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

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Keywords: Tetrahydrobenzo[b]thiophene, pyridazines, pyrazoles, antimicrobial activity

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 3e, 5c and 13b towards C. albicans CECT 1394.

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. They have been reported to exert antitubercular, anti-inflammatory, antimicrobial and antianxiety properties. A survey of the literature also reveals that substituted 2-aminothiophenes are potent and selective inhibitors of human leukocyte elastase, kinesin spindle protein (KSP), tubulin and tyrosine kinases of the fibroblast growth factor receptors (FGRF), as well as adenosine A1 receptor allosteric enhancers. Antifungal and antitumor 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, β-attack, Gewald type reaction, 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, we focused our efforts to synthesize 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. 1H-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]thiophen-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-chlorophenyl)-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 NaNO₂ (0.7 g, 0.01 mol) solution to a cold solution of 2-amino-4,5,6,7-tetrahydrobenzo[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[θ]thiophen-2-yl]-
hydrazono]-N-phenyl-acetamide (3a)

Reddish brown crystals, m.p. 98–100 °C, yield: 2.51 g (72%); Anal. for C_{19}H_{17}N_5OS (363.44), (% Calcd./Found): 90%; Anal. for C_{21}H_{17}N_7OS (415.47), (% Calcd./Found): 84%; Anal. for C_{23}H_{22}N_6O_3S (462.52), (% Calcd./Found): 91%; Anal. for C_{35}H_{39}N_8O_3S (636.44), (% Calcd./Found): 94%.

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 a 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[θ]thiophen-2-yl]-6-imino-1,6-dihydro-pyridazine-3-carboxylic acid phenylamide (5a)

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

5-Amino-2-[3-cyano-4,5,6,7-tetrahydrobenzo[θ]thiophen-2-yl]-3-imino-6-phenylcarbamoyl-2,3-dihydro-pyridazine-4-carboxylic acid ethyl ester (5b)
(N), 7.13/6.78 (S); IR (ν, cm⁻¹): 3329-3209 (2NH, NH₂), 3090 (CH aromatic), 2922-2850 (CH₂), 2260, 2199 (CN), 1685 (C=O), 1520, 1435 (C=C); ¹H-NMR (δ, ppm): 1.16-2.40 (m, 4H, cyclohexene 2CH₂), 2.56-2.89 (m, 4H, cyclohexene 2CH₂), 3.57 (s, 2H, NH₂), 7.15-7.76 (m, 4H, C₆H₄), 8.29 (s, 1H, NH), 9.40 (s, 1H, NH); MS m/z (%): 451 [M+1] (12.50), 450 [M⁺] (10.96), 76 [C₄H₆]⁺ (35.77), 57 (100.00).

5-Amino-6-(4-chlorophenylcarbamoyl)-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 C₂₂H₂₁N₆O₃SCl (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 (ν, cm⁻¹): 3433 (2NH, NH₂), 3100 (CH aromatic), 2927 (CH₃, CH₂), 2207 (CN), 1684, 1682 (2C=O), 1610, 1438 (C=C); ¹H-NMR (δ, ppm): 1.05 (s, 3H, CH₃), 1.21-2.33 (m, 4H, cyclohexene 2CH₂), 2.63-2.95 (m, 4H, cyclohexene 2CH₂), 3.57 (s, 2H, NH₂), 7.15-7.76 (m, 4H, C₆H₄); MS m/z (%): 496 [M⁻1] (0.72), 495 [M⁻2] (0.96), 105 (100.00), 76 [C₄H₆]⁺ (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 (4)

Brown crystals, m.p. over 300 °C, yield: 3.43 g (77%); Anal. for C₁₄H₁₄N₇O₂ClS (445.50), (% Calcd./Found): 59.31/58.99 (C), 4.30/3.90 (H), 7.20/6.80 (S); IR (ν, cm⁻¹): 3431 (2NH, NH₂), 3100 (CH aromatic), 2930 (CH₂), 2200, 2190 (2CN), 1687 (C=O), 1590, 1431 (C=C); ¹H-NMR (δ, ppm): 1.22 (s, 3H, CH₃), 1.82-2.40 (m, 4H, cyclohexene 2CH₂), 4.30 (q, 2H, CH₂), 6.00 (s, 1H, NH), 6.60 (s, 1H, NH), 6.90-7.60 (m, 4H, C₆H₄); MS m/z (%): 445 [M⁺] (10.75), 444 [M⁻1] (2.04), 76 [C₄H₆]⁺ (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-pyridazine-4-carboxylic acid ethyl ester (5f)

To a solution 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-dioxiane (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.

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-e

Reddish brown crystals, m.p. 203–205 °C, yield: 3.49 g (72%); Anal. for C₂₁H₁₈N₄O₃S₂ (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 (ν, cm⁻¹): 3429 (NH), 3090 (CH aromatic), 2930 (CH₂), 2207 (CN), 1686, 1682 (2C=O), 1593, 1439 (C=C), 1320, 1257 (C=S); ¹H-NMR (δ, ppm): 1.03 (s, 3H, CH₃), 1.14-1.98 (m, 4H, cyclohexene 2CH₂), 2.03-2.89 (m, 4H, cyclohexene 2CH₂), 4.30 (q, 2H, CH₂), 6.61-7.80 (m, 10H, 2CH₃), 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 [C₄H₆]⁺ (32.01), 78 (100.00).
Brown crystals, m.p. 104–106 °C, yield: 3.56 g (91%); Anal. For C_{20}H_{17}N_{7}S (391.45), (% Calcd./Found): 61.37/60.99 (C), 4.83/4.00 (H), 17.89/17.55 (N), 8.19/7.80 (S); IR (ν, cm^{-1}): 3431 (NH, NH), 3050 (CH aromatic), 2931 (CH₂), 2213 (CN), 1685, 1682 (C=O), 1545, 1437 (C=C); ¹H-NMR (65.88/65.20 (C), 4.82/4.77 (H), 22.31/21.98 (N), 7.30/7.70 (S); IR (ν, cm^{-1}): 3426 (NH, NH), 3050 (CH aromatic), 2928 (CH₂), 2205 (CN), 1590, 1431 (C=C), 1513 (N=N-H); ¹H-NMR (δ, ppm): 1.81-2.49 (m, 4H, cyclohexene 2CH₂), 2.79-2.94 (m, 4H, cyclohexene 2CH₂), 3.57 (s, 2H, NH), 6.00-7.80 (m, 10H, C₆H₅), 8.20 (s, 1H, NH), 8.90 (s, 1H, NH); MS m/z (%): 441 [M⁺] (0.20), 440 [M⁺] (0.15), 438 [M⁻] (0.26), 77 [C₆H₅]⁺ (5.10), 57 (100.00).

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

Brown crystals, m.p. 158–160 °C, yield: 2.02 g (72%); Anal. For C₁₉H₁₈N₈O₃S (422.46), (% Calcd./Found): 49.82/50.20 (C), 3.83/4.20 (H), 24.21/23.83 (N), 11.08/11.44 (S); IR (ν, cm^{-1}): 3432-3196 (2NH, OH), 2933 (CH₂), 2210 (CN), 1685 (C=O), 1600, 1402 (C=C); ¹H-NMR (δ, ppm): 1.71-2.49 (m, 4H, cyclohexene 2CH₂), 2.57-2.89 (m, 4H, cyclohexene 2CH₂), 7.10 (s, 1H, pyrazole NH), 7.30 (s, 1H, NH), 8.29 (s, 1H, OH); MS m/z (%): 291 [M⁺] (0.82), 290 [M⁺] (0.32), 289 [M⁻] (0.33), 288 [M⁻] (0.10), 150 (100.00).

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

Brown crystals, m.p. 213–215 °C, yield: 2.37 g (65%); Anal. For C₁₉H₁₈N₈O₃S (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 (ν, cm^{-1}): 3426 (NH, OH), 3050 (CH aromatic), 2931-2859 (CH₂), 2206 (CN), 1687 (C=O), 1592, 1438 (C=C),1530 (N=N-H); ¹H-NMR (δ, ppm): 1.51-2.32 (m, 4H, cyclohexene 2CH₂), 2.61-2.89 (m, 4H, cyclohexene 2CH₂), 6.93 (s, 1H, NH), 7.10-7.55 (m, 5H, C₆H₅), 8.00 (s, 1H, OH); MS m/z (%): 366 [M⁺] (2.94), 365 [M⁻] (0.58), 364 [M⁻] (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

Brown crystals, m.p. 243–245 °C, yield: 3.69 g (87%); Anal. For C₁₉H₁₈N₈O₃S (422.46), (% Calcd./Found):
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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 (NH2), 1177-2.32 (m, 4H, cyclohexene 2CH2), 2.62-2.89 (m, 4H, cyclohexene 2CH2), 3.60 (s, 2H, NH2), 4.00 (s, 2H, NH), 6.91-7.62 (m, 5H, C6H5), 8.30 (s, 1H, NH); MS m/z (%): 424 [M⁺^2] (5.85), 423 [M⁺^1] (2.66), 422 [M⁺] (7.81), 77 [C₆H₄]^⁺ (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 C₃₂H₃₃N₅O₃S (574.66), (% Calcd./Found): 64.79/64.40 (C), 5.46/6.00 (H), 19.50/19.88 (N), 5.58/5.98 (S); IR (υ, cm⁻¹): 3437 (3NH), 3100 (CH aromatic), 2930–2850 (C-H), 2207 (CN), 1688, 1620 (2C=O), 1563, 1440 (C=C); ¹H-NMR (δ, ppm): 1.17-1.91 (m, 4H, cyclohexene 2CH2), 2.08-2.89 (m, 4H, cyclohexene 2CH2), 6.92-7.44 (m, 10H, 2C₆H₅), 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 cyanocinnamate (3.83 g, 0.01 mol) or ethyl cyanoacinnamate (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.

2-(3-Cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-5-imino-3-phenyl-2,5-dihydro-pyridazine-3-carboxylic acid (4-chloro-phenyl)-amide (17a)

Brown crystals, m.p. 223–225 °C; yield: 3.58 g (70%); Anal. for C₂₅H₂₄N₅O₃Cl (511.00), (% Calcd./Found): 57.35/57.42 (C), 7.35/7.42 (H), 16.54/16.60 (N), 16.54/16.60 (S); IR (υ, cm⁻¹): 3434 (2NH), 3040 (CH aromatic), 2930 (CH₃), 2255, 2202 (2CN), 1688 (C=O), 1593, 1435 (C=C); ¹H-NMR (δ, ppm): 1.71-2.40 (m, 4H, cyclohexene 2CH₂), 2.49-2.89 (m, 4H, cyclohexene 2CH₂), 7.08-7.98 (m, 9H, C₆H₅, C₆H₄), 8.29 (s, 1H, NH), 8.70 (s, 1H, NH); MS m/z (%): 511 [M⁺] (0.17), 510 [M⁺-1] (0.35), 77 [C₆H₄]^+ (7.15), 69 (100.00).

6-(4-Chloro-phenoxyacarbamoyl)-2-(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-5-imino-3-phenyl-2,5-dihydro-pyridazine-4-carboxylic acid ethyl ester (17b)

Dark brown crystals, m.p. 228-230 °C; yield: 3.96 g (71%); Anal. for C₃₉H₃₈N₇O₅Cl (604.90), (% Calcd./Found): 58.24/58.24 (C), 7.15/7.15 (H), 12.05/12.05 (N), 15.80/15.85 (S); IR (υ, cm⁻¹): 3430 (2NH), 3040 (CH aromatic), 2931 (CH₃), 2255, 2202 (2CN), 1688 (C=O), 1593, 1435 (C=C); ¹H-NMR (δ, ppm): 1.18-1.91 (m, 4H, cyclohexene 2CH₂), 2.06-2.91 (m, 4H, cyclohexene 2CH₂), 4.36 (q, 2H, CH₃), 6.00-8.19 (m, 9H, C₆H₅, C₆H₄), 8.29 (s, 1H, NH), 8.40 (s, 1H, NH); MS m/z (%): 558 [M⁺] (19.35), 557 [M⁺-1] (15.87), 77 [C₆H₄]^+ (63.09), 55 (100.00).

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

General procedure: To a solution of 3e (3.83 g, 0.01 mol) in 1,4-dioxane (30 mL) containing triethylamine (1.00 mL), either α-cyanoacinnamitrole (1.54 g, 0.01 mol) or ethyl cyanoacinnamate (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 C₃₀H₂₇N₆O₅Cl (558.05), (% Calcd./Found): 62.42/62.11 (C), 7.35/7.42 (H), 16.54/16.60 (N), 16.54/16.60 (S); IR (υ, cm⁻¹): 3434 (2NH), 3040 (CH aromatic), 2930 (CH₃), 2255, 2202 (2CN), 1688 (C=O), 1593, 1435 (C=C); ¹H-NMR (δ, ppm): 1.71-2.40 (m, 4H, cyclohexene 2CH₂), 2.49-2.89 (m, 4H, cyclohexene 2CH₂), 7.08-7.98 (m, 9H, C₆H₅, C₆H₄), 8.29 (s, 1H, NH), 8.70 (s, 1H, NH); MS m/z (%): 511 [M⁺] (0.17), 510 [M⁺-1] (0.35), 77 [C₆H₄]^+ (7.15), 69 (100.00).

6-(4-Chloro-phenolcarbamoyl)-2-(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-5-imino-3-phenyl-2,5-dihydro-pyridazine-4-carboxylic acid ethyl ester (17b)

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 were heated under reflux for 5 h. The reaction mixture, in each case, was poured 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_{5}O_{2}S_{2} (333.32), (% Calcd./Found): 49.21/49.60 (C), 55.52/55.20 (N), 39.21/38.90 (S); IR (υ, cm⁻¹): 3423 (3NH), 2932 (CH2, CH3), 2208 (CN), 1689, 1683-1680 (2C=O), 1564, 1435 (C=C); 1H-NMR (δ, ppm): 1.76-1.78 (m, 4H, cyclohexene 2CH2), 2.60-2.79 (m, 4H, cyclohexene 2CH2), 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_{19}H_{15}N_{5}O_{3}S_{2} (387.41), (% Calcd./Found): 46.83/47.22 (C), 3.33/3.70 (H), 21.00/21.70 (N), 19.24/18.88 (S); IR (υ, cm⁻¹): 3423 (3NH), 2930 (CH3), 2205 (CN), 1690, 1683 (2C=O), 1564, 1435 (C=C), 1278 (C=S); 1H-NMR (δ, ppm): 1.43-2.50 (m, 4H, cyclohexene 2CH2), 2.60-2.72 (m, 4H, cyclohexene 2CH2), 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_{32}H_{24}N_{10}O_{9}S (696.51), (% 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 (CH2, CH3), 2210 (CN), 1723, 1684 (2C=O), 1600, 1436 (C=C); 1H-NMR (δ, ppm): 1.03 (t, 3H, CH3), 1.19 (t, 3H, CH3) 1.51-2.27 (m, 4H, cyclohexene 2CH2), 2.58-2.95 (m, 4H, cyclohexene 2CH2), 3.85 (q, 2H, CH2), 4.15 (q, 2H, CH2), 8.29 (s, 1H, OH); MS m/z (%): 391 [M+2] (25.39), 387 [M⁺-2] (17.02), 78 (100.00).
X=CN, Y=CONH[CH_{2}(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_{14}N_{5}O_{2}Cl (m/z 415 [M+]), C_{19}H_{17}N_{5}O_{2}S (m/z 349 [M']), C_{19}H_{19}N_{3}O_{4}SCl (m/z 382 [M'-1]), C_{19}H_{19}N_{3}O_{4}S (m/z 379 [M']), and C_{19}H_{17}N_{3}O_{2}S (m/z 363 [M']), respectively which confirmed their structures. Compounds 3a, 3c and 3d reacted with the cyanomethylene reagents (XCH_{2}Y, X=CN, Y=CO_{2}Et) (4a, b) to give the iminopyridazine derivatives 5a-f (Scheme 2).

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_{4}O_{3}S_{2}. Treatment of 3a with acetic anhydride/AcOH mixture under refluxing conditions gave pyridazine-3-one derivative 9.

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, 1H-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 1.74-2.43 ppm and δ 2.57-2.94 ppm for phenyl moiety and a singlet at δ 8.30 and 8.40 for 2NH protons. Mass spectra of 5a, 5b, 5e, 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.

Scheme 1. Synthesis of the hydrazone derivatives 3a-e

Scheme 2. Synthesis of the pyrazidine derivatives 5a-f

Scheme 3. Synthesis of the triazine 7a-c, pyrazidineone 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. 1H-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 phenyl moiety and at δ 8.30 and 8.40 for 2NH protons. The mass spectrum of 9 exhibited a molecular ion m/z 391 [M'] corresponding to molecular formula C_{21}H_{18}N_{4}O_{3}S_{2}. Compound 3d underwent ready hydrolysis in HCl/EtOH to give the diamido derivative 10. Microanalysis, IR and 1H-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 (H2NNHR, 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](image)

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

Thus, 1H-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 CH2 protons, δ 3.39 ppm for singlet 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](image)

**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 H2S 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 C13H13N3O2S2 and C25H30N6O2S2.

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](image)

**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 cinnaminitrile derivatives (16a, b) (PhCH=CN(X), X=CN; X=CO2Et) with the aim of formation of biologically active pyridazine derivatives.23-26 Thus, the reaction of 3c with either α-cyanocinnaminitrile (16a) or ethyl cyanocinnamate (16b) in refluxing 1,4-dioxane containing a catalytic 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.

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>E. coli ECT 101</th>
<th>B. Cereus CECT 148</th>
<th>B. subtilis CECT 498</th>
<th>C. albicans CECT 1394</th>
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</table>

Thus, ¹H-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, ¹H-NMR spectrum of 19a showed two multiplets about δ 1.76-1.78 ppm and δ 2.60-2.72 ppm for four cyclohexene CH₂ 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 (XCH₂Cl, X=CO₂Et; X=COCH₃) 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. 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, 5e, 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=CO2Et) showed higher inhibitory effect against Gram-positive bacteria and fungi used than 13a (X=CONHPh). Similarly for compounds 15a and 15b it is obvious that compound 15a (Y=CONHPh) showed higher inhibitory activity than 15b (Y=CONH[C₆H₄(4-Cl)]).

On the other hand, compound 17a (X=CN) showed high inhibitory effect towards all tested bacteria than 17b (X=CO2Et). 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-diazou-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.

References

Synthesis and antimicrobial evaluations of dyes synthesized from 4,5,6,7-tetrahydrobenzo[b]thiophene  


Received: 26.03.2013.
Accepted: 16.04.2013.