CYTOTOXICITY OF NOVEL 4,5,6,7-TETRAHYDRO-BENZO[*b*]THIOPHENE DERIVATIVES AND THEIR USES AS ANTI-LEISHMANIAL AGENTS

Rafat M. Mohareb[a]*, **Abdelgawad A. Fahmy[a]**

This work has been carried out to investigate some reactions of 4,5,6,7-tetrahydrobenzo[*b*]thiophene derivatives **1a,b** to give synthesis a series of novel heterocyclic products like N-ethoxymethino derivatives (**2a, 2b**), N-phenylaminomethino derivatives (**3a, 3b**), hydrazine derivatives (**5a-d**), pyrazole derivatives (**7a-d, 10a-d, 11a, 11b**) and N-methinonitrilo derivatives (**9a-d**). The antitumor evaluation of the newly synthesized compounds against the three human tumor cells lines namely breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) showed that some of these compounds exhibit much higher inhibitory effects towards the three tumor cell lines than the positive control doxorubicin. Moreover, they were tested against normal cells namely diploid normal human fibroblast (WI-38), Normal prostate epithelial cells (PrEC) and normal human mucosal epithelial cells (NCM 460). The anti-leishmanial evaluations of the obtained compounds were also performed. Compounds **7d** and **10b** were the most active towards tumor cell lines while compounds **3a**, **3b**, **9b** and **9d** were the most active compounds as anti-leishmanial. Docking of these most active compounds was demonstrated.

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

Introduction

Aromatic thiophenes are the most promising small-molecule selective protein kinase inhibitors.[1-4] Recently,[5] a tricyclic compound tetrahydropyridothieno[2,3-*d*]pyrimidine was identified as an initial hit with an enzyme inhibition $IC_{50} = 2.6$ μM.
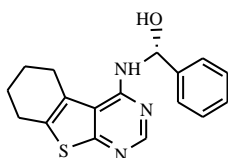
In recent years, thiophenes and their fused derivatives showed promising results as anticancer agents.[6-9] Moreover, Mohareb et al.[10] reported that 2-acylamino-4,5,6,7-tetrahydro-1-benzothiophene derivatives were tested against the three human tumor cells lines namely breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268). The results showed that most of the tested compounds exhibit high inhibitory effects towards the three tumor cell lines. To our knowledge, limited research has been done on the variation of substitution of the 2 and 3 positions of the tetrahydro-1-benzothiophene nucleus.[8,11-13] In the light of these observations, our efforts towards drug discovery prompted us to design, synthesize, and evaluate the cytotoxicity of some new tetrahydro-1-benzothiophene derivatives. In the present work, a new series of thiophene derivatives were synthesized by incorporation of a variety of ring systems such as pyridine, thiazole and thiophene at the 2nd position

of the benzothiophene ring and either cyano or ethyl carboxylate group at the 3rd position. The newly synthesized products were evaluated as to their anti-tumor activity against three cancer cell lines, namely breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460), and CNS cancer (SF-268) besides testing some of them towards three human normal cell lines. The comparison between the anti-tumor evaluation and normal cell lines for the newly synthesized thiophene products of different molecular structures will direct the future research towards the synthesis of good anti-cancer agents.

In an attempt to obtain an anti-tumor agent with high activity, the substitution pattern at the positions 2 and 3 of the thiophene pharmacophore was selected in order to alter the electronic environment and thus affect the lipophilicity of the target molecules. Our aim was the acylation of the 2-amino groups in the known tetrahydro-1-benzothiophene nucleus **1a,b** and the introduction 1,3-dicarbonyl,[14] α,β-unsaturated carbonyl groups,[15] a nitrogen or sulfur heterocyclic ring[16] that are known to contribute to the enhancement of antitumor activity. The incorporation of a heterocyclic ring at the 2nd position of the thiophene pharmacophore was of great importance in order to increase the lipophilicity and thus radically modify the bioavailability and efficacy of the synthesized compounds. Therefore, in the present work we want to provide a comparison between some previously reported[17-20] tetrahydro[*b*]benzothiophenes **1a,b** and our newly synthesized compounds derived from the synthons **1a** and **1b**.

Materials and Methods

The melting points were determined using an Electrothermal digital melting point apparatus in an open capillary tube and are uncorrected. Infrared studies were carried out on KBr pellets, using a Perkin-Elmer Paragon

1000 FT-IR, and expressed as cm^{-1}. The ^{1}H NMR spectra were measured using Varian EM-390-200 MHz in DMSO-*d6* as solvent, using TMS as internal standard and chemical shifts are expressed as δ ppm. The Mass Spectra were recorded by Shimadzu Qp-2010 Plus instrument. Microanalyses were performed at the Microanalytical Data Unit at Cairo University, Giza, Egypt, and the Chemistry Department at the American University in Cairo, New Cairo, Egypt.

Ethyl N-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-formimide (2a) and ethyl 2-((ethoxymethylene) amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (2b)

General procedure: To a solution of either 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene (**1a**) (3.50 g, 0.002 mol) or ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**1b**) (4.50 g, 0.02 mol) in 1,4-dioxane (40 mL), triethyl orthoformate (2.96 mL, 0.02 mol) was added. The reaction mixture, in each case was heated under reflux for 3 h then poured onto iced water and the formed solid product was collected by filtration.

Compound **2a:** Crystallized from 1,4-dioxane, pale yellow crystals. Mp 94 °C in 73 % yield. Analysis for C$_{12}$H$_{14}$N$_2$OS, Mol. Wt. (234.32): Calcd: C, 61.51; H, 6.02; N, 11.96; S, 13.68. Found: C, 61.34; H, 5.89; N, 12.04; S, 13.42. IR (ν cm^{-1}): 2927 (CH$_2$), 2197 (C≡N), ^{1}H NMR (DMSO) δ: 6.90 (s, 1H, CH), 3.31-3.56 (q, 2H, J= 7.66 Hz, CH$_2$), 2.31-2.34 (m, 4H, 2CH$_2$), 2.59-2.63 (m, 4H, 2CH$_2$), 1.63 (t, 3H, J = 7.66 Hz, CH$_3$). m/z (EI, 70 eV): 234 (M$^+$).

Compound **2b**: Crystallized from 1,4-dioxane, pale yellow crystals, Mp 70 °C in 76 % yield. Analysis for C$_{14}$H$_{19}$NO$_3$S, Mol. Wt. (281.37). Calcd: C, 59.76; H, 6.81; N, 4.98; S, 11.40. Found: C, 60.02; H, 6.93; N, 5.04; S, 11.28. IR (ν cm^{-1}): 2981-2935 (CH$_2$), 1655 (C=O). ^{1}H NMR (DMSO) δ: 7.20 (s, 1H, CH), 4.10, 4.15 (2q, 4H, 2CH$_2$), 2.38-2.29 (m, 4H, 2CH$_2$), 2.56-2.59 (m, 4H, 2CH$_2$), 1.23, 1.68 (2t, 6H, 2CH$_3$). m/z (EI, 70 eV): 281 (M$^+$).

N'-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-N-phenylformamidine (3a) and ethyl 2-(formamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (3b)

General procedure: To a solution of either **2a** (1.17 g, 0.005 mol) or **2b** (2.81 g, 0.01 mol) in 1,4-dioxane (30 mL), aniline (0.93 mL, 0.01 mol) was added. Each reaction mixture was heated under reflux for 3 h then poured onto ice/water and the formed solid product, in each case, was then collected by filtration. The collected filtrate was boiled in ethanol (40 mL), left to cool down to room temperature, then re-filtered.

Compound **3a:** Crystallized from 1,4-dioxane, buff crystals, Mp: 98-100 °C in 71 % yield. Analysis for C$_{16}$H$_{15}$N$_3$S, Mol. Wt. (281.38). Calcd: C, 68.30; H, 5.37; N, 14.93; S, 11.40. Found: C, 68.55; H, 5.47; N, 15.04; S, 11.29. IR (ν cm^{-1}): 3430-3320 (NH), 3059 (CH aromatic), 2935 (CH$_2$), 2220 (CN). ^{1}H NMR (DMSO) δ: 8.26 (s, 1H, NH), 7.30-7.38 (m, 5H, C$_6$H$_5$), 6.95 (s, 1H, CH), 1.62-1.67 (m, 4H, 2CH$_2$), 2.70-2.75 (m, 4H, 2CH$_2$), m/z (EI, 70 eV): 282 (M$^+$).

Compound **3b:** Crystallized from ethanol, buff crystals. Mp 76-79 °C in 85 % yield. Analysis for C$_{18}$H$_{20}$N$_2$O$_2$S, Mol. Wt. (328.43). Calcd: C, 65.83; H, 6.14; N, 8.53; S, 9.76. Found: C, 65.93; H, 6.22; N, 8.82; S, 10.05. IR (ν cm^{-1}): 3405-3298 (NH), 3066 (CH aromatic), 2936 (CH$_2$), 1688 (C=O). ^{1}H NMR (DMSO) δ: 8.49 (s, 1H, NH), 7.28-7.33 (s, 5H, C$_6$H$_5$), 7.18 (s, 1H, CH), 4.27 (q, 2H, J =7.08 Hz, CH$_2$), 1.65-1.67 (m, 4H, 2CH$_2$), 2.70-2.73 (m, 4H, 2CH$_2$), 1.28 (t, 3H, J = 7.08 Hz, CH$_3$). m/z (EI, 70 eV): 328 (M$^+$).

N''-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-yl)-formimido-hydrazide (5a), N''-(3-cyano-4,5,6,7-tetra-hydrobenzo[*b*]thiophene-2-yl)phenylform-imidohydrazide (5b), ethyl 2-((hydrazinylmethylene)-amino)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylate (5c) and ethyl 2-(((phenyl-hydrazinyl)methylene)amino)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylate (5d)

General procedure: To a solution of either 2a (2.34 g, 0.01 mol) or 2b (2.81 g, 0.01 mol) in 1,4-dioxane (40 mL) either hydrazine hydrate (0.50 mL, 0.01 mol) or phenylhydrazine (1.08 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 2.0 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 **5a:** Crystallized from 1,4-dioxane, yellow crystals, Mp: 95 °C in 80 % yield. Analysis for C$_{10}$H$_{12}$N$_4$S, Mol. Wt. (220.29). Calcd: C, 54.52; H, 5.49; N, 25.43; S, 14.56. Found: C, 54.77; H, 5.39; N, 25.29; S, 14.81. IR (ν cm^{-1}): 3443-3326 (NH, NH$_2$), 2926 (CH$_2$), 2220 (CN), 1580 (C=N). ^{1}H NMR (DMSO) δ: 6.90 (s, 1H, CH), 4.98 (s, 1H, D$_2$O exchangeable, NH), 3.29 (s, 2H, D$_2$O exchangeable, NH$_2$), 1.69- 1.72 (m, 4H, 2CH$_2$), 2.51-2.54 (2m, 4H, 2CH$_2$). m/z (EI, 70 eV): 220 (M$^+$).

Compound **5b:** Crystallized from 1,4-dioxane, yellow crystals, Mp: 160-162 °C in 77 % yield. Analysis for C$_{16}$H$_{16}$N$_4$S, Mol. Wt. (296.39). Calcd: C, 64.84; H, 5.44; N, 18.90; S, 10.82. Found: C, 64.84; H, 5.44; N, 19.27; S, 11.16. IR (ν cm^{-1}): 3477-3319 (NH, NH$_2$), 2929 (CH$_2$), 2222 (CN), 1583 (C=N). ^{1}H NMR (DMSO) δ: 8.22, 3.29 (2s, 2H, 2NH), 7.28-7.39 (m, 5H, C$_6$H$_5$), 6.93 (s, 1H, CH), 5.11 (s, 1H, D$_2$O exchangeable, NH), 1.63- 1.75 (m, 4H, 2CH$_2$), 2.50-2.58 (2m, 4H, 2CH$_2$). m/z (EI, 70 eV): 296 (M$^+$).

Compound **5c:** Crystallized from 1,4-dioxane, white crystals. Mp: 101 °C in 64 % yield. Analysis for C$_{12}$H$_{17}$N$_3$O$_2$S, M. Wt. (267.35). Calcd: C, 53.91; H, 6.41; N, 15.72; S, 11.99. Found: C, 53.79; H, 6.26; N, 15.84; S, 12.04. IR (ν cm^{-1}): 3455-3268 (NH, NH$_2$), 2935 (CH$_2$), 1684 (C=O). ^{1}H NMR (DMSO) δ: 7.17 (s, 1H, CH), 4.17 (s, 1H, D$_2$O exchangeable, NH), 3.30 (s, 2H, D$_2$O exchangeable, NH$_2$), 4.22 (q, 2H, J = 7.42 Hz, CH$_2$), 1.65-1.68 (m, 4H, 2CH$_2$), 2.60-2.67 (m, 4H, 2CH$_2$) 1.30 (t, 3H, J = 7.42 Hz, CH$_3$). m/z (EI, 70 eV): 269 (M$^+$).

Compound **5d:** Crystallized from 1,4-dioxane, white crystals. Mp: 114-116 °C in 73 % yield. Analysis for C$_{18}$H$_{21}$N$_3$O$_2$S, M. Wt. (343.44). Calcd: C, 62.95; H, 6.16; N, 12.23; S, 9.34. Found: C, 63.27; H, 5.95; N, 12.42; S, 9.57. IR (ν cm^{-1}): 3455-3268 (NH, NH$_2$), 2935 (CH$_2$), 1684 (C=O). ^{1}H NMR (DMSO) δ: 8.01, 3.34 (2s, 2H, 2NH),

7.29-7.38 (s, 5H, C$_6$H$_5$), 7.19 (s, 1H, CH), 4.22 (s, 1H, D$_2$O exchangeable, NH), 4.21 (q, 2H, J = 7.55 Hz, CH$_2$), 1.62-1.66 (m, 4H, 2CH$_2$), 2.63-2.69 (m, 4H, 2CH$_2$) 1.33 (t, 3H, J = 7.55 Hz, CH$_3$). m/z (EI, 70 eV): 343 (M$^+$).

2-((3,5-diamino-1H-pyrazol-1-yl)methyleneamino)-4,5,-6,7-tetrahydro-benzo[*b*]thiophene-3-carbonitrile (7a), 2-((3-amino-5-hydroxy-1H-pyrazol-1-yl)methyleneamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (7b), ethyl 2-((3,5-diamino-1H-pyrazol-1-yl)methylene-amino)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carbo-xylate (7c) and ethyl 2-((3-amino-5-hydroxy-1H-pyrazol-1-yl)methyleneamino)-4,5,6,7-tetrahydrobenzo[*b*]thio-phene-3-carboxylate (7d)

General procedure: To a solution of either **5a** (2.20 g, 0.01 mol) or **5b** (2.96 g, 0.01 mol) in 1,4-dioxane (40 mL) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4h then evaporated under vacuum. The remaining product, in each case, was triturated with diethyl ether and the formed solid product was collected by filtration.

Compound **7a:** Crystallized from ethanol, orange crystals, Mp: 230-233 °C in 80 % yield. Analysis for C$_{13}$H$_{14}$N$_6$S, Mol. Wt. (286.36). Calcd: C, 54.53; H, 4.93; N, 29.35; S, 11.20. Found: C, 54.66; H, 5.02; N, 29.53; S, 11.08. IR (ν cm^{-1}): 3443-3326 (2 NH$_2$), 2926 (CH$_2$), 2220 (CN), 1580 (C=N). ^{1}H NMR (DMSO) δ: 6.90 (s, 1H, CH), 7.11 (s, 1H, pyrazole H-4), 4.98, 3.29 (2s, 4H, D$_2$O exchangeable, 2NH$_2$), 1.69-1.72 (m, 4H, 2CH$_2$), 2.51-2.54 (2m, 4H, 2CH$_2$). m/z (EI, 70 eV): 220 (M$^+$).

Compound **7b:** Crystallized from 1,4-dioxane, yellow crystals, Mp: 183-185 °C in 83 % yield. Analysis for C$_{13}$H$_{13}$N$_5$OS, Mol. Wt. (287.34). Calcd: C, 54.34; H, 4.56; N, 24.37; S, 11.16. Found: C, 54.28; H, 4.71; N, 24.16; S, 10.85. IR (ν cm^{-1}): 3595-3328 (OH, NH$_2$), 2963 (CH$_2$), 2220 (CN), 1582 (C=N). ^{1}H NMR (DMSO) δ: 10.22 (s, 1H, D$_2$O exchangeable, OH), 6.90 (s, 1H, CH), 7.12 (s, 1H, pyrazole H-4), 3.24 (s, 2H, D$_2$O exchangeable, NH$_2$), 1.61-1.79 (m, 4H, 2CH$_2$), 2.52-2.57 (2m, 4H, 2CH$_2$). m/z (EI, 70 eV): 287 (M$^+$).

Compound **7c:** Crystallized from ethanol, yellow crystals. Mp: 211-213 °C in 79 % yield. Analysis for C$_{15}$H$_{19}$N$_5$O$_2$S, M. Wt. (333.41). Calcd: C, 54.04; H, 5.74; N, 21.01; S, 9.62. Found: C, 54.37; H, 5.84; N, 20.93; S, 9.83. IR (ν cm^{-1}): 3520-3318 (2 NH$_2$), 2935 (CH$_2$), 1688 (C=O). ^{1}H NMR (DMSO) δ: 6.88 (s, 1H, CH), 7.11 (s, 1H, pyrazole H-4), 3.30, 4.22 (2s, 4H, D$_2$O exchangeable, 2NH$_2$), 4.24 (q, 2H, J = 7.53 Hz, CH$_2$), 1.62-1.68 (m, 4H, 2CH$_2$), 2.63-2.68 (m, 4H, 2CH$_2$) 1.32 (t, 3H, J = 7.53 Hz, CH$_3$). m/z (EI, 70 eV): 333 (M$^+$).

Compound **7d:** Crystallized from 1,4-dioxane/EtOH, yellow crystals. Mp: 222-225 °C in 78 % yield. Analysis for C$_{15}$H$_{18}$N$_4$O$_3$S, M. Wt. (343.39). Calcd: C, 53.88; H, 5.43; N, 16.75; S, 9.59. Found: C, 53.92; H, 5.68; N, 16.83; S, 9.34. IR (ν cm^{-1}): 3555-3248 (OH, NH$_2$), 2935 (CH$_2$), 1686 (C=O). ^{1}H NMR (DMSO) δ: 10.20 (s, 1H, D$_2$O exchangeable, OH), 6.81 (s, 1H, CH), 7.11 (s, 1H, pyrazole H-4), 3.39 (s, 2H, D$_2$O exchangeable, NH$_2$), 4.24 (q, 2H, J

= 7.55 Hz, CH$_2$), 1.60-1.66 (m, 4H, 2CH$_2$), 2.62-2.67 (m, 4H, 2CH$_2$) 1.34 (t, 3H, J = 7.55 Hz, CH$_3$). m/z (EI, 70 eV): 334 (M$^+$).

Synthesis of 2-(2,2-dicyanoethylideneamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (9a), (E)-ethyl 3-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-ylimino)-2-cyanopropanoate (9b), ethyl 2-(2,2-dicyanoethylideneamino)-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-carboxylate (9c) and ethyl 2-(2-(etho-xycarbonyl)-2-cyanoethylideneamino)-4,5,6,7-tetra-hydrobenzo[*b*]thiophene-3-carboxylate (9d)

General procedure: To a solution of either **2a** (1.17 g, 0.005 mol) and **2b** (2.81 g, 0.01 mol) in 1,4-dioxane (20 and 40 mL respectively), either malononitrile (0.33 g, 0.005 mol or 0.66 g, 0.01 mol (for **9a** and **9c**) or ethyl cyanoacetate (0.57 mL, 0.005 mol or 1.13 mL, 0.01 mol) (for **9b** and **9d**) were added. The reaction mixture was heated in each case under reflux for 1 h then poured onto iced water and the formed solid product was collected by filtration.

Compound **9a**: Crystallized from 1,4-dioxane, pale brown crystals. Mp: 79 °C in 78 % yield. Analysis for C$_{13}$H$_{10}$N$_4$S, Mol. Wt. (254.31). Calcd: C, 61.40; H, 3.96; N, 22.03; S, 12.61. Found: C, 61.22; H, 4.21; N, 21.79; S, 12.49. IR (ν cm^{-1}): 2935 (CH$_2$), 2227-2200 (3 CN). ^{1}H NMR (DMSO) δ: 5.90, 6.86 (2d, 2H, 2CH), 1.69-1.72 (m, 4H, 2CH$_2$), 2.51-2.56 (m, 4H, 2CH$_2$). m/z (EI, 70 eV): 254 (M$^+$).

Compound **9b:** Crystallized from 1,4-dioxane, yellow crystals. Mp: 85 °C in 70 % yield. Analysis for C$_{15}$H$_{15}$N$_3$O$_2$S, Mol. Wt. (301.36). Calcd: C, 59.78; H, 5.02; N, 13.94; S, 10.64. Found: C, 59.73; H, 4.88; N, 14.29; S, 10.37 %. IR (ν cm^{-1}): 2936 (CH$_2$), 2197 (C≡N), 2225, 2220 (2CN) 1689 (C=O). ^{1}H NMR (DMSO) δ: 5.90, 6.86 (2d, 2H, 2CH), 4.21 (q, 2H, J = 7.67 Hz, CH$_2$), 1.55-1.58 (m, 4H, 2CH$_2$), 2.56-2.59 (m, 4H, 2CH$_2$), 1.21 (t, 3H, J = 7.67 Hz, CH$_3$). m/z (EI, 70 eV): 301 (M$^+$).

Compound **9c:** Crystallized from 1,4-dioxane, yellow crystals. Mp: 120 °C in 74 % yield. Analysis for C$_{15}$H$_{15}$N$_3$O$_2$S, Mol. Wt. (301.36). Calcd: C, 59.78; H, 5.02; N, 13.94; S, 10.64. Found: IR (ν cm^{-1}): 2936 (CH$_2$), 2197 (C≡N), 2225, 2220 (2CN) 1689 (C=O). ^{1}H NMR (DMSO) δ: 5.92, 6.88 (2d, 2H, 2CH), 4.25 (q, 2H, J = 7.67 Hz, CH$_2$), 1.56-1.58 (m, 4H, 2CH$_2$), 2.52-2.59 (m, 4H, 2CH$_2$), 1.30 (t, 3H, J = 7.67 Hz, CH$_3$). m/z (EI, 70 eV): 301 (M$^+$).

Compound **9d**: Crystallized from 1,4-dioxane, Pale yellow crystals. Mp: 70-72 °C in 90 % yield. Analysis for C$_{17}$H$_{20}$N$_2$O$_4$S, Mol. Wt. (348.42). Calcd: C, 58.60; H, 5.79; N, 8.04; S, 9.20. Found: C, 58.79; H, 5.83; N, 7.84; S, 8.91 %. IR (ν cm^{-1}): 2938 (CH$_2$), 2220 (CN), 1705, 1688 (2CO), 1638 (C=C). ^{1}H NMR (DMSO) δ: 5.22, 6.49 (2d, 2H, 2CH), 4.17, 4.30 (2q, 4H, 2CH$_2$), 1.66-1.69 (m, 4H, 2CH2), 2.60-2.66 (m, 4H, 2CH$_2$), 1.26, 1.32 (2t, 6H, 2CH$_3$). m/z (EI, 70 eV): 348 (M$^+$).

Synthesis of 2-(2,2-dicyanoethylideneamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (10a), ethyl 3-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-ylimi-no)-2-cyanopropanoate (10b), ethyl 2-(2,2-dicyano-

ethylideneamino)-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-carboxylate (10c) and ethyl 2-(2-(ethoxycarbonyl)-2-cyanoethylideneamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (10d)

General procedure: To a solution of either **9a** (3.81 g, 0.01mol), **9b** (3.01 g, 0.01 mol), **9c** (3.01 g, 0.01 mol), and **9d** (3.48 g, 0.01 mol) in 1,4-dioxane (30 mL), hydrazine hydrate (0.50 mL, 0.01 mol) was added. The reaction mixture was heated, in each case, under reflux for 1 h then poured onto iced water, containing few drops of hydrochloric acid (till pH 6), and the formed solid product was collected by filtration.

Compound **10a**: Crystallized from 1,4-dioxane,whit crystals. Mp: 120 °C, in 67 % yield. Analysis for $C_{13}H_{14}N_6S$, Mol. Wt. (286.36). Calcd: C, 54.53; H, 4.93; N, 29.35; S, 11.20. Found: C. 54.32; H, 4.78; N, 29.06; S, 10.94 %. IR (ν cm^{-1}): 3333-3428-3328 (2NH$_2$), 2941 (CH$_2$), 2220 (CN), 1640 (C=N), 1633 (C=C). ^{1}H NMR (DMSO) δ: 6.89 (s, 1H, CH), 6.34 (s, 1H, CH in pyrazole), 2.49, 3.34 (2s, 4H, 2NH$_2$), 1.71- 1.74 (m, 4H. 2CH$_2$), 2.41-2.46 (m, 4H, 2CH$_2$). m/z (EI, 70 eV): 286 (M$^+$).

Compound **10b**: Crystallized from 1,4-dioxane, Pale brown crystals. Mp: 93-95 °C in 68 % yield. Analysis for $C_{13}H_{13}N_5OS$, Mol. Wt. (287.34). Calcd: C, 54.34; H, 4.56; N, 24.37; S, 11.16. Found: C, 54.11; H, 4.75; N, 24.49; S, 11.07 %. IR (ν cm^{-1}): 3488-3328 (OH, NH$_2$), 2938 (CH$_2$), 2222 (CN), 1650 (C=N). ^{1}H NMR (DMSO) δ: 9.93 (s, 1H, OH), 6.88 (s, 1H, CH), 6.35 (s, 1H, CH in pyrazole), 3.34 (s, 2H, NH$_2$), 1.61-1.64 (m, 4H, 2CH$_2$), 1.70-1.74 (m, 4H. 2CH$_2$), m/z (EI, 70 eV): 287 (M$^+$).

Compound **10c**: Crystallized from 1,4-dioxane, Pale brown crystals. Mp: 113-115 °C in 73 % yield. Analysis for $C_{15}H_{19}N_5O_2S$, Mol. Wt. (333.41). Calcd: C, 54.04; H, 5.74; N, 20.01; S, 9.62. Found: C, 54.11; H, 4.75; N, 24.49; S, 11.07 %. IR (ν cm^{-1}): 3488-3328 (OH, NH$_2$), 2938 (CH$_2$), 2222 (CN), 1650 (C=N). ^{1}H NMR (DMSO) δ: 7.17 (s, 1H, CH), 6.33 (s, 1H, CH in pyrazole), 4.24 (q, 2H, J = 7.05 Hz, CH$_2$), 3.36, 3.66 (2s, 4H, 2NH$_2$), 1.60- 1.64 (m, 4H, 2CH$_2$), 1.73-1.77 (m, 4H. 2CH$_2$), 1.13 (t, 3H, J = 7.05 Hz, CH$_3$), m/z (EI, 70 eV): 333 (M$^+$).

Compound **10d**: Crystallized from 1,4-dioxane, Pale brown crystals. Mp: 144-146 °C in 81 % yield. Analysis for $C_{15}H_{18}N_4O_3S$, Mol. Wt. (334.39). Calcd: C, 53.88; H, 5.43; N, 16.75; S, 9.59. Found: C, 54.28; H, 5.61; N, 16.55; S, 9.43 %. IR (ν cm^{-1}): 3468-3329 (OH, NH$_2$), 2938 (CH$_2$), 1646 (C=N). ^{1}H NMR (DMSO) δ: 9.02 (s, 1H, OH), 6.79 (s, 1H, CH), 6.33 (s, 1H, CH pyrazole), 4.23 (q, 2H, J = 6.79 Hz, CH$_2$), 3.30 (s, 2H, NH$_2$), 1.62- 1.64 (m, 4H, 2CH$_2$), 1.72-1.75 (m, 4H. 2CH$_2$), 1.16 (t, 3H, J = 6.79 Hz, CH$_3$), m/z (EI, 70 eV): 334 (M$^+$).

Synthesis of 2-((3,5-dimethyl-1H-pyrazol-1-yl)methyleneamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (11a) and ethyl 2-((3,5-dimethyl-1H-pyrazol-1-yl)methyleneamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (11b)

General procedure: To a solution of either **5a** (2.20, 0.01 mol) or **5c** (2.96 g, 0.01 mol) in 1,4-dioxane (50 mL), acetyl

acetone (1.0 g, 0.01 mol) was added. The reaction mixture was heated, in each case, under reflux for 3.5 h then poured onto ice/water and the formed solid product was collected by filtration.

Compound **11a**: Crystallized from 1,4-dioxane, colorless crystals. Mp: 124 °C, in 88 % yield. Analysis for $C_{15}H_{16}N_4S$, M. Wt. (284.38). Calcd: C, 63.35; H, 5.67; N, 19.70; S, 11.28. Found : C, 63.63; H, 5.82; N, 19.59; S, 11.36. IR (ν cm^{-1}): 3055 (CH pyrazole), 2934 (CH$_2$), 2221 (CN). ^{1}H NMR (DMSO) δ: 6.94 (s, 1H, CH), 3.56 (s, 1H, pyrazole H-5), 2.67, 3.34 (2s, 6H, 2CH$_3$), 1.68-1.72 (m, 4H, 2CH$_2$), 2.51-2.55 (m, 4H, 2CH$_2$). m/z (EI, 70 eV): 284 (M$^+$).

Compound **11b**: Crystallized from 1,4-dioxane, pale yellow crystals. Mp: 95 °C in 0.23% yield. Analysis for $C_{17}H_{21}N_3O_2S$, Mol. Wt. (331.43). Calcd: C, 61.61; H, 6.39; N, 12.68; S, 9.67.Found: C, 61.48; H, 6.83; N, 12.68; S, 9.64 %. IR (ν cm^{-1}): 2938 (CH$_2$), 1689, 1684 (2 CO), 1644 (C=C) %. ^{1}H NMR (DMSO) δ: 6.62 (s, 1H, CH), 6.17 (s, 1H, pyrazole H-4), 4.20 (q, 2H, CH$_2$), 2.65, 3.33 (2s, 6H, 2CH$_3$), 2.33-2.36 (m, 4H, 2CH$_2$), 1.72-1.77 (m, 4H, 2CH$_2$), 1.21-1.26 (t, 3H, CH$_3$). m/z (EI, 70 eV): 331 (M$^+$).

Antitumor activity tests

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

Tumor Cell cultures

Three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (human glioblastoma cells) were used. 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 were grown as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 µM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 µg/mL), at 37ºC 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 compounds **2a**, **2b**, **3a**, **3b**, **5a-d**, **7a-d**, **9a-d**, **10a-d**, **11a** and **11b** on the *in vitro* growth of human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the 'In vitro Anticancer Drug Discovery Screen' that uses the protein-binding dye sulforhodamine B to assess cell growth. Briefly,

exponentially, cells growing in 96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 μM. Following this exposure period adherent cells were fixed, washed, and stained. The bound stain was solubilized and the absorbance was measured at 492 nm in a plate reader (Bio-Tek Instruments Inc., Powerwave XS, Wincoski, USA). For each test compound and cell line, a dose–response curve was obtained and the minimum concentration inhibition of 50% (IC_{50}), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth, was calculated as described elsewhere[14]. For our newly synthesized products we selected the three cancer cell lines the breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) as our compounds are electron rich systems substituted with electronegative groups and many reports from previous work[21,22] used such cell lines together with the use of doxorubicin which was showed to be the best positive control against the three cell lines.

Normal Cell Cultures

A diploid normal human fibroblast (WI-38), Normal prostate epithelial cells (PrEC),were purchased from the American Type Culture Normal colon mucosal, NCM 460 cells were obtained from In Cell Corporation LLC. All cell lines were tested regularly for *Mycoplasma* contamination by the DNA hybridization method using a Gen-Probe kit. To further characterize the possible differential effects of the obtained compounds on tumor and normal cells, we compared cell viability (scored as membrane integrity by the trypan blue exclusion assay) after the compound treatment.

Normal PrEC cells showed minimal loss of viability up to at least 25 μM of the tested compound (i.e. about 75 x IC_{50}) even after a 24-hr continuous treatment. Other normal cell lines showed similar marginal decreases in cell viability after the 4/20 hr scheme.

Anti-leishmanial activity tests

BALB/c mice (4-6 weeks) were injected intravenously with freshly transformed promastigotes of L. donovani (2 X 10^7/mouse). One-month post-infection, 4-week-infected BALB/c mice were treated with the tested compound at a dose of 5.5 and 12.5 mg/kg body weight (doses were chosen as 1/22nd and 1/10th, respectively, of LD_{50} value of the compound) intraperitoneally two times weekly for one month. Each mouse received a total of 8 intraperitoneal administrations of each compound for one month. Mice in the untreated group were received vehicle control (0.2% Tween 80 in PBS) by the same route. Therapy was compared with the standard antileishmanial drug sodium antimony gluconate (or any reference drug as indicated through the submitted table), which was administered to another group of mice at a dose of 250 mg/kg body weight. Mice in all groups were sacrificed on one month post-treatment. The splenic and liver parasites were determined by impression smear of Giemsa staining. Levels of organ parasite load were determined and expressed as the total parasite burden per organ, using the formula:[23] (organ weight in mg the number of amastigotes per cell nucleus X 2 X 10^5).

Results and discussion

Chemical synthesis

The use of thiophenes and their derivatives as potential anticancer agents prompted us to synthesize a new series of 4,5,6,7-tetrahydrobenzo[b]thiophene derivatives by incorporating through the reaction of either 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophene (**1a**), or ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (**1b**) with ethyl orthoformate to form the corresponding N-ethoxymethino derivatives **2a** and **2b**, respectively. The analytical and spectral data of **2a** and **2b** were the basis of their structure elucidation. In the present work the reactivity of compounds **2a** and **2b** were used through different chemical reactions to form new thiophene products together with their evaluation towards human tumor, normal cell lines. Moreover, the obtained products were evaluated as anti-leishmanial agents.

Scheme (1)
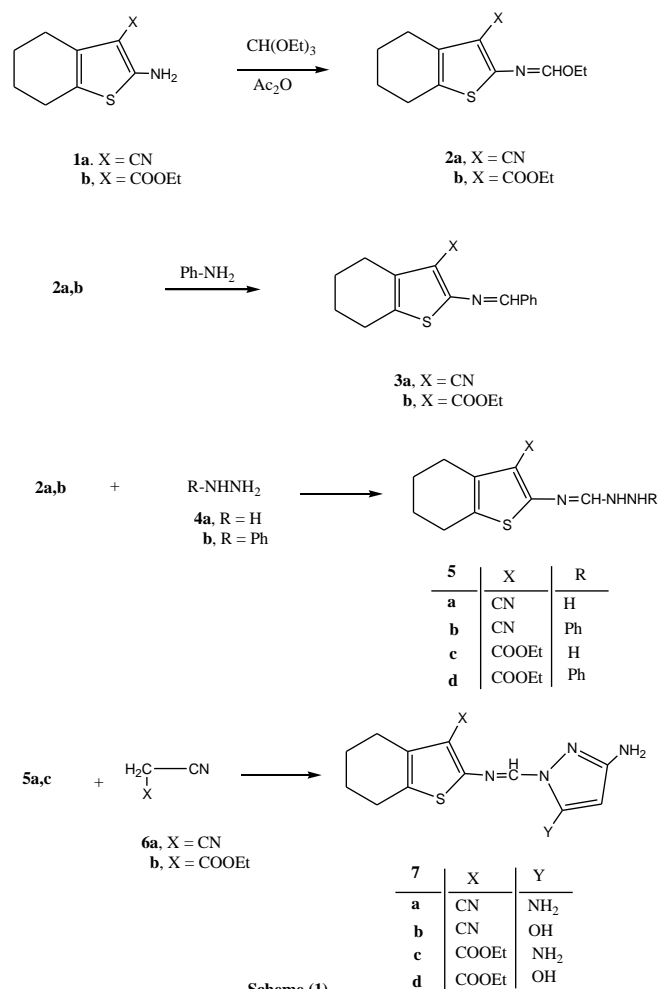
The reaction of either **2a** or **2b** with aniline gave the N-phenylaminomethino derivatives **3a** and **3b**, respectively. The structures of the latter products were established on the basis of analytical and spectral data. Thus, the mass spectrum of **3a** showed m/z   282 corresponding to M⁺ peak and the ¹H NMR spectrum showed two multiplets at δ 1.62-1.67, 2.70-2.75 ppm corresponding to the four cyclohexene

CH$_2$ groups, a singlet at δ 6.95 ppm indicating the CH group, a multiplet at δ 7.30-7.38 corresponding to the phenyl protons and a singlet at δ 8.26 (D$_2$O exchangeable) according to the NH group. On the other hand, the reaction of either **3a** or **3b** with either hydrazine hydrate (**4a**) or phenylhydrazine (**4b**) to give the hydrazino derivatives **5a-d**, respectively. The high yield of compounds **5a** and **5b** encouraged us to study their further reactivity towards cyano methylene reagents. Thus, the reaction of either **5a** or **5c** with either malononitrile (**6a**) or ethyl cyanoacetate (**6b**) gave the pyrazole derivatives **7a-d**, respectively. The analytical and spectral data of the obtained products were in agreement with the assigned structures. Thus the ^{1}H NMR spectrum of **7a** (as an example) showed beside the expected signals of the cyclohexene moiety, two singlets at δ 4.98, 3.29 ppm (D$_2$O exchangeable) corresponding to the two NH$_2$ groups, a singlet at δ 6.90 ppm indicating the CH group and a singlet at δ 7.11 ppm confirming the pyrazole H-4.

Scheme (2)
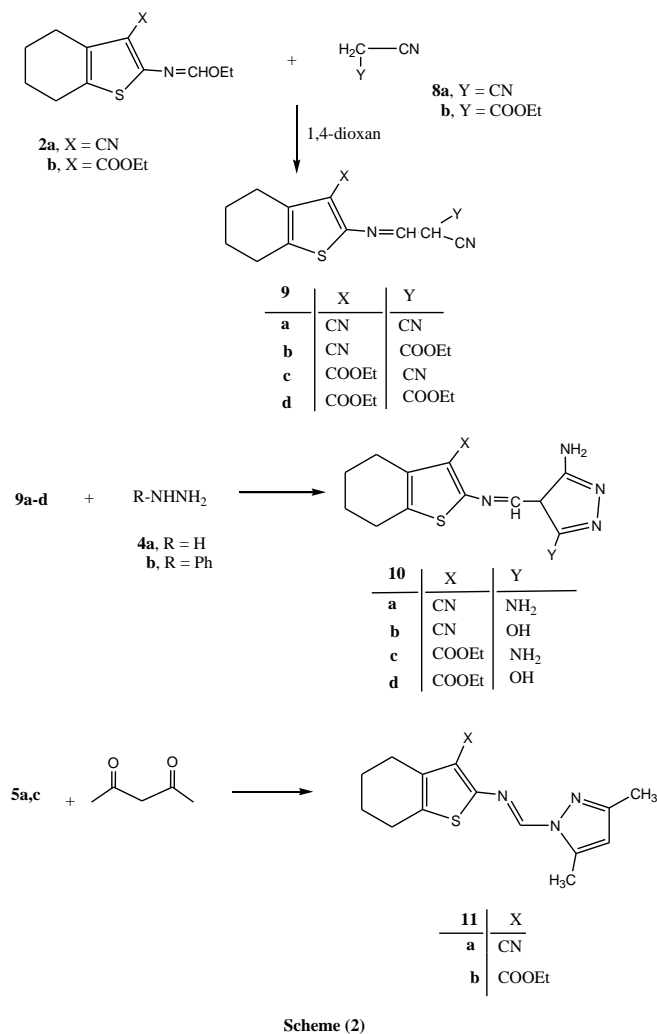
Compounds **2a** and **2b** reacted with either malononitrile or ethyl cyanoacetate to give the N-methinonitrilo derivatives **9a-d**, respectively. On the other hand, the reaction of either **9a**, **9b**, **9c** or **9d** with hydrazine hydrate gave the pyrazole derivatives **10a-d**, respectively. The analytical and spectral data were consistent with the proposed structures. The reaction of compounds **5a** and **5c** with acetylacetone gave the pyrazole derivatives **11a** and **11b**, respectively. (Scheme 2).

Antitumor activity

Compared with the positive control doxorubicin and with the normal cell lines; compounds **3a**, **5b**, **7b**, **7d** and **10b** showed higher antitumor activity in all three tested malignant cell lines.

Table 1. Effect of the obtained compounds on the growth of three human tumor cell lines

Compound	IC_{50}, µmol L^{-1}		
	MCF-7	**NCI-H460**	**SF-268**
2a	2.0 ± 1.0	4.4 ± 1.2	1.8 ± 0.8
2b	10.0 ± 2.2	6.3 ± 2.4	4 ± 1.6
3a	0.02 ± 0.009	0.01 ± 0.002	0.02 ± 0.006
3b	2.2 ± 0.6	4.7 ± 0.4	1.2 ± 0.1
5a	4.2 ± 2.5	2.8 ± 1.2	3.0 ± 1.4
5b	0.03 ± 0.006	0.03 ± 0.004	0.03 ± 0.008
5c	8.0 ± 4.2	6.3 ± 2.6	8.0 ± 1.8
5d	0.2 ± 0.09	0.8 ± 0.08	0.2 ± 0.06
7a	2.2 ± 0.9	1.7 ± 0.04	3.2 ± 1.2
7b	0.02 ± 0.01	0.08 ± 0.01	0.06 ± 0.02
7c	2.2 ± 0.8	4.6 ± 0.4	1.2 ± 0.8
7d	0.01 ± 0.005	0.02 ± 0.002	0.05 ± 0.008
9a	2.0 ± 0.4	1.3 ± 0.5	2.5 ± 1.0
9b	30.4 ± 2.8	20.1 ± 4.6	36.3 ± 4.5
9c	22.2 ± 4.8	10. 1 ± 2.6	2.8 ± 0.8
9d	10.8 ± 2.0	4.2 ± 2.4	6.2 ± 2.6
10a	0.4 ± 0.1	0.2 ± 0.01	0.1 ± 0.02
10b	0.02 ± 0.002	0.01 ± 0.006	0.02 ± 0.008
10c	4.2 ± 0.8	2.6 ± 0.4	4.2 ± 1.8
10d	0.08 ± 0.001	0.2 ± 0.06	0.5 ± 0.08
11a	6.0 ± 0.4	2.3 ± 0.5	4.5 ± 1.0
11b	20.2 ± 2.8	8.1 ± 1.6	1.8 ± 0.8
Doxorubicin	0.04±0.008	0.09±0.008	0.09±0.007

Table 2. Effect of the obtained compounds on the growth of three normal cell lines

Compound	IC_{50}, µmol L^{-1}		
	WI-38	**PrEC**	**NCM 460**
2a	0.11 ± 0.08	0.81 ± 0.70	0.21 ± 0.12
2b	4.18 ± 0.08	3.87 ± 0.02	2.74 ± 0.02
3a	1.14 ± 0.06	1.03 ± 0.02	3.04 ± 0.08
3b	4.33 ± 1.23	2.42 ± 0.92	6.28 ± 1.41
5a	2.16 ± 1.30	4.18 ± 1.54	1.31 ± 0.63
5b	0.36 ± 0.02	0.22 ± 0.02	0.42 ± 0.01
5c	2.18 ± 0.32	1.29 ± 0.21	2.43 ± 0.14
5d	0.08 ± 0.01	0.33 ± 0.19	0.06 ± 0.01
7a	3.18 ± 1.24	2.13 ± 1.12	3.28 ± 1.11
7b	0.21 ± 0.01	0.38 ± 0.05	0.49 ± 0.08
7c	4.77 ± 1.28	4.81 ± 0.93	6.22 ± 2.64
7d	0.47 ± 0.28	0.45 ± 0.03	0.09 ± 0.01
9a	2.18 ± 0.84	1.13 ± 0.72	3.28 ± 1.01
9b	0.28 ± 0.02	0.18 ± 0.02	0.19 ± 0.08
9c	4.44 ± 1.08	6.81 ± 2.13	2.22 ± 1.14
9d	0.17 ± 0.06	0.15 ± 0.01	0.09 ± 0.04
10a	3.18 ± 1.14	2.23 ± 1.04	4.38 ± 0.99
10b	0.41 ± 0.02	0.68 ± 0.14	0.29 ± 0.13
10c	5.78 ± 2.32	4.55 ± 1.63	2.62 ± 0.84
10d	6.47 ± 2.48	3.46 ± 1.60	4.25 ± 2.02
11a	0.18 ± 0.08	0.13 ± 0.01	0.28 ± 0.11
11b	1.08 ± 0.31	2.33 ± 0.19	1.06 ± 0.11

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

Compounds **10a** and **10d** also showed higher antitumor activity in all three tested cell lines compared with the normal cell lines, but not with doxorubicin. The highest antitumor activity in MCF-7 was achieved by compound **7d** while the highest activities in NCI-H460 and SF-268, were achieved by compound **3a**. However, compound **10b** showed the second highest antitumor activity in all the three malignant cell lines. On the other hand, compound **9b** showed the lowest antitumor activity in all the three malignant cell lines, which was even lower than its cytotoxicity in the normal cell lines.

Anti-leishmanial activity

All the tested compounds showed high to moderate antileishmanial inhibition activity with no consistent pattern with respect to the cyano or ester substitution.

Table 3. Anti-leishmanial activity of compounds **2a,b-11a,b** at 50 μM against L. donovani axenic amastigotes

No.	Average inhibition,%	GI_{50}^{a} (μM)	No.	Average inhibition, %	GI_{50}^{a} (μM)
2a	40	12	**9a**	44	10
2b	50	-	**9b**	98	10
3a	98	10	**9c**	60	10
3b	98	30	**9d**	98	28
5a	60	18	**10a**	55	16
5b	70	-	**10b**	66	10
5c	85	-	**10c**	78	-
5d	40	-	**10d**	80	-
7a	60	18	**11a**	97	10
7b	95	12	**11b**	90	18
7c	40	-	*d*	98	
7d	97	20	*e*	0	

a-GI_{50} = concentration for 50% growth inhibition; b-Amphotericin B (1 μM), c-Culture medium and DMSO; *d*-positive control; *e*-negative control

Structural activity relationship (SARS)

SAR of the antitumor activity

The cyano containing compounds **2a, 3a, 5a, 5b** and **11a** showed higher antitumor activity than their COOEt-containing counterparts **2b, 3b, 5c, 5d** and **11b** respectively, in the three malignant cell lines due to the higher cytotoxicity of their CN groups relative to the COOEt group. However, only compounds **3a** and **5b** showed significantly higher cytotoxicity to the malignant cell lines Compared with the normal cell lines, except for NCM 460 in case of compound **3b**. Compounds **7a** and **7c** did not show significant difference in their activities on both malignant and normal cell lines, except for higher antitumor activity in case of NCI-H460, while both compounds **7b** and **7d** showed significantly higher cytotoxicity to the malignant cell lines compared with the normal cell lines due to the presence of the OH group. Compounds **10a, 10b** and **10d** all

showed higher antitumor activity compared with the normal cell lines. The CN-containing compounds **10a** and **10b** showed higher cytotoxicity than the COOEt-containing compounds **10c** and **10d**.

SAR of the anti-leishmanial activity

The phenyl containing counterpart compounds **3a**, and **3b**, as well as the OH-containing counterpart compounds **9b** and **9d** all showed the highest inhibition activity of 98%, comparable to the positive control Amphotericin, while the cyano containing counterpart compounds **9a** and **9c** showed moderate inhibition activities of 44% and 60% respectively. The pyrazole containing compounds **7d** and **11a** showed the same high inhibition activity of 97% in spite of their structural differences concerning the cyano versus the ester group, as well as the differences in their pyrazole substitution. On the other hand, their counterparts, compounds **7b** and **11b** showed different, but still high, inhibition activities of 95% and 90% respectively. However, the two other pyrazole containing counterparts **7a** and **7c** showed moderate inhibitions of 60% and 40% respectively. In case of the pyrazole containing compounds **10a-d**, the ester containing compounds **10c** and **10d** showed close inhibition activities of 80% and 78% respectively, while their cyano containing counterparts **10a** and **10b** showed moderate inhibition activities of 55% and 66% respectively. Both compounds **2a** and **2b** showed moderate inhibition activities of 40% and 50%, being higher in the presence of the cyano group in that particular case.

Preparation for docking

Docking was carried out on an Intel Pentium 1.6 GHz processor, 512 MB memory with windows XP operating system with Molecular Operating Environment (MOE 2008.10; Chemical Computing Group, Canada) as the computational software. All the minimizations were performed with MOE until a root mean square deviation (RMSD) gradient of 0.05 Kcal mol^{-1}A$^{0-1}$ with MMFF94x force-field and the partial charges were automatically calculated. The 3D structure of the Protein Cyclin Dependent Kinase2 (CDK2) complexed with Thiophene Carboxamide was obtained from the Protein Data Bank (PDB ID: 1EVE) at Research Collaboration for Structural Bioinformatics (RCSB) protein data bank base 60 with 2.5A^{0} resolution.

Scoring: Poses generated by the placement methodology were scored using the London dG scoring function implemented in MOE, which estimates the free energy of binding of the ligand from the given pose. The top 10 poses for each ligand were output in MOE database. Each resulting ligand pose was then subjected to MMFF94x energy minimization. The minimized docking conformations were then re-scored using London dG scoring method. Validation of the function implemented in MOE was done by docking the native ligand (thiophene carboxamide) into its binding site; the docked results of the previous mentioned ligand were compared to the crystal structure of the bound ligand-protein complex. The RMSD of the docked ligand was 2.5Å as it seems exactly superimposed on the native bound one. These results indicate the accuracy of the MOE in comparison with the biological methods.

Compound **3a**, energy = -20.5 kJ mol^{-1}
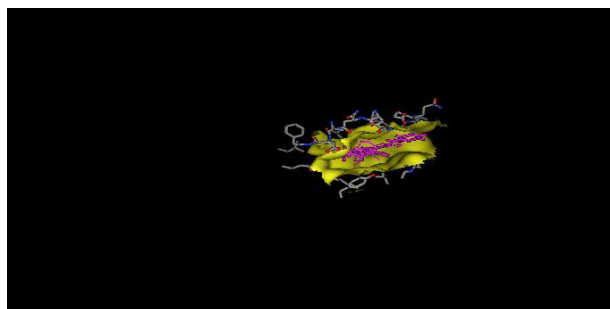
Compound **3b** energy =- 25.5 kJ mol^{-1}
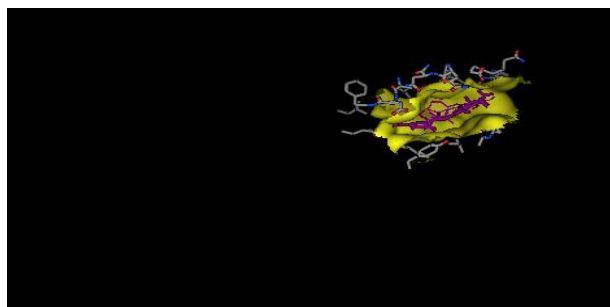
Compound **7b** energy= -28.5 kJ mol^{-1}
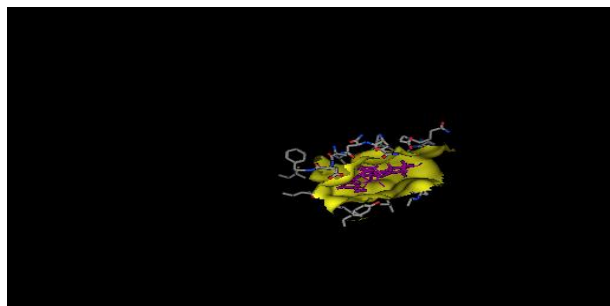
Compound **9b** energy = -19.5 kJ mol^{-1}
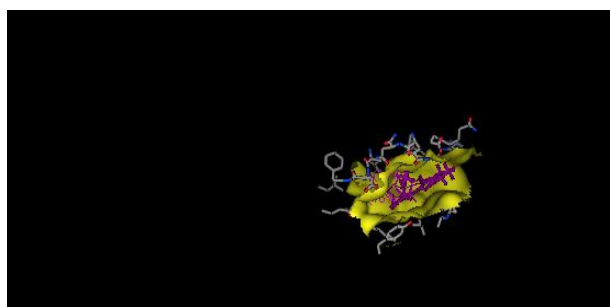
Compound **11b** energy = -23.5 kJ mol^{-1}
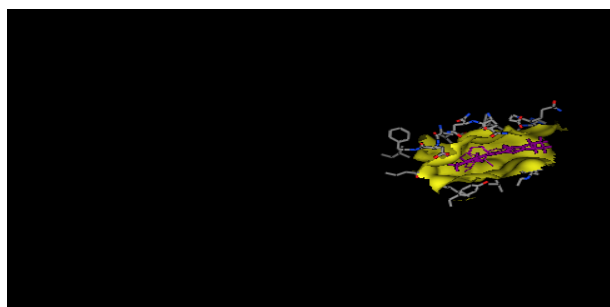
In the present work all new compounds were docked using the rigid receptor/ flexible ligand approach adopting five energy maps which are hydrophobicity, electrostatic, hydrogen bond formation and two Van der Waals parameters. The docking scores were expressed in energy terms. The lower the binding energy, the better binding affinity. The docking study displayed showed that most of the designed compounds have a promising affinity to inhibit CDK2. Docking of compounds **3a, 3b, 7b** and **11b** are demonstrated.

Acknowledgment

References

[1]Janetka, J. W., Almeida, L., Ashwell, S., Brassil, P. J., Daly, K., Deng, C., Gero, T., Glynn, R. E., Horn, C. L., Ioannidis, S., Lyne, P., Newcombe, N. J., Oza, V. B., Pass, M., Springer, S. K., Su, M., Toader, D., Vasbinder, M. M., Yu, D., Yu, Y., Zabludoff, S. D., *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 4242.

[2]Ye, D., Zhang, Y., Wang, F., Zheng, M., Zhang, X., Luo, X., Shen, X., Jiang, H., Liu, H., *Bioorg. & Med. Chem.,* **2010**, *18***,** 1773.

[3]Malmström, J., Viklund, J., Slivo, C., Costa, A., Maudet, M., Sandelin, C., Hiller, G., Olsson, L. L., Aagaard, A., Geschwindner, S., Xue, Y., Vasänge, M., *Bioorg. Med. Chem. Lett.*, **2012**, *22*, 5919.

[4]Emmitte, K. A., Adjebang, G. M., Andrews, C. W., Alberti, J. G. B., Bambal, R., Chamberlain, S. D., Davis-Ward, R. G., Dickson, H. D., Hassler, D. F., Hornberger, K. R., Jackson, J. R., Kuntz, K. W., Lansing, T. J., Mook Jr., R. A., Nailor, K. E., Pobanz, M. A., Smith, S. C., Sung, C.-M., Cheung, M., *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 1694.

[5]Wu, C.-H., Coumar, M. S., Chu, C.-Y., Lin, W.-H., Chen, Y.-R., Chen, C.-T., Shiao, H.-Y., Rafi, S., Wang, S.-Y., Hsu, H., Chen, C.-H., Chang, C.-Y., Chang, T.-Y., Lien, T.-W., Fang, M.-Y., Yeh, K.-C., Chen, C.-P., Yeh, T.-K., Hsieh, S.-H., Hsu, J. T.-A., Liao, C.-C., Chao, Y.-S., Hsieh, H.-P., *J. Med. Chem.*, **2010**, *53*, 7316.

[6]Jennings, L. D., Kincaid, S. L., Wang, Y. D., Krishnamurthy, G., Beyer, C. F., McGinnis, J. P., Miranda, M., Discafani, C. M., Rabindran S. K., *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 4731.

[7]Romagnoli, R., Baraldi, P. G., Jung, M. K., Iaconinoto, M. A., Carrion, M. D., Remusat, V., Preti, D., Tabrizi, M. A., Francesca, F., Clercq, E. D., Balzarini, J., Hamel, E., *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 4048.

[8]Nakhi, A., Adepu, R., Rambabu, D., Kishore, R., Vanaja, G. R., Kalle, A. M., Pal, M., *Bioorg. Med. Chem. Lett.*, **2012**, *22*, 4418.

[9]Al-Said, M. S., Bashandy, M. S., Al-qasoumi, S. I., Ghorab, M. M., *Eur. J. Med. Chem.*, **2011**, *46*, 137.

[10]Shams, H. Z., Mohareb, R. M., Helal, M. H., Mahmoud, A. E., *Molecules*, **2011**, *16*, 52.

[11]Gupta, S. V., Baheti, K., Bora, R., Dekhane, D., Chhabría, M., Shingare, M., Pawar, S., Shishoo, C. J., Thore, S. N., *Eur. J. Med. Chem.*, **2009**, *44*, 4721.

[12]Ashalatha, B. V., Narayana, B., Raj, K. V., Kumari, N. S., *Eur. J. Med. Chem.*, **2007**, *42*, 719.

[13]Campaigne, E., *Compr. Heterocycl.. Chem.*, **1984**, *4*, 863.

[14]Bose, G., Bracht, K., Bednarski, P. J., Lalk, M., Langer, P., *Bioorg Med. Chem.*, **2006**, *14*, 4694.

[15]Behbehani, H., Ibrahim, H.M., Makhseed, S., Elnagdi, M. H., Mahmoud, H., *Eur. J. Med. Chem.*, **2012**, *52*, 51.

[16]Jennings, L. D., Kincaid, S. L., Wang, Y. D., Krishnamurthy, G., Beyer, C. F., McGinnis, J. P., Miranda, M., Discafani, C. M., Rabindran, S. K., *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 4731.

[17]Gewald, K., Schinke, E., Böttcher, H., *Chem. Ber.*, **1966**, *99*, 94.

[18]Gewald, K. Z., *Chem.*, **1962**, *2*, 305.

[19]Nikolakopoulos, G., Figler, H., Linden, J., Scammells, P. J., *Bioorg. Med. Chem.*, **2006**, *14*, 2358.

[20]Amr, A. E. E., Sherif, M. H., Assy, M. G., Al-Omar, M. A., Ragab, I., *Eur. J. Med. Chem.*, **2010**, *45*, 5935.

[21]Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenny, S., Boyd, M. R., *J. Natl. Cancer Inst.*, **1990**, *82*, 1107.

[22]Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paul, K., Vistica, D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A., Gray-Goodrich, M., Campbell, H., Mayo, J., Boyd, J. M., *J. Natl. Cancer Inst.*, **1991**, *83*, 757.

[23]Stauber, L. A., Franchino, E. N., Grun, J., *J. Protozool.*, **1958**, *5*, 269.