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Synthesis of Some New Arylazothiophene and Arylazopyrazole Derivatives as Antitumor Agents

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ABSTRACT

The starting 1-phenylbutane-1,3-dione (**1**) was used as key intermediate for the synthesis of several new thiophene and pyrazole derivatives. The newly synthesized compounds were evaluated for *in vitro* cytotoxicity against an Ehrlich ascites cells and *in vivo* cytotoxicity for compound **10d** using EAC assay and 5-fluorouracil is used as reference drug. Compounds **7c**, **e** and **10c**, **d** showed significant activity in certain cancer cell and have been targeted for further studies, compound **10d** is more effective and showed the highest activity. Structures of the newly prepared compounds were confirmed by both spectral, analytical data and molecular calculations.

Keywords: Benzoylacetone; Phenylisothiocyanate; Thiophene; Pyrazole; Thiocarbamoyl; Cytotoxic Agents

1. Introduction

Aryl isothiocyanates are versatile reagents which have been used as synthetic intermediate to prepare biologically active heterocyclic compounds [1]. As a part of our program of developing new, simple and efficient procedures for the synthesis of new aromatic compounds using readily available aryl isothiocyanates, we have recently affected recyclization of thiocarbamoyl into pyrazoles and thiazoles [2-5]. This procedure appears to be a fundamental type of type of thiocarbamoyl transformation into pyrazole ring.

In continuation of our studies on the chemistry of thiocarbamoyl and active methylene compounds [6,7] and as a part of our program directed toward developing new approaches to a variety of heterocycles exhibited antitumor activities [8,9], we report here the scope and applicability of 1-phenylbutane-1,3-dione (**1**) as a unique precursor for the synthesis of some new pyrazole, thiophene and thiocarbamoyl derivatives and their behavior towards different reagents.

2. Experimental

2.1. General

All melting points are in degree centigrade and were determined on Gallenkamp electric melting point apparatus. The IR spectra ν/cm^{-1} (KBr) were recorded on Perkin Elmer Infrared Spectrophotometer Model 157, Grating. The ^{1}H-NMR spectra were obtained on a Varian Spectrophotometer at 200 MHz using TMS as an internal reference and DMSO-d_6 as solvent. The mass spectra (EI) were recorded on 70 eV with Kratos MS equipment and/or a Varian MAT 311 A Spectrometer. Elemental analyses (C, H, and N) were carried out at the Micro Analytical Center of Cairo Univ., Giza, Egypt.

2.1.1. Synthesis of Thiocarbamoyl Derivative 3

To a cold suspension of potassium hydroxide (1.4 g, 25 mmol) in DMF (30 mL) was added the benzoyl acetone (4.05 g, 25 mmol), followed by phenyl isothiocyanate (3.375 g, 25 mmol). The mixture was stirred overnight at room temperature and then poured onto ice-cold water. Acidification using dilute HCl until the medium becomes acidic gave solid product **3** which was filtered off, washed with water, dried and crystallized from aqueous ethanol to give compound **3**.

Yield 100%; mp 140˚C; IR (KBr): ν/cm^{-1} = 3229, 3125, 1600, 1283 cm^{-1} (OH, NH, C=O, C=S); ^{1}H NMR (DMSO): δ_{ppm} = 2.5 (s, 3H, CH_3), 7.1 - 7.5 (m, 10H, Ar), 11.9 (s, 1H, NH); EIMS (*m/z*) (%) = 297 (M^+, 14), 255 (17), 222 (25). Anal. for $C_{17}H_{15}NO_2S$ (297.4): calcd.: C 68.66, H 5.08%; found: C 68.50, H 4.90%.

2.1.2. Coupling of 3 with Aromatic Diazonium Salts: Formation of Monoazothiocarbamoyl Derivatives 4a-e

General procedure: A well stirred solution of aromatic amines (20 mmol) in concentrated HCl (6 mL) and water

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(4 mL) was cooled in an ice bath and diazotized with a solution of sodium nitrite (1.39 g, 20 mmol) in water (5 mL).

The above cold diazonium solution was added drop wise to a well stirred cold solution of **3** in ethanol (10 mL) containing sodium acetate (1.75 g, 20 mmol). The reaction mixture was stirred for 1 - 2 h until reach complete coupling reaction. The crude product was filtered off, dried well and recrystallized from ethanol.

4a, Yield 80%; mp 140˚C; IR (KBr): ν/cm^{-1} = 3250, 1640, 1280 cm^{-1} (NH, C=O, C=S); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 6.8 - 7.8 (m, 15H, Ar), 13.9 and 16.4 (s, 2 H, 2NH); EIMS (*m/z*) (%) = 359 (M^+, 19), 239 (13), 161 (4.5), 105 (80), 77 (100). Anal. for $C_{21}H_{17}N_3OS$ (359.4): calcd.: C 70.17, H 4.77%; found: C 70.00, H 4.60%.

4b, Yield 88%; mp 174˚C; IR (KBr): ν/cm^{-1} = 3250, 2919, 1640, 1280 cm^{-1} (NH, CH_3, C=O, C=S); ^{1}H NMR (DMSO): δ_{ppm} = 2.3 (s, 3H, CH_3), 7.1 - 8.2 (m, 14H, Ar), 10.7 and 12.3 (s, 2H, 2NH); EIMS (*m/z*) (%) = 373 (M^+, 15), 239 (12), 105 (73), 77 (96). Anal. for $C_{22}H_{19}N_3OS$ (373.5): calcd.: C 70.75, H 5.13%; found: C 70.75, H 5.08%.

4c, Yield 85%; mp 172˚C; IR (KBr): ν/cm^{-1} = 3250, 2937, 1640, 1280 cm^{-1} (NH, CH_3, C=O, C=S); ^{1}H NMR (DMSO): δ_{ppm} = 4.0 (s, 3H, CH_3), 7.0 - 8.7 (m, 14H, Ar), 12.6 and 16.4 (s, 2H, 2NH); EIMS (*m/z*) (%) = 389 (M^+, 24), 239 (15), 161 (18), 105 (80), 77 (100). Anal. for $C_{22}H_{19}N_3O_2S$ (389.5): calcd.: C 67.84, H 4.92%; found: C 67.83, H 4.80%.

4d, Yield 78%; mp 218˚C; IR (KBr): ν/cm^{-1} = 3250, 1640, 1550, 1280 cm^{-1} (NH, C=O, NO_2, C=S); ^{1}H NMR (DMSO): δ_{ppm} = 6.8 - 7.9 (m, 14 H, Ar), 12.5 and 14.8 (s, 2 H, 2NH); EIMS (*m/z*) (%) = 404 (M^+, 18), 402 (7), 239 (30), 161 (10), 105 (100), 77 (97). Anal. for $C_{21}H_{16}N_4O_3S$ (404.4): calcd.: C 62.36, H 3.99%; found: C 62.30, H 3.99%.

4e, Yield 72%; mp 200˚C; IR (KBr): ν/cm^{-1} = 3250, 1640, 1280 cm^{-1} (NH, C=O, C=S); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 7.0 - 7.6 (m, 14H, Ar), 10.2 and 14.6 (s, 2H, 2NH); EIMS (*m/z*) (%) = 393 (M^+, 17), 239 (20), 125 (10), 105 (75), 77 (100). Anal. for $C_{21}H_{16}N_3OSCl$ (393.9): calcd.: C 64.03, H 4.09%; found: C 64.00, H 4.03%.

2.1.3. Synthesis of the Acyclic Intermediate 8a, 9a, 11a and 13a

Equimolecular quantities of **4a** (3.59 g, 10 mmol) in ethanol containing potassium carbonate (1.39 g, 10 mmol) and phenacyl bromide and/or ethyl bromoacetate and/or chloroacetonitrile and/or chloroacetone were stirred for 6 hrs at room temperature, then left to stand at the same temperature for 24 h. The separated solid product was washed with water, dried and crystallized from ethanol to give **8a**, **9a**, **11a** and **13a**, respectively.

8a, Yield 58%; mp 153˚C; IR (KBr): ν/cm^{-1} = 3150, (1640 and 1623), 1600 cm^{-1} (NH, two C=O, N=N); ^{1}H NMR (DMSO): δ_{ppm} = 4.2 (s, 2 H, CH_2), 6.8 - 7.9 (m, 20H, Ar), 10.5 (s, 1H, NH). Anal. for $C_{29}H_{23}N_3O_2S$ (477.5): calcd.: C 72.94, H 4.85%; found: C 72.94, H 4.79%.

9a, Yield 60%; mp 170˚C; IR (KBr): ν/cm^{-1} = 3150, (170 and 1625), 1600 cm^{-1} (NH, two C=O, N=N); ^{1}H NMR (DMSO): δ_{ppm} = 1.3 (t, 3H, CH_3), 3.7 (s, 2H, CH_2), 4.3 (q, 2H, CH_2), 6.8 - 7.7 (m, 15H, Ar), 12.9 (s, 1H, NH); EIMS (*m/z*) (%) = 445 (M^+, 10). Anal. for $C_{25}H_{23}N_3O_3S$ (445.5): calcd.: C 67.40, H 5.20%; found: C 67.30, H 5.00%.

11a, Yield 60%; mp 160˚C; IR (KBr): ν/cm^{-1} = 3200, 2220, 1695 cm^{-1} (NH, CN, C=O); ^{1}H NMR (DMSO): δ_{ppm} = 3.23 (s, 2H, CH_2), 7.5 - 8.0 (m, 15H, Ar), 14.2 (s, 1H, NH); EIMS (*m/z*) (%) = 398 (M^+, 90). Anal. for $C_{23}H_{18}N_4OS$ (398.48): calcd.: C 69.33, H 4.55%; found: C 69.33, H 4.55%.

13a, Yield 76%; mp 178˚C; IR (KBr): ν/cm^{-1} = 3250, 1660, 1600 cm^{-1} (NH, C=O, N=N); ^{1}H NMR (DMSO): δ_{ppm} = 2.5 (s, 3H, CH_3), 3.2 (s, 2H, CH_2), 7.5 - 8.0 (m, 15H, Ar), 14.5 (s, 1H, NH). Anal. for $C_{23}H_{19}N_3O_2S$ (401.49): calcd.: C 68.81, H 4.77%; found: C 68.80, H 4.76%.

2.1.4. Synthesis of Thiophene Derivatives

Pathway 1) A mixture of equimolecular amounts of **4a-e** and α-halo compounds (10 mmol) was stirred in DMF (20 mL) containing potassium carbonate (1.39 g, 10 mmol) overnight. The reaction mixture was poured onto ice-cold water, acidified by dilute HCl, filtered off and recrystallized from ethanol to give the corresponding thiophene derivatives.

Pathway 2) Refluxing the acyclic intermediate **8a**, **9a**, **11a** and **13a** in ethanol (20 mL) containing a catalytic amount of TEA for 3 h afforded the corresponding thiophene derivatives **7a**, **10a**, **12a** and **14a**.

Compounds **7a-e**, **10a-e**, **12a-e** and **14a-e** were obtained according to **pathway 1**.

7a, Yield 68%; mp 180˚C; IR (KBr): ν/cm^{-1} = 3150, 1660, 1600 cm^{-1} (NH, C=O, N=N); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 7.4 - 8.0 (m, 20H, Ar), 10.2 (s, 1H, NH); EIMS (*m/z*) (%) = 459 (M^+, 10), 428 (18), 105(20), 77 (80). Anal. for $C_{29}H_{21}N_3OS$ (459.6): calcd.: C 75.79, H 4.61%; found: C 75.79, H 4.59%.

7b, Yield 60%; mp 200˚C; IR (KBr): ν/cm^{-1} = 3321, 2919, 1660, 1602 cm^{-1} (NH, CH_3, C=O, N=N); ^{1}H NMR (DMSO): δ_{ppm} = 2.3 (s, 3H, CH_3), 7.0 - 7.8 (m, 19H, Ar), 14.2 (s, 1H, NH); EIMS (*m/z*) (%) = 473 (M^+, 70), 472 (18), 239 (20), 105 (25), 77 (80). Anal. for $C_{30}H_{23}N_3OS$ (473.6): calcd.: C 76.08, H 4.90%; found: C 76.00, H 4.80%.

7c, Yield 64%; mp 210˚C; IR (KBr): ν/cm^{-1} = 3344, 2923, 1684, 1598 cm^{-1} (NH, CH_3, C=O, N=N); ^{1}H NMR

(DMSO): δ_{ppm} = 3.9 (s, 3H, CH_3), 6.9-7.5 (m, 19H, Ar), 12.3 (s, 1H, NH); EIMS (*m/z*) (%) = 489 (M^+, 68), 458 (40), 368 (20), 105 (28), 77 (100). Anal. for $C_{30}H_{23}N_3O_2S$ (489.6): calcd.: C 73.60, H 4.74%; found: C 73.60, H 4.60%.

7d, Yield 74%; mp 226˚C; IR (KBr): ν/cm^{-1} = 3342, 1680, 1549, 1590 cm^{-1} (NH, C=O, NO_2, N=N); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 7.0 - 8.4 (m, 19H, Ar), 10.8 (s, 1H, NH); EIMS (*m/z*) (%) = 504 (M^+, 80), 501 (24), 456 (16), 105 (70), 77 (90). Anal. for $C_{29}H_{20}N_4O_3S$ (504.6): calcd.: C 69.30, H 4.00%; found: C 69.00, H 4.00%.

7e, Yield 55%; mp 160˚C; IR (KBr): ν/cm^{-1} = 3320, 1660, 1600 cm^{-1} (NH, C=O, N=N); ^{1}H NMR (DMSO): δ_{ppm} = (7.0 - 7.6) (m, 19H, Ar), 14.2 (s, 1H, NH); EIMS (*m/z*) (%) = 494 (M^+, 60), 492 (18), 105 (70). Anal. for $C_{29}H_{20}N_3OSCl$ (494): calcd.: C 70.51, H 4.08%; found: C 70.51, H 4.08%.

10a, Yield 55%; mp 180˚C; IR (KBr): ν/cm^{-1} = 3327, 1700, 1594 cm^{-1} (NH, C=O, N=N); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 1.1 (t, 3H, CH_3), 4.1 (q, 2H, CH_2), 7.0 - 7.6 (m, 15H, Ar), 14.3 (s, 1H, NH); EIMS (*m/z*) (%) = 427 (M^+, 80), 105 (70), 77 (90). Anal. for $C_{25}H_{21}N_3O_2S$ (427.5): calcd.: C 70.24, H 4.95%; found: C 70.19, H 4.95%.

10b, Yield 75%; mp 170˚C; IR (KBr): ν/cm^{-1} = 3427, 1734, 1580 cm^{-1} (NH, C=O, N=N); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 1.1 (t, 3H, CH_3), 2.3 (s, 3H, CH_3), 4.2 (q, 2H, CH_2), 7.0 - 7.6 (m, 14H, Ar), 14.1 (s, 1H, NH); EIMS (*m/z*) (%) = 441 (M^+, 50), 105 (M^+, 70). Anal. for $C_{26}H_{23}N_3O_2S$ (441.5): calcd.: C 70.72, H 5.25%; found: C 70.72, H 5.25%.

10c, Yield 62%; mp 140˚C; IR (KBr): ν/cm^{-1} = 3344, 1684, 1608 cm^{-1} (NH, C=O, N=N); ^{1}H NMR (DMSO): δ_{ppm} = 1.3 (t, 3H, CH_3), 3.9 (s, 3H, CH_3), 4.2 (q, 2H, CH_2), 7.0 - 7.6 (m, 14H, Ar), 14.2 (s, 1H, NH); EIMS (*m/z*) (%) = 457 (M^+, 10), 426 (12), 105 (70). Anal. for $C_{26}H_{23}N_3O_3S$ (457.5): calcd.: C 68.25, H 5.07%; found: C 68.25, H 5.07%.

10d, Yield 70%; mp 190˚C; IR (KBr): ν/cm^{-1} = 3345, 1718, 1540, 1594 cm^{-1} (NH, C=O, NO_2, N=N); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 1.1 (t, 3H, CH_3), 4.1 (q, 2H, CH_2), 7.3 - 8.4 (m, 14H, Ar), 14.9 (s, 1H, NH); EIMS (*m/z*) (%) = 472 (M^+, 10), 426 (24), 105 (77), 77 (89). Anal. for $C_{25}H_{20}N_4O_4S$ (472.5): calcd.: C 63.55, H 4.27%; found: C 63.55, H 4.00%.

10e, Yield 57%; mp 180˚C; IR (KBr): ν/cm^{-1} = 3316, 1715, 1690 cm^{-1} (NH, C=O, N=N); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 1.1 (t, 3H, CH_3), 4.1 (q, 2H, CH_2), 7.2 - 7.6 (m, 14H, Ar), 14.1 (s, 1H, NH); EIMS (*m/z*) (%) = 462 (M^+, 80). Anal. for $C_{25}H_{20}N_3O_2SCl$ (462): calcd.: C 65.00, H 4.36%; found: C 65.00, H 4.30%.

12a, Yield 52%; mp 204˚C; IR (KBr): ν/cm^{-1} = 3327, 2196, 1595 (NH, CN, N=N); ^{1}H NMR (DMSO): δ_{ppm} = 7.31 - 7.56 (m, 15H, Ar), 14.2 (s, 1H, NH); EIMS (*m/z*) (%) = 380 (M^+, 86), 365 (15), 105 (77), 77 (100). Anal. for $C_{23}H_{16}N_4S$ (380.5): calcd.: C 72.61, H 4.24%; found: C 72.61, H 4.00%.

12b, Yield 58%; mp 176˚C; IR (KBr): ν/cm^{-1} = 3324, 2199 cm^{-1} (NH, CN); ^{1}H NMR (DMSO): δ_{ppm} = 2.3 (s, 3H, CH_3), 7.3 - 7.6 (m, 14H, Ar), 13.8 (s, 1H, NH); EIMS (*m/z*) (%) = 394 (M^+, 88), 379(18), 105 (78). Anal. for $C_{24}H_{18}N_4S$ (394.3): calcd.: C 73.07, H 4.60%; found: C 73.00, H 4.50%.

12c, Yield 53%; mp 184˚C; IR (KBr): ν/cm^{-1} = 3345, 2201cm^{-1} (NH, CN); ^{1}H NMR (DMSO): δ_{ppm} = 4.2 (s, 3H, CH_3), 7.2 - 8.0 (m, 14H, Ar), 14.2 (s, 1H, NH); EIMS (*m/z*) (%) = 410 (M^+, 88), 381 (70), 105 (70). Anal. for $C_{24}H_{18}N_4OS$ (410.5): calcd.: C 70.22, H 4.42%; found: C 70.00, H 4.00%.

12d, Yield 57%; mp 210˚C; IR (KBr): ν/cm^{-1} = 3319, 2202, 1593, 1531 cm^{-1} (NH, CN, N=N, NO_2); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 7.3 - 8.0 (m, 14H, Ar), 14.3 (s, 1H, NH); EIMS (*m/z*) (%) = 425 (M^+, 100), 379 (24), 105 (70). Anal. for $C_{23}H_{15}N_5O_2S$ (425.5): calcd.: C 64.93, H 3.55%; found: C 64.80, H 3.40%.

12e, Yield 83%; mp 190˚C; IR (KBr): ν/cm^{-1} = 3420, 2198, 1568 cm^{-1} (NH, CN, N=N); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 7.2 - 8.5 (m, 14H, Ar), 14.3 (s, 1H, NH); EIMS (*m/z*) (%) = 414 (M^+, 68). Anal. for $C_{23}H_{15}N_4SCl$ (414.9): calcd.: C 66.58, H 3.55%; found: C 66.58, H 3.50%.

14a, Yield 74%; mp 140˚C; IR (KBr): ν/cm^{-1} = 3337, 1645, 1597 cm^{-1} (NH, C=O, N=N); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 2.1 (s, 3H, CH_3), 7.3 - 7.8 (m, 15H, Ar), 14.2 (s, 1H, NH); EIMS (*m/z*) (%) = 397 (M^+, 78). Anal. for $C_{24}H_{19}N_3OS$ (397.5): calcd.: C 72.52, H 4.82%; found: C 72.40, H 4.70%.

14b, Yield 50.5%; mp 140˚C; IR (KBr): ν/cm^{-1} = 3345, 1645, 1598 (NH, C=O, N=N); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 1.9 (s, 3H, CH_3), 2.3 (s, 3H, CH_3), 7.1 - 7.6 (m, 14H, Ar), 14.1 (s, 1H, NH); EIMS (*m/z*) (%) = 411 (M^+, 15). Anal. for $C_{25}H_{21}N_3OS$ (411.5): calcd.: C 72.97, H 5.14%; found: C 72.97, H 5.00%.

14c, Yield 63%; mp 192˚C; IR (KBr): ν/cm^{-1} = 3345, 1643, 1600 cm^{-1} (NH, C=O, N=N); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 1.9 (s, 3H, CH_3), 4.2 (s, 3H, CH_3), 7.2 - 8.0 (m, 14H, Ar), 14.1 (s, 1H, NH); EIMS (*m/z*) (%) = 427 (M^+, 25). Anal. for $C_{25}H_{21}N_3O_2S$ (427.5): calcd.: C 70.24, H 4.95%; found: C 70.24, H 4.95%.

14d, Yield 86%; mp 160˚C; IR (KBr): ν/cm^{-1} = 3445, 1645, 1515 cm^{-1} (NH, C=O, NO_2); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 1.9 (s, 3H, CH_3), 7.3 - 7.58 (m, 14H, Ar), 14.7 (s, 1H, NH); EIMS (*m/z*) (%) = 442 (M^+, 12). Anal. for $C_{24}H_{18}N_4O_3S$ (442.5): calcd.: C 65.14, H 4.10%; found: C 65.14, H 4.00%.

14e, Yield 69.5%; mp 140˚C; IR (KBr): ν/cm^{-1} = 3321, 1622, 1594 cm^{-1}(NH, C=O, N=N); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 1.9 (s, 3H, CH_3), 7.2 - 7.6 (m, 14H, Ar), 13.85 (s, 1H, NH); EIMS (*m/z*) (%) = 431 (M^+, 78). Anal. for $C_{24}H_{18}N_3OSCl$ (431.9): calcd.: C 66.74, H 4.20%; found:

C 66.74, H 4.00%.

2.1.5. 1-Phenylamino-1-hydrazone-2-(arylazo)-2-benzoylethylene (15a)

A mixture of **4a** (3.55 g, 50 mmol) and hydrazine hydrate (1.6 g, 50 mmol) in ethanol (20 mL) was stirred for 4 h at room temperature. The reaction mixture was then cooled and the solid product was filtered off and recrystallized from ethanol to give compound **15a**.

Yield 65%; mp 168˚C; IR (KBr): ν/cm^{-1} = 3550, 3340, 1640 cm^{-1} (NH_2, NH, C=O); ^{1}H NMR (DMSO): δ_{ppm} = 6.4 (s, 2H, NH_2), 7.2 - 7.8 (m, 15H, Ar), 12.7 and 14.0 (s, 2H, 2NH). Anal. for $C_{21}H_{19}N_5O$ (357.4): calcd.: C 70.57, H 5.35%; found: C 70.50, H 5.30%.

2.1.6. Synthesis of Arylazopyrazoles 16a-e

Method A: A mixture of **4a-e** (50 mmol), hydrazine hydrate (1.6 g, 50 mmol) in DMF was refluxed for 4 h. The reaction mixture then poured onto ice water (200 mL). The solid product was filtered off and recrystallized from ethanol-DMF (1:1) to give the corresponding derivatives **16a-e**, respectively.

Method B: Refluxing the acyclic intermediate **15a** in ethanol (20 mL) containing a catalytic amount of TEA for 3 h gave the pyrazole derivative **16a**.

16a, Yield 48%; mp 256˚C; IR (KBr): ν/cm^{-1} = 3235 and 3192, 1595 cm^{-1} (two NH, N=N); ^{1}H NMR (DMSO): δ_{ppm} = 7.3 - 8.0 (m, 15H, Ar), 10.0 and 12.0 (s, 2H, 2NH); EIMS (*m/z*) (%) = 339 (M^+, 12). Anal. for $C_{21}H_{17}N_5S$ (339.4): calcd.: C 74.32, H 5.05%; found: C 74.32, H 5.00%.

16b, Yield 38%; mp 228˚C; IR (KBr): ν/cm^{-1} = 3229 and 3189, 1597 cm^{-1} (two NH, N=N); ^{1}H NMR (DMSO): δ_{ppm} = 2.3 (s, 3H, CH_3), 7.0 - 8.2 (m, 14H, Ar), 10.1 and 12.0 (s, 2H, 2NH); EIMS (*m/z*) (%) = 353 (M^+, 21). Anal. for $C_{22}H_{19}N_5$ (353.4): calcd.: C 74.77, H 5.42%; found: C 74.77, H 5.42%.

16c, Yield 40%; mp 210˚C; IR (KBr): ν/cm^{-1} = 3230 and 3195, 1597 (two NH, N=N); ^{1}H NMR ($CDCl_3$): δ_{ppm} = 4.1 (s, 3H, CH_3), 7.0 - 7.7 (m, 14H, Ar), 10.2, 13.1 (s, 2H, 2NH). Anal. for $C_{22}H_{19}N_5O$ (369.4): calcd.: C 71.53, H 5.18%; found: C 71.53, H 5.00%.

16d, Yield 43%; mp 200˚C; IR (KBr): ν/cm^{-1} = 3343 and 3231, 1597 cm^{-1} (two NH, N=N); ^{1}H NMR (DMSO): δ_{ppm} = 7.3 - 7.8 (m, 14H, Ar), 13.8 and 14.2 for two (s, 1H, NH); EIMS (*m/z*) (%) = 384 (M^+, 24). Anal. for $C_{21}H_{16}N_6O_2$ (384.4): calcd.: C 63.62, H 4.20%; found: C 63.62, H 4.20%.

16e, Yield 33%; mp 230˚C; IR (KBr): ν/cm^{-1} = 3187 and 3117, 1594 cm^{-1} (two NH, N=N); ^{1}H NMR (DMSO): δ_{ppm} = 7.3 - 8.0 (m, 14H, Ar), 13.7 and 13.9 (s, 2H, 2NH); EIMS (*m/z*) (%) = 373 (M^+, 37). Anal. for $C_{21}H_{16}N_5Cl$ (373.8): calcd.: C 67.47, H 4.31%; found: C 67.47, H 4.31%.

2.2. Antitumor Activity

Cells of Ehrlich ascites tumor were obtained from National Cancer Institute, Cairo, Egypt. Animals, adult Swiss male albino mice (20 - 25 g) were procured from Pharmacology Faculty, Mansoura University, Egypt and used throughout this study. They were housed in microlon boxes in a controlled environment (temperature 25˚C + 2˚C and 12 h dark/light cycle) with standard laboratory diet and water ad libitum (5-Fluorouracil → 20 mg/kg).

2.2.1. Animals Were Divided into Seven Groups as Follows

All animals were inoculated with 2 × 10^6 cells/mouse on day "0" except normal group and treatment started 24 h after inoculation, at 2 doses of 50 and 250 mg/kg/day, i.p. The control group was treated with the same volume of 0.9% sodium chloride solution. All the treatments were given for nine days.

2.2.2. Tumor Reducing Activity

Ehrlich ascites tumor cells (0.2 mL) were injected into the mice peritoneal cavity and different concentrations (1 mg/mL to 0.1 mL/mice on day) of the drug (5 mice/group) were injected from day-1 to day-9 every day. Animals were observed for the development as ascites tumor and death due to tumor burdon. Life span % I LS = (T-C)/C × 100, where T is the number of days the treated animals survived and C is the number of days control animals survived. % I LS more than 25% was considered as significant [10].

3. Results and Discussion

3.1. Chemistry

We have been particularly interested in studying if reactions of such thiocarbamoyl might be extended to include more general synthesis of other classes of organic compounds and its utility as synthetic intermediate for the synthesis of new heterocyclic compounds. The present work reports on the synthesis of several new arylazothiophene and arylazopyrazole derivatives by the reaction of thiocarbamoyl of the type **4** with compounds containing an active methylene group in the presence of a base. Reactions of this type have not been reported previously, but they were found to give products in excellent yields under very mild conditions. Moreover, the resulting thiophene and pyrazole derivatives have latent functional substituents which have potential for further chemical transformations and new routes for the preparation of substituted thiophene and pyrazole derivatives with possible biological activity. Now, we have extended our synthetic program to the synthesis of otherwise inaccessible heterocyclic ring system utilizing phenyl isothiocyanate as a key starting material. It is known that a great variety

of reactants bearing the N=C=S fragment undergoes cyclization on reaction with α-halocarbonyl compounds to afford thiazoles, 2,3-dihydrothiazoles [11], which have been shown to exhibit antiprotozoal [12], and fungicidal properties [13].

Thus, the base-prompted reaction of the acidic methylene compound **1** with phenyl isothiocyanate in dry DMF at room temperature in basic medium led to the formation of the non-isolable intermediate **2** which gave thiocarbamoyl derivative **3** upon treatment with dilute HCl. Treatment of **3** with aromatic diazonium salts in the presence of sodium acetate affected acetyl cleavage with the formation of arylazothiocarbamoyl derivatives **4a-e** rather than the expected product **5**. This result is in agreement with the previously reported work [14-18], (**Scheme 1**).

Compound **3** was obtained according to the proposed following mechanism (**Figure 1**):

Assignment of the product **3** was based on elemental analysis, IR and ^{1}H-NMR spectral data. ^{1}H NMR spectrum of **3** displayed multiplet signals at δ 7.1 - 7.5 ppm for aromatic protons and exchangeable proton at 11.9 ppm for NH proton. On the other hand, the structure of the newly prepared hydrazone derivatives **4a-e** was based on their correct elemental analysis and spectral data. The ^{1}H NMR spectrum of compound **4a** displayed multiplet signals at δ 6.8 - 7.8 ppm for aromatic protons and two exchangeable protons at δ 13.9 and 16.4 ppm for two NH protons. Compounds **4a-e** were obtained according to the proposed following mechanism (**Figure 2**):

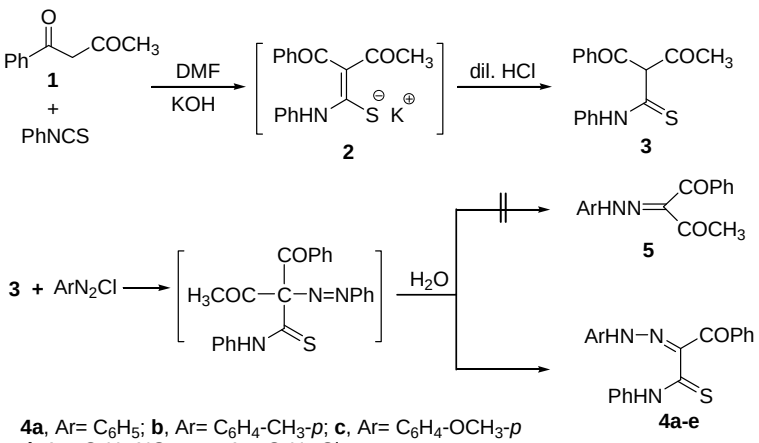
In this paper, we describe a generally applicable extension of this synthetic approach, first reported by Hantzsch and Weber [19]. Thus, the base-prompted reaction of compounds **4a** with potassium carbonate in dry DMF at room temperature affords the non-isolable intermediate **6a**. Stirring of **6** with phenacyl bromide in DMF overnight yielded a product **7a**, which analyzed correctly for $C_{29}H_{21}N_3OS$. The structure **7a** was inferred from its spectral data. Its ^{1}H NMR spectrum showed two multiplet signals integrated for (20H) centered at 7.4 and 8.0 (aromatic protons) and a singlet (1H) at δ 10.2. On shaking the compound with D_2O, the broad band signal at δ 10.2 disappeared. Based on the foregoing data, structure **7a** was assigned to this product. The structure **7a** was further confirmed by alternative synthesis. Thus, it was found that, stirring of **4a** with phenacyl bromide in the presence of potassium carbonate in ethanol at room temperature produced acyclic intermediate **8a**. Structure **8a** was suggested for the reaction product on the basis of both elemental and spectral analyses (*c.f.* Experimental part).

Refluxing **8a** in ethanol with few drops of TEA led to the formation of a product identical in all respects (m.p. mixed m.p., IR) to **7a**. Similarly, compounds **7b-e** were synthesized by **pathway** (**1**), (**Scheme 2**).

The addition of two or more equivalents of ethyl bromoacetate, chloroacetonitrile, chloroacetone leads only to thiophenes **10**, **12**, and **14** in good yields. Thus, condensation of the intermediate salt with an equimolar amount of chloroacetyl chloride or with chloroacetic acid in ethanol, a product that analyzed for $C_{25}H_{23}N_3O_3S$ was isolated in each case in good yield. The acyclic structure **9a** was established on the basis of its IR spectrum that showed bands related to NH and CO functions (*c.f.* Experimental part).

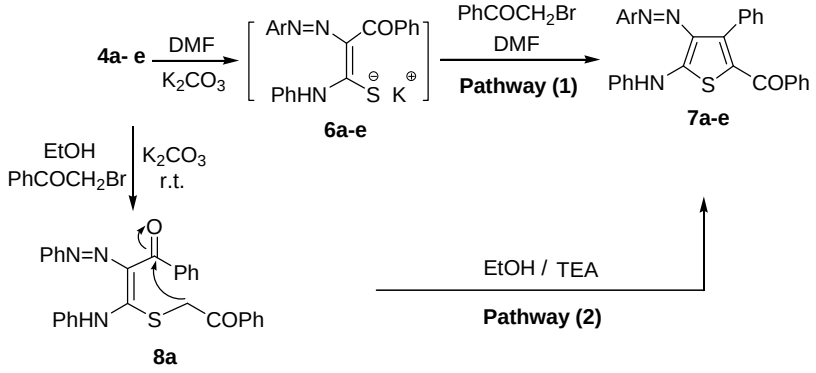
Alternatively, treatment of the intermediate **6a** with ethyl bromoacetate in ethanol gives a single product, which is identical in all respects to **9a** (m.p. mixed m.p. and IR spectrum) (*c.f.* Experimental part). Refluxing of **9a** in ethanol with a catalytic amount of TEA or leaving it in DMF containing potassium carbonate at room temperature overnight afforded the corresponding thiophene derivative **10a**. In a similar manner, compounds **10a-e** were prepared by **pathway 1)**.

Similarly, when the intermediate potassium salt **6a** is stirred with chloroacetonitrile in ethanol at room tem-



4a, Ar= C_6H_5; **b**, Ar= C_6H_4-CH_3-*p*; **c**, Ar= C_6H_4-OCH_3-*p*
d, Ar= C_6H_4-NO_2-*p*; **e**, Ar= C_6H_4-Cl-*p*

Scheme 1. Synthesis of thiocarbamoyl 3 and hydrazone derivatives 4a-e.



Scheme 2. Synthesis of thiophene derivatives 7a-e.

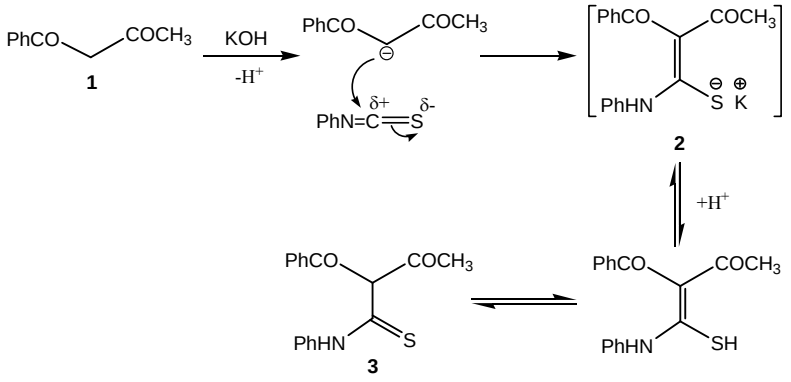


Figure 1. The proposed mechanism of formation of thiocarbamoyl derivative 3.

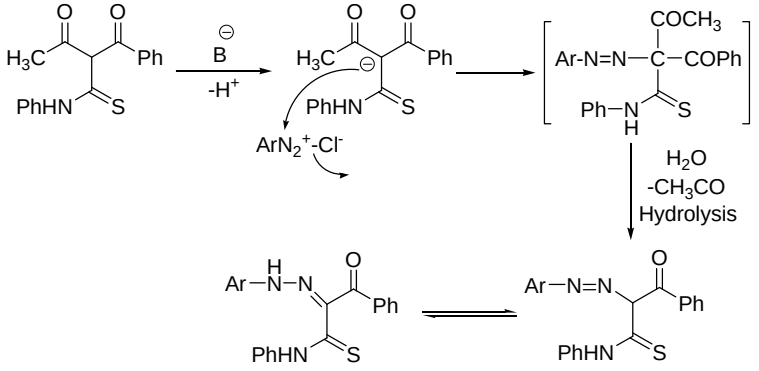
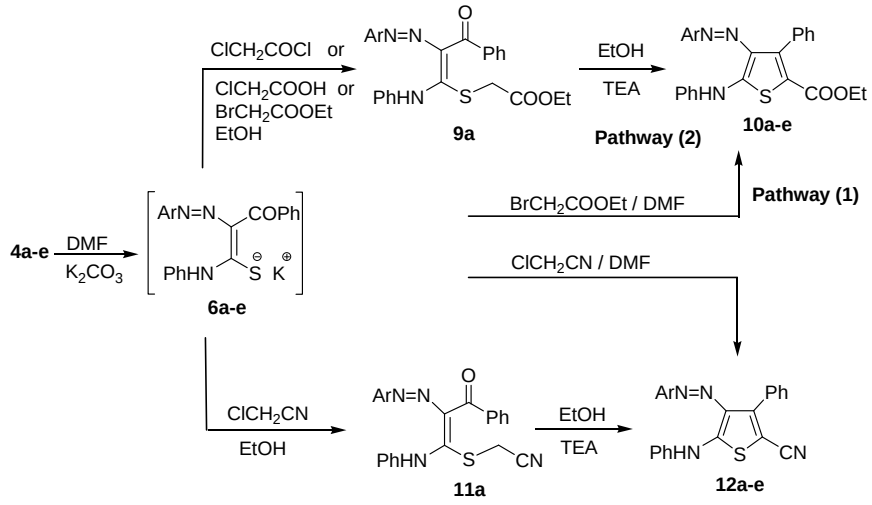


Figure 2. The proposed mechanism of formation of hydrazone derivatives 4a-e.

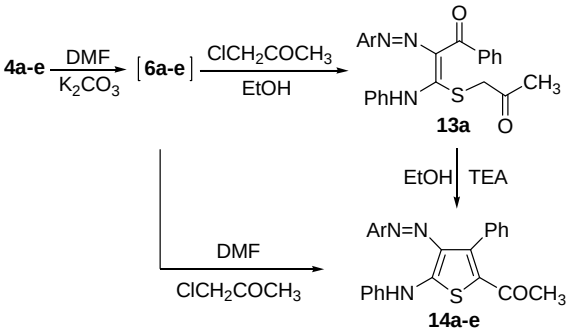
perature, the corresponding acyclic intermediate **11a** is exclusively isolated in good yield. The structure **11** has been confirmed on the basis of elemental and spectral data (*c.f.* Experimental part). Furthermore, heating of the intermediate **11a** in ethanol containing a catalytic amount of TEA affords the thiophene derivative **12a**. The thiophene structure **12a** was established on the basis of its IR spectrum which showed bands related to NH and CN functions. Its ^{1}H NMR spectrum reveals broad signals at δ 14.2 ppm (1H, NH). On the other hand, it has been found that **12a-e** are directly formed by treatment of **6a-e** with chloroacetonitrile in dimethylformamide and in the presence of potassium carbonate at room temperature overnight by **pathway (1)**, (**Scheme 3**).

Compound **6a** reacted readily with chloroacetone in the presence of ethanol at room temperature to afford the acyclic intermediate **13a** by (KCl) elimination. Refluxing **13** in ethanol with a catalytic amount of TEA gave the thiophene derivative **14a** whose structure was confirmed by its alternative synthesis. Thus, stirring **6a-e** with chloroacetone in DMF overnight affords the thiophene derivative **14a-e** in reasonably good yield, (**Scheme 4**).

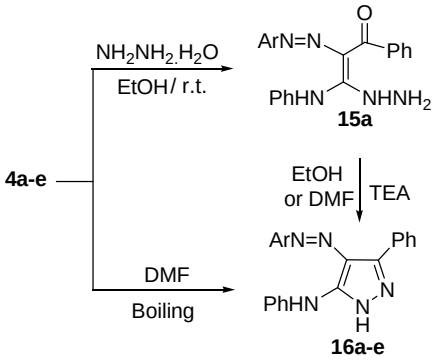
On the other hand, treatment of the key intermediate **4b** with hydrazine hydrate in DMF gave a single product



Scheme 3. Synthesis of thiophene derivatives 10a-e and 12a-e.



Scheme 4. Synthesis of thiophene derivatives 14a-e.



8, **9**, **11**, **13**, **15a**, Ar= Ph

7, **10**, **12**, **14**, **16a**, Ar= C_6H_5; **b**, Ar= C_6H_4-CH_3-*p*; **c**, Ar= C_6H_4-OCH_3-*p*; **d**, Ar= C_6H_4-NO_2-*p*; **e**, Ar= C_6H_4-Cl-*p*

Scheme 5. Synthesis of arylazopyrazole derivatives 16a-e.

which analyzed correctly for $C_{22}H_{19}N_5$ (**16b**). The structure of **16b** was inferred from its spectral data. The ^{1}H NMR spectrum revealed a singlet at δ 2.3 assigned for the methyl protons, a broad band located at δ 10.1 and 12.0 assignable to the NH protons, and a multiplet at δ 7.0 - 8.2, assigned for aromatic protons. The formation of **16a-e** is assumed to proceed *via* the replacement of the SH group by the hydrazine moiety to give the intermediate **15** which then cyclized *via* the carbonyl group to afford the final isolable products **16a-e**. In fact, the structure of **16** was further confirmed by alternative synthesis. Thus, it has been found that treatment of **4a** with hydrazine hydrate for long time in ethanol produced the intermediate **15a**. Refluxing **15a** in ethanol containing a catalytic amount of TEA or in DMF lead to the formation of a product identical in all respects (m.p., mixed m.p., IR) with **16a** [20]. Structure **15a** is suggested for the reaction product on the basis of both elemental and spectral analyses. The ^{1}H NMR spectrum revealed a broad signal at δ 6.4 assigned for NH_2 protons, a multiplet at δ 7.2 - 7.8 for aromatic protons, and a singlet at δ 12.7 and 14.0 ppm for NH protons, (**Scheme 5**).

These results indicate that the reaction of thiocarbamoyls and bifunctional reagents provides an excellent route for the synthesis of five, otherwise not easily accessible arylazothiophenes and pyrazoles. The compounds synthesized will be subjected to biological testing.

3.2. Biological Activity

3.2.1. Discussion

3.2.1.1. Effect of Drugs on the Viability of Ehrlich Ascites Cells *in Vitro*

To examine whether these substances have a direct cytotoxic effect on Ehrlich ascites cells (EAC) viability, the percentage of viable cells was estimated by the trypan blue [21], exclusion test. The desired concentration of tumor cells (2×10^6 cells per 0.2 mL) was obtained by dilution with saline (0.9% sodium chloride solution). Viability of tumor cells obtained and used in this experiment was always higher than 90%. Below this percentage, the cells were discarded and the entire procedure was

repeated. Twenty five sulfur containing compounds were tested for cytotoxicity against EAC *in vitro*. Results for the ($ED_{50\times10^3}$), ($ED_{25\times10^3}$) and ($ED_{10\times10^3}$) values of the active compounds are summarized in **Table 1**. Compounds **4a**, **c**, **7c** and **10c** displayed moderate cytotoxicity, whereas **7e** and **10d** were the most potent with ($ED_{25\times10^3}$). The other rest of compounds showed weak to no activity. Thus, it would appear that introducing thiophene moiety enhances the cytotoxic properties. By comparing the cytotoxicity results in **Table 1**, the following structure-activity relationships (SARs) were drawn: 1) Converting the thiocarbamoyl moiety to the corresponding thiophene (**7c**, **e** and **10c**, **d**) led to increase cytotoxicity against EAC. Compounds **7e** and **10d** were the most active, whereas, compounds **7c** and **10c** exhibited good activity. Thus, the ring substituents affected the activity in the thiophene derivatives; 2) The most of compounds containing electronedonating groups were very weak or completely inactive at ($ED_{25\times10^3}$) than the compounds containing electronewithdrawing groups. Thus, the position and nature of substituents on the structure of thiophene derivatives seem to modulate antitumor activity. The reliable criteria for judging the value of any anticancer drug are prolongation of life span and decrease of WBC from blood [8,9]. The results of the present study showed an antitumor effect of the compounds against EAC in Swiss albino mice (**Table 2**).

3.2.1.2. Effect of Compound **10d** on Survival Time [22]

The mean survival time (MST) of each group consisting of seven mice was noted. The antitumor efficacy was compared with that of 5-fluorouracil (5-FU), for 9 days. The MST of the treated groups was compared with that of the control group using the following calculation: % Increase in life span over control = (MST of treated group-MST of control group) × 100 - 100, where MST= survival time (days of each mouse in a group)/Total no. of mice (**Table 2**).

3.2.1.3. Effect of the Sulfur Containing Compounds on Hematological Parameters

To examine the influence of the tested compounds on the hematological status of EAC-bearing mice, a comparison was made among the following groups (n = 7) of mice on the 14th day after inoculation. The groups comprised of 1) tumor-bearing mice; 2) tumor-bearing mice treated for the first 9 days; and 3) control mice (normal). Blood was drawn from each mouse by the retro orbital plexus method and the white blood cell count (WBC), red blood cell count (RBC) and hemoglobin were determined. From each group take 100 µM sample of Ehrlich ascites cells (from three mice) and make 20 fold dilutions in saline. The cells were stained by Giemsa stain and measure the number of viable cells under microscope. The

Table 1. Effect of sulfur containing compounds on the viability of Ehrlich ascites cells (EAC) *in vitro* assay.

Compound	Dead %		
	$ED_{50\times10^3}$ µM	$ED_{25\times10^3}$ µM	$ED_{10\times10^3}$ µM
5-flu	96.3%	0%	0%
4a	100%	9.1%	0%
4b	100%	0%	0%
4c	100%	9.1%	3.2%
4d	100%	4.5%	0%
4e	100%	0%	0%
7a	100%	0%	10%
7b	100%	0%	0%
7c	100%	11.8%	0%
7d	100%	0%	0%
7e	100%	53.8%	3.3%
10a	100%	0%	0%
10b	100%	0%	0%
10c	100%	21%	0%
10d	100%	53.8%	3.8%
10e	68.4%	4%	0%
12a	100%	0%	0%
12b	100%	8%	0%
12c	100%	0%	0%
12d	100%	0%	0%
12e	100%	3.6%	0%
14a	94.4%	0%	0%
14b	100%	0%	0%
14c	100%	0%	0%
14d	100%	4.3%	0%
14e	100%	0%	0%

Where, ED is the concentration of compounds in µM.

Table 2. *In vivo* cytotoxicity of 10d using EAC assay.

Test Group	Hb 12 - 16 g/dl	HCT 35% - 50% (hematocrit)	WBCs 4000 - 11,000 /cmm	Ehrlish cells count mil/ml	MST/day
*Normal	13.9	53.6	8.4	-	-
**Control	8.7	35.5	38.6	220.0	9.1
5-FU	10.0	42.3	13.8	123.0	16.7
Compound (10d)	9.0	39.7	26.0	172.8	12.8

*Normal test: without EAC; **Control test: with EAC.

viable cells those, which did not take up the stain, where the dead cells (stained cells).

From **Table 2**; it was noticed that, 1) in the 5th day after inoculation of Ehrlich cells in mice, increase in body weight and ascites was observed clearly, also the mice became slow and inactive; 2) Mice received compound **10d** and 5-FU were more protected against ascites and increase in weight than control mice; 3) Mice received compound **10d** showed slight toxic symptoms such as dizziness, dirty look; erection of tail, slow movement. Also the liver of animals was pale and slightly enlarged; 4) Percentage increase of life span over control showed to be high in mice treated with total extracts; 5) Mice that received compound **10d** showed comparable results to 5-FU; 6) Hematological parameters of tumor bearing mice on Day 14 showed significant changes when compared with normal mice; 7) Total WBC count was found to increase with a reduction in the hemoglobin content and increase of RBCs; 8) Compound **10d** and 5-FU showed minimal ascites and slight increase in body weight unlike control group.

4. Conclusion

Modification of thiocarbamoyl produced thiophene compounds with potential for further development as anticancer agents. Based on these preliminary screening results, compounds **7c**, **e** and **10c**, **d** showed significant activity in certain cancer cell and have been targeted for further studies, compound **10d** is more effective and showed the highest activity. Additional research, including mode of action studies, is planned to accurately establish relative activity for (SAR) and rational design. Recently, the cancer chemopreventive effects of thiophene have been intensively investigated. Thiophene exhibited pronounced antitumor activity by triggering apoptosis inhuman tumor cells [23]. Studies are underway to investigate the apoptosis inducing activity of compounds found to be cytotoxic in this study.

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