); Anal. for $C_{21}H_{18}N_4O_3S_2$ (438.52), (% Calcd./Found): 57.52/57.15 (C), 4.14/4.13 (H), 12.78/13.18 (N), 14.62/14.65 (S); IR (υ, cm^{-1}): 3429 (NH), 3090 (CH aromatic), 2930 (CH_2), 2207 (CN), 1686, 1682 (2C=O), 1593, 1439 (C=C), 1320, 1257 (C=S); ^{1}H-NMR (δ, ppm): 1.03 (t, 3H, CH_3), 1.14-1.98 (m, 4H, cyclohexene $2CH_2$), 2.03-2.89 (m, 4H, cyclohexene $2CH_2$), 4.30 (q, 2H, CH_2), 6.61-7.80 (m, 10H, $2C_6H_5$), 8.29 (s, 1H, NH); MS *m/z* (%): 440 [M^++2] (18.07), 439 [M^++1] (18.99), 438 [M^+] (16.85), 437 [M^+-1] (25.88), 77 $[C_6H_5]^+$ (32.01), 78 (100.00).

2-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-5-imino-4-phenyl-3-thioxo-2,3,4,5-tetrahydro-[1,2,4]triazine-6-carboxy-lic acid (4-methoxy-phenyl)-amide (7c)

Reddish brown crystals, m.p. 188–190 °C, yield: 3.76 g (73%); Anal. for $C_{26}H_{22}N_6O_2S_2$ (514.62), (% Calcd./Found): 60.68/60.33 (C), 4.31/4.00 (H), 16.33/15.99 (N), 12.46/12.15 (S); IR (υ, cm^{-1}): 3430 (2NH), 3100 (CH aromatic), 2929 (CH_2, CH_3), 2204 (CN), 1686 (C=O), 1595, 1436 (C=C), 1320, 1242 (C=S); ^{1}H-NMR (δ, ppm): 1.14 (s, 3H, CH_3), 1.16-1.98 (m, 4H, cyclohexene $2CH_2$), 2.04-2.73 (m, 4H, cyclohexene $2CH_2$), 6.53-7.50 (m, 9H, C_6H_4, C_6H_5), 8.29 (s, 1H, NH), 9.80 (s, 1H, NH); MS *m/z* (%): 516 [M^++2] (32.45), 515 [M^++1] (23.51), 514 [M^+] (0.99), 70 (100.00).

Synthesis of 4-amino-1-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]-thiophen-2-yl)-6-oxo-1,6-dihydropyridazine-3-carboxylic acid phenylamide (9)

To a solution of **3a** (3.49 g, 0.01 mol) in acetic acid/acetic anhydride (10: 3 mL) was added. The reaction mixture was heated under reflux for 1 h. The solid product formed upon pouring onto ice/water mixture was collected by filtration, washed with water and crystallized from 1,4-dioxane.

Brown crystals, m.p. 104–106 °C, yield: 3.56 g (91%); Anal. For $C_{20}H_{17}N_5O_2S$ (391.45), (% Calcd./Found): 61.37/60.99 (C), 4.38/4.00 (H), 17.89/17.55 (N), 8.19/7.80 (S); IR (υ, cm^{-1}): 3431 (NH, NH_2), 3050 (CH aromatic), 2931 (CH_2), 2213 (CN), 1685, 1682 (2C=O), 1545, 1437 (C=C); ^{1}H-NMR (δ, ppm): 1.74-2.43 (m, 4H, cyclohexene $2CH_2$), 2.57-2.96 (m, 4H, cyclohexene $2CH_2$), 3.42 (s, 2H, NH_2), 3.90 (s, 1H, pyridazine CH-5), 7.12-7.82 (m, 5H, C_6H_5), 8.29 (s, 1H, NH); MS *m/z* (%): 393 [M^++2] (0.39), 392 [M^++1] (4.92), 391 [M^+] (3.60), 381 (100.00).

Synthesis of 2-[(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)hydrazono]-*N*-(4-methoxy-phenyl)-malonamide (10)

Equimolar amounts of **3d** (3.79 g, 0.01 mol) in ethanolic hydrochloric acid (30: 10 mL) were heated under reflux for 10h. The solid product formed upon pouring onto ice/water mixture was collected by filtration, washed with water and crystallized from ethanol.

Brown crystals, m.p. 168–170 °C, yield: 3.10 g (78%); Anal. for $C_{19}H_{19}N_5O_3S$ (397.45), (% Calcd./Found): 57.42/57.47 (C), 4.82/4.55 (H), 17.62/17.22 (N), 8.07/8.40 (S); IR (υ, cm^{-1}): 3412 (2NH, NH_2), 3110 (CH aromatic), 2930-2850 (CH_2, CH_3), 2211 (CN), 1689, 1680 (2C=O), 1513, 1440 (C=C); ^{1}H-NMR (δ, ppm): 1.20 (s, 3H, CH_3), 1.75-2.50 (m, 4H, cyclohexene $2CH_2$), 2.51-2.86 (m, 4H, cyclohexene $2CH_2$), 3.60 (s, 2H, NH_2), 6.86-7.34 (m, 4H, C_6H_4), 8.29 (s, 1H, NH), 9.30 (s, 1H, NH); MS *m/z* (%): 397 [M^+] (14.52), 369 [M^++1] (15.26), 395 [M^+-2] (5.88), 80 (100.00).

Synthesis of 4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile derivatives 12a-d

General procedure: To a solution of compound **3a** (3.49 g, 0.01 mol) or **3b** (3.49 g, 0.01 mol) in 1,4-dioxane (25 mL), either hydrazine hydrate **11a** (0.50 g, 0.01 mol), or phenyl hydrazine **11b** (1.08 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 5 h. The solid products formed, in each case, upon pouring onto ice/water mixture containing few drops of hydrochloric acid were collected by filtration, and crystallized from 1,4-dioxane.

2-[*N'*-(3-Amino-5-phenylimino-1,5-dihydro-pyrazol-4-ylidene)-hydrazino]-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (12a)

Brown crystals, m.p. 153–155 °C, yield: 3.13 g (86%); Anal. for $C_{18}H_{17}N_7S$ (363.44), (% Calcd./Found): 59.49/59.22 (C), 4.71/4.70 (H), 26.98/26.58 (N), 8.82/9.20 (S); IR (υ, cm^{-1}): 3400-3328 (2NH, NH_2), 3100 (CH aromatic), 2932-2854 (CH_2), 2208 (CN), 1597, 1441 (C=C), 1520 (=N-NH); ^{1}H-NMR (δ, ppm): 1.71-2.40 (m, 4H, cyclohexene $2CH_2$), 2.61-2.91 (m, 4H, cyclohexene $2CH_2$), 6.91 (s, 1H, pyrazole NH), 7.09-7.36 (m, 5H, C_6H_5), 8.29 (s, 1H, NH); MS *m/z* (%): 365 [M^++2] (0.25), 364 [M^++1] (0.33), 361 [M^+-2] (0.31), 77 $[C_6H_5]^+$ (8.70), 80 (100.00).

2-[*N'*-(3-Amino-1-phenyl-5-phenylimino-1,5-dihydro-pyrazol-4-ylidene)hydrazino]-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (12b)

Brown crystals, m.p. 178–180 °C, yield: 2.77 g (63%); Anal. for $C_{24}H_{21}N_7S$ (439.54), (% Calcd./Found): 65.58/65.20 (C), 4.82/4.77 (H), 22.31/21.98 (N), 7.30/7.70 (S); IR (υ, cm^{-1}): 3426 (NH,NH_2), 3050 (CH aromatic), 2928 (CH_2), 2205 (CN), 1590, 1431 (C=C), 1513 (=N-NH); ^{1}H-NMR (δ, ppm): 1.81-2.49 (m, 4H, cyclohexene $2CH_2$), 2.79-2.94 (m, 4H, cyclohexene $2CH_2$), 3.57 (s, 2H, NH_2), 6.00-7.80 (m, 10H, $2C_6H_5$), 8.20 (s, 1H, NH), 8.90 (s, 1H, NH); MS *m/z* **(%):** 441 [M^++2] (0.20), 440 [M^++1] (0.15), 438 [M^+-1] (0.26), 77 $[C_6H_5]^+$ (5.10), 57 (100.00).

2-[*N'*-(3-Hydroxy-5-oxo-1,5-dihydro-pyrazol-4-ylidene)hydrazino]-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (12c)

Dark brown crystals, m.p. 158–160 °C, yield: 2.02 g (72%); Anal. For $C_{12}H_{11}N_5O_2S$ (289.31), (% Calcd./Found): 49.82/50.20 (C), 3.83/4.20 (H), 24.21/23.83 (N), 11.08/11.44 (S); IR (υ, cm^{-1}): 3432-3196 (2NH, OH), 2933 (CH_2), 2210 (CN), 1685 (C=O), 1600, 1402 (C=C); ^{1}H-NMR (δ, ppm): 1.71-2.49 (m, 4H, cyclohexene $2CH_2$), 2.57-2.89 (m, 4H, cyclohexene $2CH_2$), 7.10 (s, 1H, pyrazole NH), 7.30 (s, 1H, NH), 8.29 (s, 1H, OH); MS *m/z* (%): 291 [M^++2] (0.82), 290 [M^++1] (0.32), 289 [M^+] (0.33), 288 [M^+-1] (0.10), 150 (100.00).

2-[*N'*-(3-Hydroxy-5-oxo-1-phenyl-1,5-dihydro-pyrazol-4-ylidene)hydrazino]-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (12d)

Brown crystals, m.p. 213–215 °C, yield: 2.37 g (65%); Anal. for $C_{18}H_{15}N_5O_2S$ (365.41), (% Calcd./Found): 59.16/58.77 (C), 4.14/4.30 (H), 19.17/18.80 (N), 8.78/9.10 (S); IR (υ, cm^{-1}): 3426 (NH, OH), 3050 (CH aromatic), 2931-2859 (CH_2), 2206 (CN), 1687 (C=O), 1592, 1438 (C=C),1530 (=N-NH); ^{1}H-NMR (δ, ppm): 1.51-2.32 (m, 4H, cyclohexene $2CH_2$), 2.61-2.89 (m, 4H, cyclohexene $2CH_2$), 6.93 (s, 1H, NH), 7.10-7.55 (m, 5H, C_6H_5), 8.00 (s, 1H, OH); MS *m/z* (%): 366 [M^++1] (2.94), 365 [M^+] (0.58), 364 [M^+-1] (1.00), 51 (100.00).

Synthesis of the 2,3,4,5-tetrahydro-[1,2,4]triazine-6-carboxylic acid hydrazide and phenylhydrazide derivatives 13a,b

General procedure: Equimolar amounts of **7b** (4.38 g, 0.01 mol) and either hydrazine hydrate **11a** (0.50 g, 0.01 mol), or phenyl hydrazine **11b** (1.08g, 0.01 mol) in 1,4-dioxane (25 mL) were heated under reflux for 5 h. The reaction mixture, in each case, pouring onto ice/water mixture containing few drops of hydrochloric acid was collected by filtration, and crystallized from 1,4-dioxane.

2-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-3-hydrazono-5-oxo-4-phenyl-2,3,4,5-tetrahydro-[1,2,4]triazine-6-carboxylic acid hydrazide (13a)

Brown crystals, m.p. 243–245 °C, yield: 3.69 g (87%); Anal. for $C_{19}H_{18}N_8O_2S$ (422.46), (% Calcd./Found):

54.02/54.40 (C), 4.29/4.14 (H), 26.52/26.14 (N), 7.59/7.90 (S); IR (υ, cm^{-1}): 3424 (NH, $2NH_2$), 3100 (CH aromatic), 2930 (CH_2), 2206 (CN), 1684, 1682 (2C=O), 1557, 1436 (C=C); ^{1}H-NMR (δ, ppm): 1.17-2.32 (m, 4H, cyclohexene $2CH_2$), 2.62-2.89 (m, 4H, cyclohexene $2CH_2$), 3.60 (s, 2H, NH_2), 4.00 (s, 2H, NH_2), 6.91-7.62 (m, 5H, C_6H_5), 8.30 (s, 1H, NH); MS *m/z* (%): 424 [M^++2] (5.58), 423 [M^++1] (2.66), 422 [M^+] (7.81), 77 $[C_6H_5]^+$ (45.42), 174 (100.00).

2-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-5-oxo-4-phenyl-3-(phenyl-hydrazono)-2,3,4,5-tetrahydro-[1,2,4]triazine-6-carboxylic acid *N*′-phenyl-hydrazide (13b)

Reddish brown crystals, m.p. 183–185 °C, yield: 4.40 g (88%); Anal. for $C_{31}H_{26}N_8O_2S$ (574.66), (% Calcd./Found): 64.79/64.40 (C), 4.56/4.20 (H), 19.50/19.88 (N), 5.58/5.98 (S); IR (υ, cm^{-1}): 3427 (3NH), 3100 (CH aromatic), 2930-2850 (CH_2), 2207 (CN), 1688, 16820 (2C=O), 1563, 1440 (C=C); ^{1}H-NMR (δ, ppm): 1.17-1.91 (m, 4H, cyclohexene $2CH_2$), 2.08-2.89 (m, 4H, cyclohexene $2CH_2$), 6.92-7.44 (m, 10H, $2C_6H_5$), 7.95 (s, 1H, NH), 8.10 (s, 1H, NH), 8.30 (s, 1H, NH); MS *m/z* (%): 573 [M^+-1] (21.83), 572 [M^+-2] (24.65), 188 (100.00).

Synthesis of 2-(4-oxo-4,5-dihydro-thiazol-2-yl)-acetamide and N-phenyl-acetamide derivatives (15a, b)

General procedure: To a solution of **3a** (3.49 g, 0.01 mol) or **3c** (3.83 g, 0.01 mol) in acetic acid (30 mL), thioglycolic acid **14** (0.92 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 5 h. The solid product formed upon pouring onto ice/water mixture was collected by filtration, washed with water and crystallized from acetic acid.

2-[(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-hydrazono]2-(4-oxo-4,5-dihydro-thiazol-2-yl)-*N*-phenyl-acetamide (15a)

Brown crystals, m.p. 218–220 °C, yield: 2.79 g (66%); Anal. for $C_{20}H_{17}N_5O_2S_2$ (423.51), (% Calcd./Found): 56.72/56.37 (C), 4.05/4.27 (H), 16.54/16.22 (N), 15.14/15.06 (S); IR (υ, cm^{-1}): 3406 (2NH), 3050 (CH aromatic), 2932 (CH_2), 2211 (CN), 1688, 1681 (2C=O), 1530, 1435 (C=C); ^{1}H-NMR (δ, ppm): 1.80-2.43 (m, 4H, cyclohexene $2CH_2$), 2.57-2.99 (m, 4H, cyclohexene $2CH_2$), 3.65 (s, 2H, thiazole C5-H), 7.39-7.70 (m, 5H, C_6H_5), 8.30 (s, 1H, NH), 10.50 (s, 1H, NH); MS *m/z* (%): 423 [M^+] (0.51), 422 [M^+-1] (0.49), 421 [M^+−2] (0.75), 77 $[C_6H_5]^+$ (3.31), 69 (100.00).

***N*-(4-Chloro-phenyl)-2-[(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]-thiophen-2-yl)-hydrazono]2-(4-oxo-4,5-dihydro-thiazol-2-yl)-acetamide (15b)**

Dark brown crystals, m.p. 228-230 °C, yield: 2.98 g (65%); Anal. for $C_{20}H_{16}N_5O_2S_2Cl$ (457.96), (% Calcd./Found): 52.45/52.85 (C), 3.52/3.82 (H), 15.29/14.90 (N), 14.00/13.66 (S); IR (υ, cm^{-1}): 3428 (2NH), 3050 (CH aromatic), 2929 (CH_2), 2205 (CN), 1690, 1683 (2C=O), 1588, 1490 (C=C); ^{1}H-NMR (δ, ppm): 1.51-2.32 (m, 4H, cyclohexene $2CH_2$), 2.73-2.99 (m, 4H, cyclohexene $2CH_2$), 3.99 (s, 2H, thiazole C5-H), 6.00 (s, 1H, NH), 6.92 (s, 1H, NH), 7.03-7.80 (m, 4H, C_6H_4); MS *m/z* (%): 460 [M^++2] (3.50), 459 [M^++1] (3.08), 458 [M^+] (5.41), 135 (100.00), 76 $[C_6H_4]^+$ (4.98).

Synthesis of the pyridazine carboxylic acid amide and the ethyl ester derivatives 17a,b

General procedure: To a solution of **3c** (3.83 g, 0.01 mol) in 1,4-dioxane (30 mL) containing triethylamine (1.00 mL), either α-cyanocinnamonitrile **16a** (1.54 g, 0.01 mol) or ethyl cyanocinnamate **16b** (2.01 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 5 h, then cooled and neutralized by pouring onto ice/water mixture containing few drops of hydrochloric acid. The solid product formed, in each case, was filtered off and crystallized from 1,4-dioxane.

5-Cyano-1-(3-cyano-5-propyl-thiophen-2-yl)-4-imino-6-phenyl-1,4-dihydro-pyridazine-3-carboxylic acid (4-chloro-phenyl)-amide (17a)

Brown crystals, m.p. 223–225 °C, yield: 3.58 g (70%); Anal. for $C_{27}H_{19}N_6OSCl$ (511.00), (% Calcd./Found): 63.46/63.47 (C), 3.75/4.03 (H), 16.45/16.40 (N), 6.27/6.60 (S); IR (υ, cm^{-1}): 3434(2NH), 3040 (CH aromatic), 2930 (CH_3), 2255, 2202 (2CN), 1688 (C=O), 1593, 1435 (C=C); ^{1}H-NMR (δ, ppm): 1.71-2.40 (m, 4H, cyclohexene $2CH_2$), 2.49-2.89 (m, 4H, cyclohexene $2CH_2$), 7.08-7.98 (m, 9H, C_6H_4, C_6H_5), 8.29 (s, 1H, NH), 8.70 (s, 1H, NH); MS *m/z* (%): 511 [M^+] (0.17), 510 [M^+-1] (0.35), 77 $[C_6H_5]^+$ (7.15), 69 (100.00).

6-(4-Chloro-phenylcarbamoyl)-2-(3-cyano-4,5,6,7-tetrahydro-benzo[*b*]thiophen-2-yl)-5-imino-3-phenyl-2,5-dihydro-pyrid-azine-4-carboxylic acid ethyl ester (17b)

Dark brown crystals, m.p. 228-230 °C, yield: 3.96 g (71%); Anal. for $C_{29}H_{24}N_5O_3SCl$ (558.05), (% Calcd./Found): 62.42/62.11 (C), 4.33/3.96 (H), 12.55/12.58 (N), 5.75/6.10 (S); IR (υ, cm^{-1}): 3430 (2NH), 3100 (CH aromatic), 2931 (CH_2, CH_3), 2205 (CN), 1690, 1683 (2C=O), 1555, 1438 (C=C); ^{1}H-NMR (δ, ppm): 1.03 (t, 3H, CH_3); 1.18-1.91 (m, 4H, cyclohexene $2CH_2$), 2.06-2.91 (m, 4H, cyclohexene $2CH_2$), 4.36 (q, 2H, CH_2), 6.00-8.19 (m, 9H, C_6H_4, C_6H_5), 8.29 (s, 1H, NH), 8.40 (s, 1H, NH); MS *m/z* (%): 558 [M^+] (19.35), 557 [M^+-1] (15.87), 77 $[C_6H_5]^+$ (63.09), 55 (100.00).

Synthesis of the 4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile derivatives 19a,b

General procedure: Equimolar amounts of 3b (3.49 g, 0.01 mol) and either urea 18a (0.60 g, 0.01 mol), or thiourea 18b (0.76 g, 0.01 mol) in sodium ethoxide solution [prepared by adding metallic sodium (0.23 g, 0.01 mol) in absolute ethanol (30 mL)] were heated under reflux for 5 h. The reaction mixture, in each case, pouring onto ice/water mixture containing few drops of hydrochloric acid and the

formed solid product was collected by filtration and crystallized from 1,4-dioxane.

2-[N$^{/-}$(2,4,6-Trioxo-tetrahydro-pyrimidin-5-ylidene)hydrazino]-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (19a)

Brown crystals, m.p. 243-245 °C, yield: 2.44 g (77%); Anal. for $C_{13}H_{11}N_5O_3S$ (317.32), (% Calcd./Found): 49.21/49.60 (C), 3.49/3.89 (H), 22.07/21.70 (N), 10.10/10.46 (S); IR (υ, cm^{-1}): 3430 (3NH), 2927 (CH_3), 2210 (CN), 1685, 1683-1680 (3C=O), 1510, 1434 (C=C); ^{1}H-NMR (δ, ppm): 1.76-1.78 (m, 4H, cyclohexene 2CH_2), 2.60-2.72 (m, 4H, cyclohexene 2CH_2), 6.99 (s, 1H, NH), 7.16 (s, 1H, NH), 7.33 (s, 1H, NH); MS *m/z* (%): 319 [M$^+$+2] (0.13), 318 [M$^+$+1] (0.15), 317 [M$^+$] (0.13), 64 (100.00).

2-[*N*$^{/-}$(,4,6-Dioxo-2-thioxo-tetrahydro-pyrimidin-5-ylidene)-hydrazino]-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (19b)

Brown crystals, m.p. 203-205 °C, yield: 3.00 g (90%); Anal. for $C_{13}H_{11}N_5O_2S_2$ (333.39), (Calcd./Found): 46.83/47.22 (C), 3.33/3.70 (H), 21.01/20.70 (N), 19.24/18.88 (S); IR (υ, cm^{-1}): 3423 (3NH), 2930 (CH_3), 2205 (CN), 1690, 1683 (2C=O), 1564, 1435 (C=C), 1320, 1278 (C=S); ^{1}H-NMR (δ, ppm): 1.43-2.50 (m, 4H, cyclohexene 2CH_2), 2.60-2.72 (m, 4H, cyclohexene 2CH_2), 7.34 (s, 1H, NH), 7.77 (s, 1H, NH), 8.29 (s, 1H, NH); MS *m/z* (%): 335 [M$^+$+2] (0.12), 334 [M$^+$+1] (0.12), 333 [M$^+$] (0.10), 78 (100.00).

Synthesis of the malonic acid diethyl ester derivatives, 21a, b

General procedure: To a solution of compound **3b** (3.49 g, 0.01 mol) in 1,4-dioxane (30 mL), either ethyl chloroacetate **20a** (1.22 g, 0.01 mol), or chloroacetone **20b** (0.92 g, 0.01 mol) was added in the presence of a catalytic amount of potassium carbonate. The reaction mixture, in each case, was heated under reflux for 5 h. The solid products formed, in each case, upon pouring onto ice/water mixture containing few drops of hydrochloric acid were collected by filtration, and crystallized from 1,4-dioxane.

1-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-4-hydroxy-1H-pyrazole-3,5-dicarboxylic acid diethyl ester (21a)

Brown crystals, m.p. 220-222 °C, yield: 3.46 g (89%); Anal. for $C_{18}H_{19}N_3O_5S$ (389.43), (% Calcd./Found): 55.52/55.23 (C), 4.92/5.30 (H), 10.79/11.10 (N), 8.23/8.60 (S); IR (υ, cm^{-1}): 3433 (OH), 2929 (CH_2, CH_3), 2210 (CN), 1723, 1684 (2C=O), 1600, 1436 (C=C); ^{1}H-NMR (δ, ppm): 1.03 (t, 3H, CH_3), 1.19 (t, 3H, CH_3) 1.51-2.27 (m, 4H, cyclohexene 2CH_2), 2.58-2.95 (m, 4H, cyclohexene 2CH_2), 3.85 (q, 2H, CH_2), 4.15 (q, 2H, CH_2), 8.29 (s, 1H, OH); MS *m/z* (%): 391 [M$^+$+2] (25.39), 387 [M$^+$-2] (17.02), 78 (100.00).

5-Acetyl-1-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-4-hydroxy-1H-pyrazole-3-carboxylic acid ethyl ester (21b)

Dark brown crystals, m.p. 148-150 °C, yield: 3.13 g (87%); Anal. for $C_{17}H_{17}N_3O_4S$ (359.40), (% Calcd./Found): 56.81/57.20 (C), 4.77/4.88 (H), 11.69/11.99 (N), 8.92/9.32 (S); IR (υ, cm^{-1}): 3429 (OH), 2929 (CH_2, CH_3), 2208 (CN), 1724, 1684 (2C=O), 1591, 1438 (C=C); ^{1}H-NMR (δ, ppm):1.03 (t, 3H, CH_3), 1.20 (s, 3H, CH_3) 1.71-2.40 (m, 4H, cyclohexene 2CH_2), 2.60-2.79 (m, 4H, cyclohexene 2CH_2), 4.10 (q, 2H, CH_2), 8.28 (s, 1H, OH); MS *m/z* (%): 360 [M$^+$+1] (11.53), 201 (93.30), 57 (100.00).

Synthesis of 2-[(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl-)hydrazono]-*N*-phenyl-malonamic acid ethyl ester (23)

To a solution of **3b** (3.49 g, 0.01 mol) in 1,4-dioxane (30 mL), aniline **22** (0.93 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 5 h. The solid product formed upon pouring onto ice/water mixture was collected by filtration, washed with water and crystallized from 1,4-dioxane.

Brown crystals, m.p. 223-225 °C, yield: 3.49 g (88%); Anal. for $C_{20}H_{20}N_4O_3S$ (396.46), (% Calcd./Found): 60.59/60.22 (C), 5.08/4.69 (H), 14.13/14.53 (N), 8.09/8.44 (S); IR (υ, cm^{-1}): 3698-3426 (2NH), 2932 (CH_2, CH_3), 2209 (CN), 1688, 1683 (2C=O), 1588, 1496 (C=C); ^{1}H-NMR (δ, ppm): 1.24 (t, 3H, CH_3); 1.76-2.43 (m, 4H, cyclohexene 2CH_2), 2.57-2.89 (m, 4H, cyclohexene 2CH_2), 3.57 (q, 2H, CH_2), 6.00 (s, 1H, NH), 6.90 (s, 1H, NH), 7.10-8.30 (m, 5H, C_6H_5); MS *m/z* (%): 397 [M$^+$+1] (0.75), 396 [M$^+$] (0.72), 77 [C_6H_5]$^+$ (14.38), 367 (100.00).

Antimicrobial activity of the newly synthesized compounds

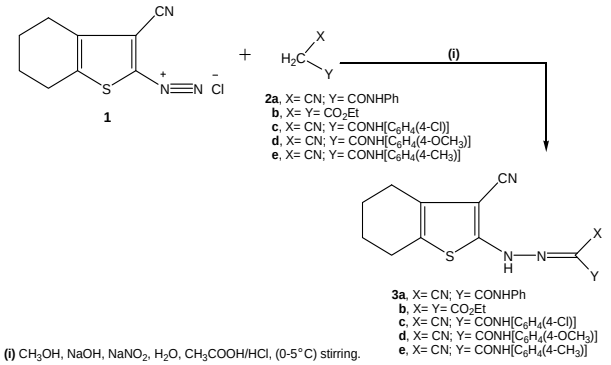
Microorganisms used were obtained from Microbial Chemistry Department, National Research Center, Cairo, Egypt. For the *in vitro* antimicrobial activity evaluation, microorganism suspensions were prepared to contain approximately 108 cfu/mL and the plates were inoculated. A stock solution of each of the synthesized compounds (1000 μg mL^{-1}) in DMSO was prepared and graded dilutions of the tested compounds were incorporated in a cavity (depth 3 mm, diameter 4 mm) made in the center of the Petri dish (nutrient agar for bacteria and Sabouraud *vs.* dextrose agar medium for fungi).

The plates were incubated in duplicates for 24 h at 37 °C (for bacteria) and at 30 °C (for fungi). A positive control using only inoculation and a negative control using only DMSO in the cavity were carried out. The results of antimicrobial screening of the synthesized and standard antibiotics are given in Table I.

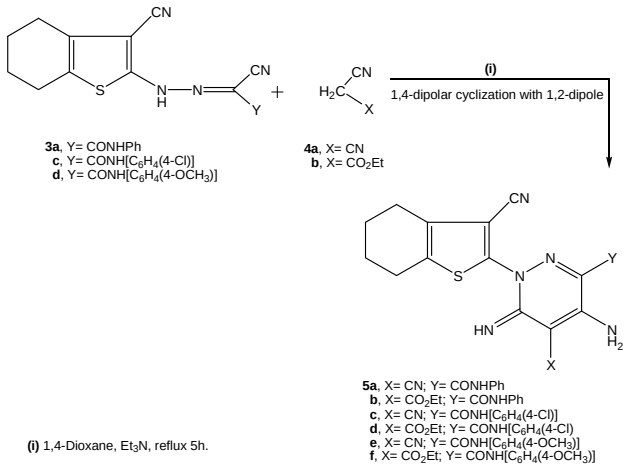
Results and discussion

3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-diazonium chloride (**1**) reacted with the active methylene reagents (XCH_2Y, X=CN, Y=CONHPh; X=Y=CO_2Et; X=CN, Y=CONH[C_6H_4(4-Cl)]; X=CN, Y=CONH[C_6H_4(4-OCH_3);

X=CN, Y=CONH[C_6H_4(4-CH_3)] (**2a-e**) to give the hydrazone derivatives **3a-e** (Scheme 1). The analytical and spectral data of the products were in analogous with their respective structures. Thus, the mass spectral data for compounds 3a-e revealed a molecular formula $C_{18}H_{15}N_5OS$ (*m/z* 349 [M^+]), $C_{16}H_{19}N_3O_4S$ (*m/z* 349 [M^+]), $C_{18}H_{14}N_5OSCl$ (*m/z* 382 [M^+-1]), $C_{19}H_{17}N_5O_2S$ (*m/z* 379 [M^+]) and $C_{19}H_{17}N_5OS$ (**m/z** 363 [M^+]), respectively which confirmed their structures. Compounds **3a, 3c** and **3d** reacted with the cyanomethylene reagents (XCH_2Y, X=Y=CN; X=CN, Y=CO_2Et) (**4a, b**) to give the iminopyridazine derivatives **5a-f** (Scheme 2) .



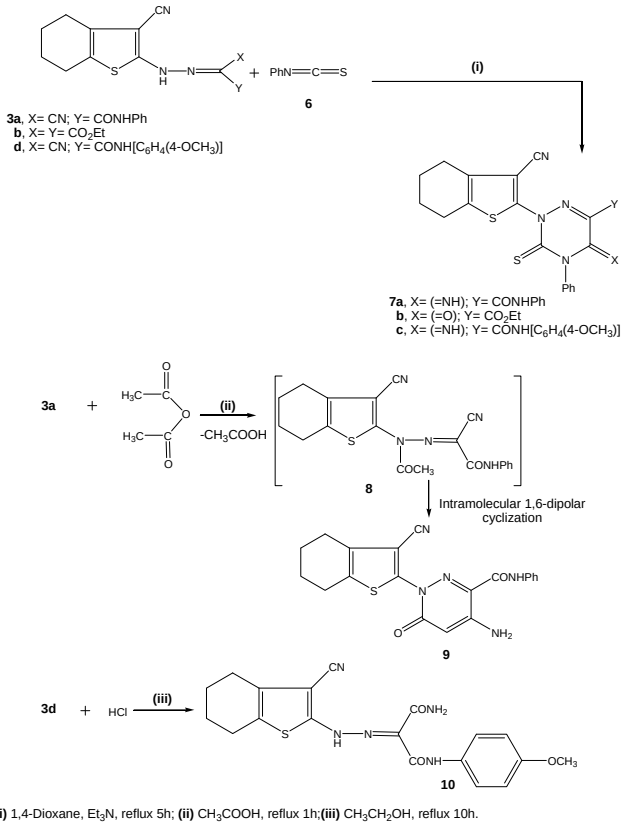
Scheme 1. Synthesis of the hydrazono derivatives **3a-e**



Scheme 2. Synthesis of the pyridazine derivatives **5a-f**

The reaction involved 1,4-dipolar cyclization of compounds **3a, 3c** and **3d** with 1,2-dipoles (**4a, b**). The structures of the latter products were based on their respective analytical and spectral data. Thus, ^{1}H-NMR spectrum of **5a** showed two multiplets about δ 1.81-2.27 ppm and δ 2.57-2.94 ppm that integrated for four cyclohexene CH_2 protons, δ 4.44 ppm for NH_2 protons, multiplets at δ 6.91-7.40 ppm for phenyl moiety and at δ 8.30 and 8.40 for 2NH protons. Mass spectra of **5a, 5b, 5c, 5d, 5e** and **5f** exhibited a molecular ions m/z 415 [M^+], *m/z* 462 [M^+], *m/z* 450 [M^+], 496 [M^+-1], *m/z* 445 [M^+] and *m/z* 492 [M^+] corresponding to their molecular formulae, respectively.

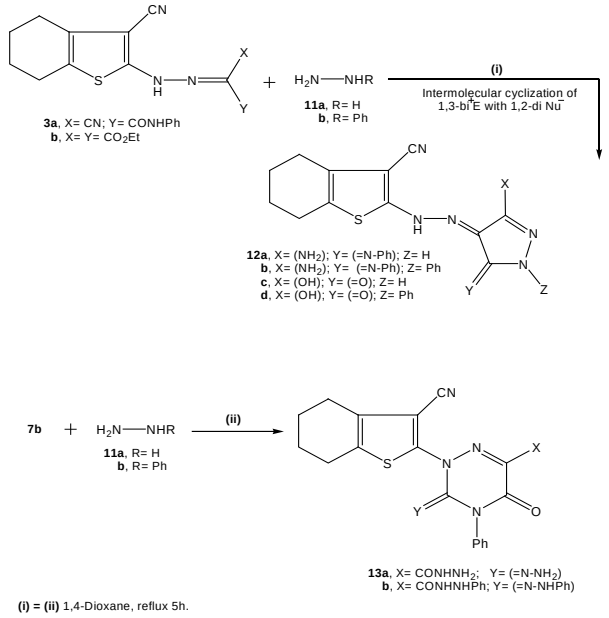
On the other hand the reaction of either compounds **3a, 3b** or **3d** with phenylisothiocyanate (**6**) gave the triazine derivatives **7a-c** (Scheme 3). The reaction took place via nucleophilic attack of NH moiety of compounds **3a,3b** or **3d** on isocyanate C=S terminal followed by 1,6-dipolar cyclization. The analytical and spectral data of **7a-c** are consistent with their corresponding structures (see experimental section). As an example, the appearance of two C=O stretching modes about 1600 and 1620 cm^{-1} region cited for triazene oxo function and ethoxy carbonyl in the IR spectrum of **7b**. Also, the mass spectrum of **7b** showed molecular ion *m/z* 438 corresponding to molecular formula $C_{21}H_{18}N_4O_3S_2$. Treatment of **3a** with acetic anyhydride/AcOH mixture under refluxing conditions gave pyridazine-3-one derivative **9**.



Scheme 3. Synthesis of the triazine **7a-c**, pyridazinone **9** and diamido **10** derivatives

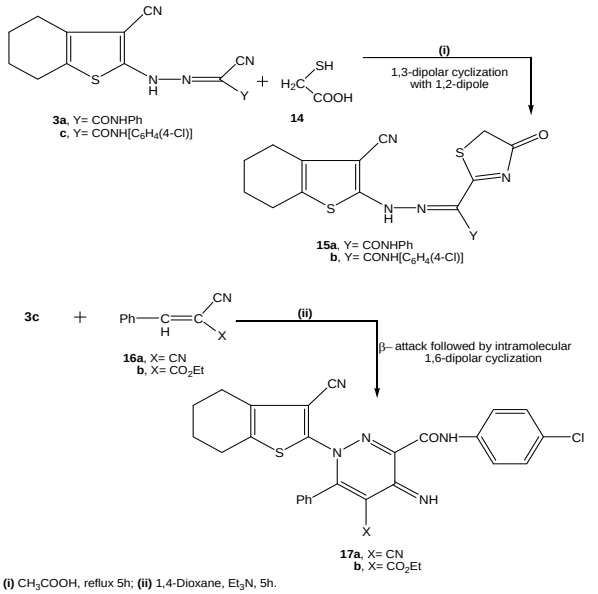
The reaction took place through formation of the intermediate **8** followed by 1,6-dipolar intramolecular cyclization to give **9**. ^{1}H-NMR spectrum of compound **9** showed two multiplets about δ 1.74-2.43 ppm and δ 2.57-2.96 ppm for four cyclohexene CH_2 protons, δ 3.42 ppm for NH_2 protons, pyridazine C5-H protons at δ 3.90 ppm, multiplets at δ 7.12-7.82 ppm for phenyl moiety and a singlet at δ 8.29 integrated for NH proton. The mass spectrum of **9** exhibited a molecular ion *m/z* 391 [M^+] corresponding to molecular formula $C_{20}H_{17}N_5O_2S$. Compound **3d** underwent ready hydrolysis in HCl/EtOH to give the diamido derivative **10**. Microanalysis, IR and ^{1}H-NMR of **10** are fully consistent with the proposed structure.

Next, we moved towards studying the reactivity of the hydrazone derivatives **3a** and **3b** towards hydrazines (H_2N-NHR, R=H; R=Ph) namely hydrazine hydrate (**11a**) and phenylhydrazine (**11b**) to afford the respective pyrazole derivatives **12a-d** (Scheme 4). The reaction involved intermolecular cyclization of 1,3-bielectrophilic compounds **3a, b** with 1,2-dinucleophiles (**11a**) and (**11b**). The analytical and spectral data of the latter products were the basis of their structural elucidation.



Scheme 4. Synthesis of pyrazole **12a-d** and triazine **13a, b** derivatives

Thus, ^{1}H-NMR spectrum of **12a** (as an example) showed two multiplets about δ 1.71-2.40 ppm and δ 2.61-2.91 ppm for four cyclohexene CH_2 protons, δ 3.39 ppm for singlet NH_2 protons, a singlet pyrazole NH proton at δ 6.92 ppm, multiplets at δ 7.09-7.36 ppm for phenyl moiety and a singlet NH proton at δ 8.29 ppm.

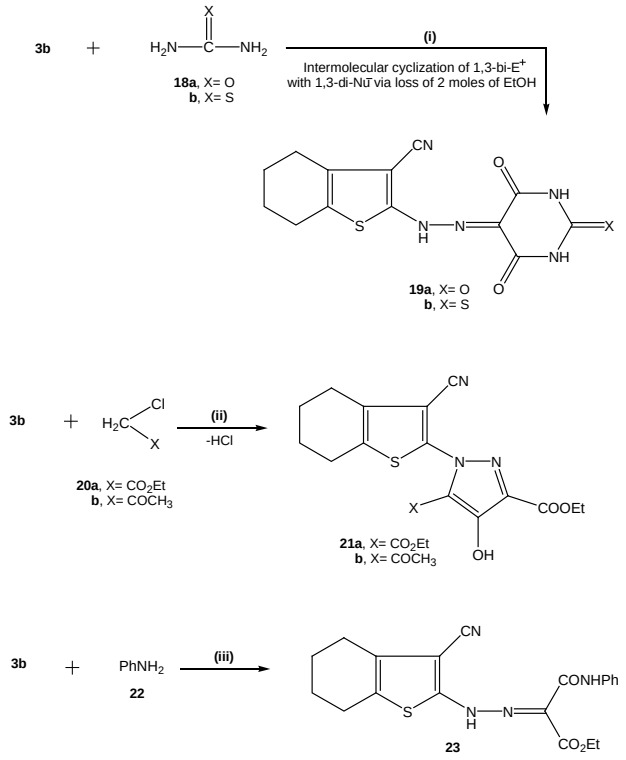


Scheme 5. Synthesis of the thiazolone **15a, b** and pyridazine **17a,b** derivatives.

In the mass spectra of **12a-d** the existing [M^++1] ions (*m/z*=364, *m/z*=440) and [M^+] ions (*m/z*=289, *m/z*=365), confirmed their respective molecular weights. The absence of C=O absorption in the 1600-1800 cm^{-1} region confirmed the assignment for pyrazole structures **12a** and **12b**. The appearance of C=O and a broad OH bands in the regions 1625, 1620 and 3432, 3426 cm^{-1}, respectively confirmed the structures of **12c** and **12d**.

The reaction of the pyridazine derivative **7b** with either hydrazine hydrate (**11a**) or phenylhydrazine (**11b**) gave the hydrazide derivatives **13a** and **13b**, respectively (Scheme 4). The reaction involved the loss of H_2S and two moles of EtOH. the mass spectra of **13a** and **13b** showed molecular ion peaks [M^+]=424 and [M^+-1]=499 corresponding to their respective molecular formulae $C_{19}H_{16}N_6O_2S_2$ and $C_{25}H_{20}N_6O_2S_2$.

Interestingly, the reaction of either of compounds **3a** or **3c** with thioglycollic acid (**14**) gave the thiazole derivatives **15a** and **15b**, respectively (Scheme 5). The reactions took place through 1,3-dipolar cyclization with 1,2-dipole via nucleophilic attack by SH group on the cyano moiety in **3a** or **3c** followed by water elimination.



Scheme 6. Synthesis of the pyrimidine **19a, b**, 4-hydroxy-pyrazole **21a, b** and hydrazono-malonamic acid ethyl ester **23** derivatives

Next, we studied the reaction of **3c** with cinnamonitrile derivatives (**16a, b**) (PhCH=C(CN)X, X=CN; X=CO_2Et) with the aim of formation of biologically active pyridazine derivatives.[23-26] Thus, the reaction of **3c** with either α-cyanocinnamonitrile (**16a**) or ethyl cyanocinnamate (**16b**) in refluxing 1,4-dioxane containing a cartalytic amount of triethylamine afforded the pyridazine derivatives **17a** and **17b**, respectively (Scheme 5). The reaction occurs via β-attack followed by 1,6-dipolar intramolecular cyclization.

Table 1. Antimicrobial activity data of the synthesized compounds in terms of MIC in μg mL^{-1}.

Compound No.	*E. coli ECT 101*	*B. Cereus CECT 148*	*B. subtilis CECT 498*	*C. albicans CECT 1394*
3a	Not active	4.62	8.39	12.62
3b	0.46	8.66	25.33	12.22
3c	Not active	12.34	6.13	0.40
3d	Not active	6.05	12.42	4.55
3e	Not active	6.22	12.89	18.42
5a	Not active	8.42	10.29	16.02
5b	2.66	4.73	12.8	11.32
5c	Not active	18.32	6.22	0.40
5d	Not active	20.15	23.16	100
5e	10.46	8.66	25.33	12.22
5f	Not active	0.08	5.23	8.44
7a	0.81	6.46	20.63	10.22
7b	Not active	7.39	4.33	12.77
7c	Not active	10.23	2.56	28.60
9	6.82	4.92	2.11	10.39
10	Not active	7.03	0.68	20.50
12a	2.77	4.66	12.33	8.41
12b	2.46	8.55	18.33	12.42
12c	12.46	10.66	2.33	10.22
12d	10.12	6.13	2.22	10.25
13a	Not active	25	23	26
13b	Not active	0.05	3.13	0.61
15a	0.86	2.44	15.92	10.11
15b	Not active	12.32	16.32	14.40
17a	16.64	0.06	6.33	50
17b	Not active	12.30	4.22	12.55
19a	8.22	5.23	0.22	16.22
19b	Not active	22.01	0.48	25.60
21a	Not active	6.25	20	30
21b	12.50	20	6.25	8.65
23	Not active	0.08	2.22	6.44
Ampicillin	6.25	3.13	12.50	-
cycloheximide	-	-	-	12.50

Thus, ^{1}H-NMR of **17a** and **17b** revealed signals due to two NH protons at about δ 8.29-8.70 ppm. Signals integrated for ester protons in compound **17b** were also observed in their respective fields. The mass spectra of **17a** and **17b** exhibited molecular ion peaks [M^+] at *m/z* 511 and *m/z* 558 respectively corresponding to their molecular formulae.

The high yield of **3b** encouraged us to synthesize biologically active systems via reaction with some chemical reagents. Thus, compound **3b** reacted with either urea (**18a**) or thiourea (**18b**) in sodium ethoxide solution to give pyrimidine derivatives **19a** and **19b**, respectively (Scheme 6). The reaction took place via 1,3-intermolecular cyclization of compound **3b** with 1,3-dinucleophiles **18a** and **18b** via loss of two moles of ethanol. The analytical and spectral data of the latter products were based on analytical and spectral data. Thus, ^{1}H-NMR spectrum of **19a** showed two multiplets about δ 1.76-1.78 ppm and δ 2.60-2.72 ppm for four cyclohexene CH_2 protons and three singlets at δ 6.99, 7.16 and 7.33 for 3NH protons. The appearance of three C=O stretching about 1600, 1634 and 1660 cm^{-1} cited for pyrimidine oxo functions and the presence of C=S stretching bands at 1320 and 1278 cm^{-1} in the IR spectra of **19a** and **19b** proved the proposed structures.

Moreover, the reaction of **3b** with α-halocarbonyl reagents (XCH_2Cl, $X=CO_2Et$; $X=COCH_3$) namely ethyl chloroacetate (**20a**) and α-chloroacetone (**20b**) gave the pyrazole derivatives **21a** and **21b**, respectively (Scheme 6). The reaction took place through 1,5-dipolar intramolecular cyclization via loss of ethanol. The mass spectra of **21a** and **21b** displayed molecular ions [M^++2] at *m/z* 391 and [M^+-1] at *m/z* 360 corresponding to their respective molecular formulae.

Finally the reaction of **3b** with aniline (**22**) gave the anilide derivative **23** (Scheme 6). The analytical and spectral data of compound **23** were in agreement with its respective structure (see experimental section).

In vitro evaluation of antibacterial and antifungal activities.

The synthesized compounds were screened in vitro for their antimicrobial activity against a variety of bacterial and

fungal isolates. Evaluation of the antibacterial activity against Gram-negative (*Escherichia coli ECT 101* and *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Bacillus subtilis CECT 498* and *Bacillus cereus CECT 148*) and the antifungal activity against *Candida albicans CECT 1394* as a representative species of fungi were assessed for the synthesized compounds. The minimal inhibitory concentration (MIC in μg mL^{-1}) was determined using an adaptation of agar streak dilution method based on radial diffusion.[27, 28] Different concentrated solutions of ampicillin (antibacterial) and cycloheximide (antifungal) were used as standards. The MIC was considered to be the lowest concentration of the tested compounds which inhibits growth of bacteria or fungi on the plate.

The results indicated that most of the synthesized compounds exhibited noticeable antimicrobial activity, and that the bacterial isolates were less active to the synthesized compounds than the fungal species.

Gram-negative bacteria (*Escherichia coli ECT 101* and *Pseudomonas aeruginosa*) showed low activity than Gram-positive bacteria (*Bacillus subtilis CECT 498* and *Bacillus cereus CECT 148*), where all the compounds tested were not active against *Pseudomonas aeruginosa* starting from DMSO solutions of 1000 μg mL^{-1} of each compound.

Compounds **3b, 7a** and **15a** exhibited the highest inhibitory activity against *Escherichia coli ECT 101*, compounds **5f**, **13b**, **17a** and **23** are highly active against *Bacillus cereus CECT 148*, compounds **10**, **19a** and **19b** showed the highest inhibitory activity towards *Bacillus subtilis CECT 498*, while compounds **3c**, **5c** and **13b** demonstrated the highest inhibitory activity against the fungal species *C. albicans CECT 1394*. It is noteworthy that all the aforementioned compounds showed higher inhibitory activity than the selected standards (ampicillin and cycloheximide).

On the other hand, compounds **5e, 12c, 12d, 17a** and **21b** showed the lowest inhibitory activity against *Escherichia coli ECT 101*, compounds **3c, 5c, 5d, 13a, 15b, 17b, 19b** and **21b** are less active towards *Bacillus cereus CECT 148*, compounds **3b, 5d, 5e, 7a, 12b, 13a, 15a, 15b** and **21a** exhibited the lowest inhibitory activity towards *Bacillus subtilis CECT 498*. Compounds **5d, 7c, 10, 13a, 17a, 19b** and **21a** showed lower inhibitory activity against *C. albicans CECT 1394* compared with the standard itself. The rest of compounds showed moderate inhibitory activity.

It was also observed that while compound **13b** is totally active against tested Gram-positive bacteria and fungi, it is inactive against Gram-negative bacteria used. Compound **5d** is totally inactive towards all tested bacteria and fungi isolates.

Comparing compounds **13a** and **13b** indicated that **13b** (X=CONHNHPh) showed higher inhibitory effect against Gram-positive bacteria and fungi used than **13a** ($X=CONHNH_2$). Similarly for compounds **15a** and **15b** it is obvious that compound **15a** (Y=CONHPh) showed higher inhibitory activity than **15b** ($Y=CONH[C_6H_4(4\text{-}Cl)]$).

On the other hand, compound **17a** (X=CN) showed high inhibitory effect towards all tested bacteria than **17b** ($X=CO_2Et$). Also, compound **19a** (X=O) indicated higher inhibitory activity than **19b** (X=S).

Conclusion

We have reported a convenient synthesis of a variety of bioactive dyes (**3a-e**) from 3-cyano-2-diazo-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**1**) which coupled with active methylene reagents (**2a-e**). The reactivity of bioactive dyes (**3a-e**) towards different chemical reagents were studied. Most of the synthesized systems were found to be promising antibacterial agents and hence deserve further pharmacological investigation. Currently, we are investigating the potential antitumor activity of the synthesized systems and related derivatives. The results of these investigation will be published in due time.

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