25 *Egypt. J. Chem.* **53**, No. 3, pp. 385- 401 (2010)

# Synthesis of New Bithiophene and Thieno [2,3-*d*] Pyrimidine Derivatives with Potent Antimicrobial Activity

**Hanaa Hosni[#], K. Ali* and E. Mustafa****
*Pesticides Chemistry Department, *Applied Organic Chemistry Department and **Microbial Chemistry Department, National Research Centre, Giza, Egypt.*

THE VERSATILE synthon 2-Amino-4-(thiophene-2-yl) thiophene-3-carbonitrile (1) was used as precursor for the synthesis of a series of bithiophene (2) and thieno [2, 3-*d*] pyrimidine (3) derivatives. The antimicrobial evaluations of the newly synthesized compounds were carried out in vitro assays for antifungal and antibacterial activities. Amongst the tested compounds, 1-(4\-cyano-2,3\-bithiophen-5\-yl)3-phenylthiourea (2b), 4-imino-3-phenyl-5-(thiophenyl-2-yl)-3,4-dihydrothieno[2,3-*d*] pyrimidine-2(1*H*)-thione (3b) and 1-phenyl-3-(5-(thiophenyl-2-yl)thieno[2,3-*d*]pyrimidin-4-yl) thiourea (5c) exhibit significant antibacterial activities against *Pseudomonas sp*, *Escherichia coli*, *Streptococcus lactis*, *Bacillus subtilis* and exhibit significant antifungal activities against *Aspergillus niger*, *Penicillium sp, Candida albicans* and *Rhodotorula ingeniosa.* From structure-activity relationship (SAR) point of view, the attachment of phenyl thioureido moiety to bithiophenes can be considered as a breakthrough in developing a new therapeutic antimicrobial agents related to bithiophene system.

**Keywords:** Phenylthiourea, Bithiophen, Heterocycles, Antimicrobial agents, Structure elucidation and Thieno [2,3-*d*] pyrimidine

Attention, which has led to the development of different synthetic procedures in the pharmaceutical industry. The amino thiophene derivatives belong to an important structural class, which is used toward the preparation of other highly functional derivatives, such as heterocyclic containing systems[1-8]. Also, Ureido and thioureido compounds are well known for their wide biological activities[9]. However, the derivatives of thieno-thiophene were developed for different purposes in the pharmaceutical field and have been tested as potential antitumor[10], antiviral[11] and antibiotic[12]. Also the derivatives of fused thiophene and pyrimidines are valued not only for their rich and varied chemistry, but also for many important biological properties[13]. Among them, the thieno pyrimidine derivatives have interesting biological applications, for example, some alkyl substituted thienopyrimidines show significant anticancer, antifungal and antibacterial activities[14-18]. From the previous reports, and as part of our ongoing research program aiming at the synthesis of variety organic compounds that of

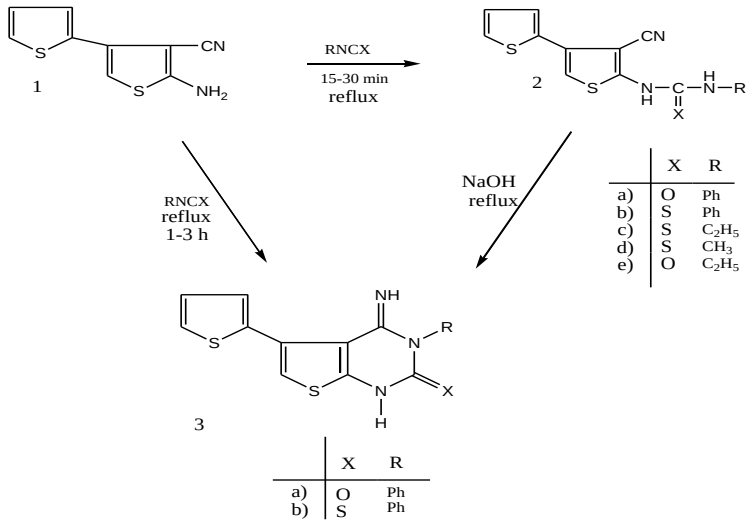
[#]Correspondence author E-mail: hanaanrc@yahoo.com

heterocyclic systems for biological and pharmacological evaluation[19-23], we report here the synthesis of organic compounds that are of biological interest starting from 2-amino-4(thiophene-2-yl) thiophene-3-carbonitrile 1 to increase the selectivity of the new compounds towards bacteria and fungi.

## Results and Discussion

*Chemistry*

Treatment of 2-amino-4-(thiophene-2-yl)-thiophene-3-carbonitrile (1) with isocyanate or isothiocyanate derivatives, in pyridine for 15-30 min, afforded the corresponding open structures of 1-(4\-cyano-2,3\-bithiophen-5\)-3-(substituted) urea or thiourea derivatives (2a-e). It was of interest that, when compound 1 was treated with ethyl isocyanate or phenyl isothiocyanate under the same reaction conditions but for longer time(3 hr), it afforded the corresponding cyclic structures of 4-imino-3-phenyl-5-(thiophenyl-2-yl)-3,4-dihydrothieno [2,3-*d*] pyrimidine-2(1*H*)-one and thione (3a,b), respectively. Compounds 3a, b could also be obtained from gentile boiling of compounds 2a, b in 1 % sodium hydroxide solution (Scheme1). The structures of products 2a-e and 3a, b were confirmed by their elemental analysis and spectral data. For example, the IR spectrum of compound 2a showed two absorption bands at 1660 and 2208 $cm^{-1}$ assignable to amide carbonyl and cyano groups, respectively. Other important bands appeared at 3331 and 3282 $cm^{-1}$ characterized for two amides NH. The $^1H$ NMR spectrum of compound 2a showed signals at δ 7.31-8.10, 8.50 and 9.70 due to 5 aromatic, 4 thiophene and 2NH protons, respectively. The lake of absorption bands of the cyano groups in the IR spectra of compounds 3a, b confirms the inte armolecular cyclization of compound 2a, b (Scheme1).



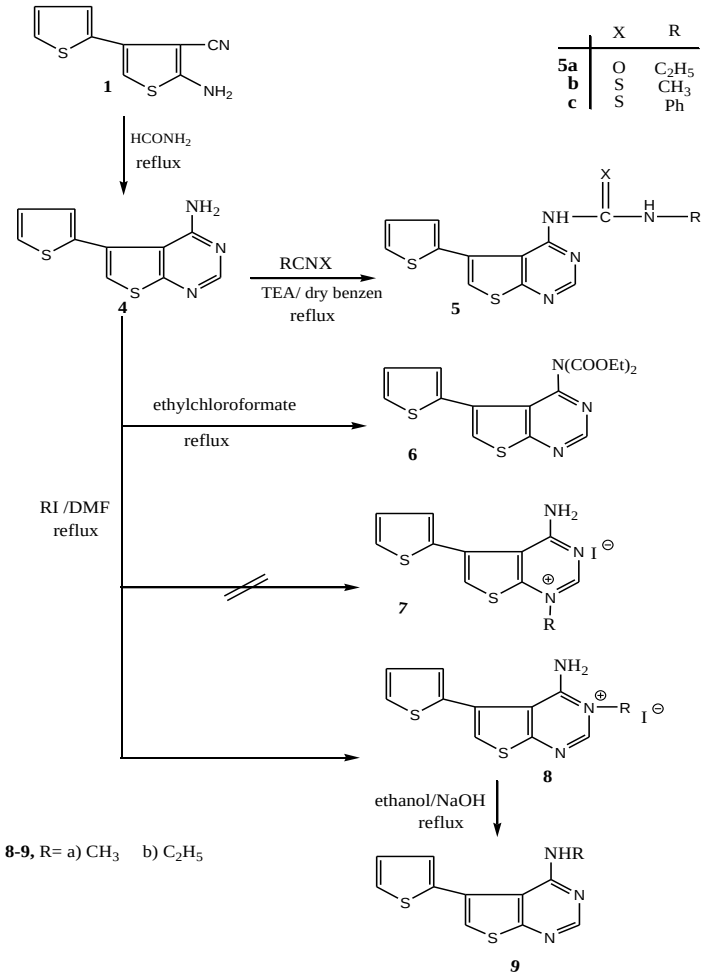

**Scheme 1**

Compound 1 condensed with formamide followed by inte armolecular cyclization afforded the 5-(thiophene-2-yl)-thieno [2, 3-*d*] pyrimidine-4-yl amine (4). The $^1$H NMR spectrum of the latter compound displayed broad signal ($D_2O$-exchangble) at δ 5.08 due to $NH_2$ protons, and C-2 proton of the formed pyrimidine ring showed at δ 8.65. Furthermore, the $^{13}$C NMR spectrum of compound 4 displayed two important signals at 166.74 and 158.26 corresponding to C-2 and C-4 of the pyrimidine ring, respectively. Treatment of compound 4 with ethyl isocyanate, methyl isothiocyanate or phenyl isothiocyanate in refluxing benzene, afforded the corresponding 1-(substituted)-3-[5-thiophene-2-yl] thieno [2,3-*d*]pyrimidin-4-yl] urea or thiourea derivatives 5a-c, respectively. The IR spectrum of compound 5a showed absorption bands at 3377, 3224 and 1670 cm$^{-1}$ due to two NH and C=O groups, respectively. The $^1$H NMR spectrum of 5a revealed signals δ 1.05-1.08 (triplet) and 3.19-3.37 (quartet) corresponding to the ethyl group and two NH ($D_2O$-exchangble) protons signals at δ 8.20 and 8.90 (Scheme 2). On the other hand, when compound 4 was refluxed with ethyl chloroformate, it afforded a product identified as ethyl *N*-[5-(2-thienyl)-3-ethoxycarbonyl]-thieno[2,3-*d*] pyrimidine-4-ylidene]carbamate (6) The. IR spectrum of compound 6 showed presence of two carbonyl (carboxylic ester) absorptions bands at 1760, 1725 cm$^{-1}$ and absence of bands corresponding to amino group. The $^1$H NMR spectrum of compound 6 showed ethyl proton signals at δ 1.15-1.30 (triplet) and 4.10-4.30 (quartet). Alkylation of the 5-(thiophene-2-yl) thieno [2,3-d]pyrimidine–4-ylamine (4) with alkyl iodides gave the corresponding 3-alkyl derivatives 8a,b and not the isomeric 1-alkyl derivatives 7. The structures of 3-alkyl derivatives 8a,b confirmed and the isomeric 1-alkyl 7 excluded on the basis of elemental analysis and spectral data of the products. For example, the$^1$H NMR spectra of products 8a,b, generally, revealed characteristic signals, beside the signals due to the incurporation of the alkyl not radical at N3- of the thienopyrimidine ring at δ 3.90.

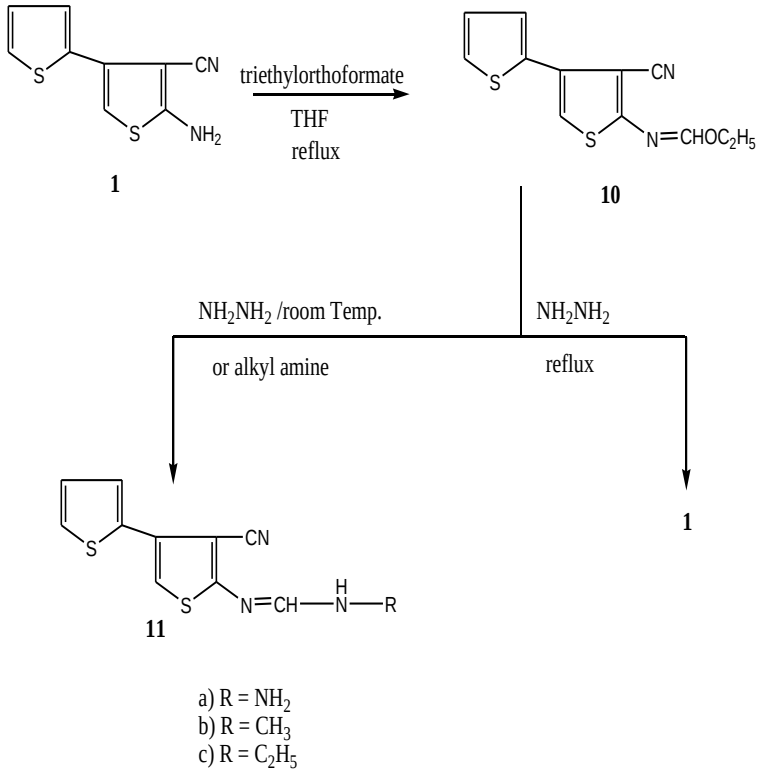
As a complementary support for the proposed structure of type 8, obtained when the pyrimidinium iodide salts 8a,b were treated with aqueous sodium hydroxide solution, the dequaternized and rearranged (Dimroth type) products N-alkyl-5-(thiophene-2-yl)thieno[2,3-d]pyrimidine 9a,b were obtained. The structures of 9a, b were confirmed by careful studying of their mass spectra which revealed *m/z* 247 ($M^+$-HI) and *m/z* 261 ($M^+$-HI), respectively. This observation suggested the splitting of HI in mass spectrometer[18] (Scheme 2).

Treatment of 2-amino-4-(thiophene-2-yl) thiophene-3-carbonitrile (1) with triethylorthoformate and trifluoroacetic acid in tetrahydrofuran afforded the ethyl *N*-4́-cyano-2,3́-bithiophen-5́-yl formimidate (10). The $^1$H NMR spectrum of the latter compound revealed ethyl proton signals as triplet and quartet at δ 1.45-1.58 and 4.39-4.42, respectively and signal at δ 8.0 which corresponds to N=CH-O

proton. However, in the present work it was found that, the formimidate 10 reacted with hydrazine hydrate and alkyl amines at room temperature to afford the corresponding *N*-substituted products 11a-c. Structure of product 11a as an example was confirmed by the presence of absorption bands at 3317, 3193, 3103, 2200 $cm^{-1}$ due to $NH_2$, NH, C≡N groups. The $^1H$ NMR spectrum of product 11a showed ($D_2O$-exchangble) singlet signals at δ 5.30, 8.60 due to $NH_2$ and NH protons, respectively and signal at δ 8.10 due to N=CH protons. On the other hand when the formimidate 10 was treated with hydrazine hydrate, in refluxing ethanol, it afforded product 1 (Scheme 3).




**Scheme 2**




**Scheme 3**

## Biological Testing

*Antimicrobial screening*

In this study, we tested the antimicrobial activities of some of the synthesized compounds. The results of the antimicrobial evaluation of the tested compounds are presented in Table 1. They showed that most of the tested compounds exhibit significant antibacterial effect against *Pseudomonas sp* (Ps), *Escherichia coli* (E.c), *Streptococcus lactis (*S.l) and *Bacillus subtilis (*B.s), while the antifungal activities of the tested compounds showed significant antifungal activities against Aspergillus's *niger* (A.n), *Penicillium sp (*P.sp), *Candida albican (C.al)* and *Rhodotorula ingeniosa (*R.i*)*. Compounds 2b, 3b and 5c exhibited potent bioactivity against bacteria and fungi as compared to the reference streptomycin and fusidic acid, respectively while compounds 1, 2d, 3a, and 11c exhibited a moderate antimicrobial activity against bacterial and fungal strains used.

**TABLE 1. Antimicrobial activity of the synthesized compounds.**

| Compound | Fungi | | | | Bacteria | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Gram -ve | | Gram +ve | |
| | *R.I* | *C. alb.* | *P.sp* | *A.n* | *P .s* | *E. c* | *S.l* | *B.s* |
| **1** | 14 | 15 | 16 | 14 | 18 | 20 | 17 | 19 |
| **2a** | 13 | 11 | 15 | 11 | 11 | 13 | 14 | 12 |
| **2b** | 18 | 19 | 17 | 19 | 22 | 22 | 24 | 23 |
| **2c** | 13 | 14 | 15 | 12 | 15 | 15 | 15 | 14 |
| **2d** | 16 | 16 | 15 | 16 | 20 | 19 | 21 | 19 |
| **2e** | 10 | 11 | 10 | 9 | 4 | 5 | 6 | 7 |
| **3a** | 16 | 17 | 16 | 16 | 18 | 17 | 18 | 17 |
| **3b** | 15 | 13 | 14 | 16 | 23 | 21 | 22 | 22 |
| **4** | 14 | 12 | 13 | 13 | 12 | 14 | 12 | 11 |
| **5a** | 5 | 4 | 6 | 7 | 15 | 17 | 17 | 15 |
| **5b** | 16 | 17 | 18 | 17 | 20 | 18 | 18 | 19 |
| **5c** | 16 | 19 | 17 | 19 | 23 | 22 | 26 | 23 |
| **8a** | 11 | 10 | 12 | 12 | 6 | 9 | 8 | 7 |
| **10** | 7 | 7 | 9 | 9 | 8 | 7 | 8 | 9 |
| **11a** | 12 | 12 | 14 | 13 | 15 | 14 | 15 | 15 |
| **11b** | 7 | 6 | 5 | 5 | 13 | 11 | 12 | 14 |
| **11c** | 15 | 16 | 16 | 15 | 20 | 21 | 20 | 19 |
| Streptomycin | - | - | - | - | 22 | 21 | 22 | 21 |
| Fusidic acid | 17 | 18 | 18 | 17 | - | - | - | - |

R.i: *Rhodotorula ingeniosa* C.alb: *Candida albicans*
P.sp: *Penicillium sp* A.n: *Aspergillus niger*
P .s: *Pseudomonas sp* E. c: *Escherichia coli*
S.l: *Streptococcus lactis* B.s: *Bacillus subtilis*

## Experimental

*Chemistry*

*General*

Melting points were determined on the Electro thermal 9100 melting point apparatus (Electro thermal, UK) and are uncorrected. Micro analytical date (Elementar, Vario El, USA) were found within ± 0.4% of the theoretical values. The IR spectra (KBr) were recorded on a FT-IR NEXCES spectrophotometer (Shimadzu, Japan). The $^1$H NMR spectra were measured with a Jeol ECA 500 MHz (Japan) in DMSO-$d_6$ and chemical shifts were recorded in δ ppm relative to TMS. Mass spectra (El) were run at 70 eV with a Finnegan SSQ 7000 spectrometer (Thermo-Instrument System Incorporation, USA). The reaction was followed by TLC (silica gel, aluminum sheets 60 $F_{254}$, Merck, Germany). Some of the analytical data was summarized in Table 2.

**TABLE 2. Analytical data of newly synthesized compounds.**

| Cpd. No. | Yield | Solv. Cryst. | mp | Mol. Formula | M.W | Elemental analysis cal/found % C H N S |
|---|---|---|---|---|---|---|
| **1** | 60 % | THF/ MeOH | 218-220 | $C_9H_6N_2S_2$ | 206.3 | 52.40,2.93,13.58, 31.09<br>52.30,2.90,13.45, 31.00 |
| **2a** | 60 % | EtOH | 218-220 | $C_{16}H_{11}N_3OS_2$ | 325.1 | 59.06,3.41,12.91, 19.71<br>59.00,3.30,12.85, 19.66 |
| **2b** | 50 % | Ethyl-acetate | 150-152 | $C_{16}H_{11}N_3S_3$ | 341.5 | 56.28,3.25,12.31, 28.17<br>56.20,3.20,12.26, 28.11 |
| **2c** | 65 % | EtOH | 228-230 | $C_{12}H_{11}N_3S_3$ | 293.4 | 49.12,3.78,14.32, 32.78<br>49.05,3.72,14.25, 32.70 |
| **2d** | 55% | Ethyl-acetate | 223-225 | $C_{11}H_9N_3S_3$ | 279.4 | 47.29,3.25,15.04, 34.43<br>47.20,3.22,15.00, 34.31 |
| **2e** | 60 % | EtOH | 288-290 | $C_{12}H_{11}N_3OS_2$ | 277.4 | 51.96,4.00,15.15, 23.12<br>51.88,3.90,15.8, 23.06 |
| **3a** | 50 % | THF/ MeOH | Above 300 | $C_{16}H_{11}N_3OS_2$ | 325 | 59.06,3.41,12.91, 19.71<br>59.00,3.35,12.80, 19.60 |
| **3b** | 65 % | THF/ MeOH | Above 300 | $C_{16}H_{11}N_3S_3$ | 341 | 56.28,3.25,12.31, 28.17<br>56.25,3.20,12.20, 28.08 |
| **4** | 75 % | Ethyl-acetate | 193-195 | $C_{10}H_7N_3S_2$ | 233.3 | 51.48,3.02,18.01, 27.49<br>51.45,2.99,17.95, 27.40 |
| **5a** | 70 % | EtOH | 163-165 | $C_{13}H_{12}N_4OS_2$ | 304 | 51.30,3.97,18.41, 21.07<br>51.23,3.90,18.28, 21.00 |
| **5b** | 70 % | EtOH | 198-200 | $C_{12}H_{10}N_4S_3$ | 306 | 47.09,3.29,18.30, 31.43<br>47.00,3.22,18.20, 31.38 |
| **5c** | 60 % | EtOH | 203-205 | $C_{17}H_{12}N_4S_3$ | 368 | 55.47,3.28,15.22, 26.14<br>55.35,3.20,15.18, 26.10 |
| **6** | 60% | n.Hexane | 148-150 | $C_{16}H_{15}N_3O_4S_2$ | 377.4 | 50.91,4.01,11.13,16.99<br>50.80,3.95,11.03,16.88 |
| **8a** | 70 % | EtOH | Above 300 | $C_{11}H_{10}N_3S_2I$ | 375.3 | 35.21,2.69,11.20, 17.09<br>35.10,2.62,11.12, 17.00 |
| **8b** | 70 % | EtOH | Above 300 | $C_{12}H_{12}N_3S_2I$ | 289.3 | 37.02,3.11,10.79, 16.47<br>37.00,3.06,10.70, 16.41 |
| **9a** | 50 % | EtOH | 252-254 | $C_{11}H_9N_3S_2$ | 247.3 | 53.42,3.67,16.99, 25.93<br>53.34,3.62,16.90, 25.88 |
| **9b** | 50 % | EtOH | 150-152 | $C_{12}H_{11}N_3S_2$ | 261.0 | 55.20,4.24,16.08, 24.56<br>55.10,4.18,16.00, 24.50 |
| **10** | 60 % | EtOH | 158-160 | $C_{12}H_{10}N_2OS_2$ | 262.6 | 54.94,3.84,10.68, 24.44<br>54.86,3.80,10.60, 24.38 |
| **11a** | 50 % | MeOH | 168-170 | $C_{10}H_8N_4S_2$ | 248.0 | 48.37,3.25,22.56, 25.82<br>48.30,3.22,22.51, 25.73 |
| **11b** | 50 % | Ethyl-acetate | 188-190 | $C_{11}H_9N_3S_2$ | 247.3 | 53.42,3.67,16.99, 25.93<br>53.30,3.60,16.94, 25.90 |
| **11c** | 50 % | Ethyl-acetate | 198-200 | $C_{12}H_{11}N_3S_2$ | 261.0 | 55.21,4.24,16.09, 24.56<br>55.15,4.20,16.00, 24.48 |

*2-Amino-4-(thiophene-2-yl) thiophene 3-carbonitrile (1)*

A mixture of 2-acetylthiophene (12.6 ml, 10 mmol), malononitrile (6.6 g, 10 mmol), ammonium acetate (0.7 g, 40 mmol) and glacial acetic acid (12 ml, 20 mmol) in dry benzene (50 ml) was refluxed for 30 hr with separation of water,

and then kept in an ice chest for 12 hr. The precipitated solid was collected by filtration, washed with water, dried, and finally crystallized from EtOH/DMF. A mixture of the obtained solid product from the pervious step, diethyl amine (2 ml) and elemental sulfur (3.2 g, 10 mmol) in ethyl alcohol (50 ml) was refluxed for 6 hr and then left to cool. The obtained solid product was collected by filtration, washed with EtOH, dried and recrystallized from THF/MeOH to afford (60 %) of compound 1.

IR (KBr): υ 3384, 3311 ($NH_2$), 2186 (C≡N) $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$)**:** δ 5.5 (2H-$NH_2$ $D_2O$ exchangeable), 7.0-8.0 (m, 4 thiophene-H). Ms *m/z* (%): 206 ($M^+$, 100, base peak).

*Preparation of 1 (4\ - cyano-2, 3\-bithiophen-5\-yl) 3-substituted urea or thiourea (2a-e)*

*General procedure*

A mixture of 1 (0.2 g, 1 mmol) and appropriate isocyanate or isothiocyanate (1.2 mmol) in pyridine (10 ml) was refluxed for 15-30 min. The solvent was removed by distillation under reduced pressure and the reminder left to cool then titrated with methanol. The precipitated solid product was collected by filtration and recrystallized from the proper solvent to afford the 3-substituted urea or thiourea derivatives (2a-e).

*1-(4\-Cyano-2,3\-bithiophen-5\-yl)-3-phenyl urea (2a)*

IR (KBr): υ 3331, 3282 (2 NH), 2208 (C≡N), 1660 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$)**:** δ 7.31-8.10 (m, 10 H, 4 thiophene-H and 5 ArH's) 8.5 (s, 1H, NH-$D_2O$ exchangeable.), 9.70 (s, 1H, NH $D_2O$ exchangeable). MS *m/*z (%): 325 ($M^+$, 100, base peak).

*1-(4\-Cyano-2,3\-bithiophen-5\-yl) 3-phenyl thiourea (2b)*

IR (KBr): υ 3368, 3200 (2 NH), 2204 (C≡N), 1514, 1245 (C=C), (C=S)) $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$)**:** δ 6.85-7.65 (m, 4 thiophene-H and 5 ArH's), 8.40 (s, 1H, NH $D_2O$ exchangeable), 9.70 (s, 1H, NH $D_2O$ exchangeable). MS *m/*z (%): 341 ($M^+$, 19) and 77 (100, base peak).

*1-(4\-Cyano-2,3\-bithiophen-5\-yl)-3-ethyl thiourea (2c)*

*IR (KBr): υ 3376, 3195 (2 NH), 2199 (C≡N)* $^1H$ NMR (DMSO-$d_6$)**:** δ 1.10-1.30 (t, 3H, $CH_3$), 3.40-3.50 (q, 2H, $CH_2$), 6.60-7.50 (m, 4 thiophene-H, NH $D_2O$ exchangeable), 10.20 (s, 1H, NH, $D_2O$ exchangeable). MS *m/*z (%): 293 ($M^+$, 35) and 205(100, base peak).

1-(4\-Cyano-2,3\-bithiophen-5\yl)-3-methyl thiourea (2d)

IR (KBr): υ 3370, 3313 (2 NH), 2190 (C≡N), 1205 (C=S) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$)**:** δ 1.10 (s, 3H, $CH_3$), 6.80-7.90 (m, 4 thiophene-H), 8.65(s, 1H, NH $D_2O$ exchangeable), 11.4 (s, 1H, NH $D_2O$ exchangeable.). MS *m/*z (%): 279 ($M^+$, 28) and 205(100, base peak).

*1-(4$^{\backslash}$-Cyano-2, 3$^{\backslash}$ -bithiophen-5$^{\backslash}$ - yl) - 3 -ethyl urea (2e)*

IR (KBr): υ 3396, 3281 (2 NH), 2210 (C≡N), 1697 (C=O) $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$)**:** δ 1.00-1.030 (t, 3H, $CH_3$ ethyl, J = 6.8 Hz), 3.35-3.4 (q, 2H, $CH_2$ ethyl, J = 6.6 Hz), 6.92-7.65 (m, 5H, 4 thiophene-H), 8.5(s, 1H, NH, $D_2O$ exchangeable), 10.30 (s, 1H, NH, $D_2O$ exchangeable.). Ms *m/z* (%): 277 ($M^+$, 20) and (206, base peak).

*Preparation of the 4-imino-3-[substituted]-5-(thiophenyl-2-yl)-3, 4-dihydro thieno [2,3-d]pyrimidine-2(1H) one or 2(1H) thione (3a,b)*

*Method A*

A mixture of 1 (0.2 g, 1 mmol) and phenyl isocyanate or isothiocyanate (1.2 mmol) in pyridine (10 ml) was heated under reflux for 1-3 hr. The reaction mixture was concentrated and left to cool then diluted with methanol. The precipitated solid product was collected by filtration and recrystallized from the proper solvent to afford 3a, b.

*Method B*

A suspension of 2a, b (10 mmol) in sodium hydroxide solution (1 %, 25 ml) was warmed on a water bath (70-80$^oC$) for one hour. The clear solution was left to cool and then acidified with diluted hydrochloric acid. The solid product separated out was filtered off, washed with water, dried and crystallized from THF/ methanol to give 3a, b.

*4-Imino-3- phenyl-5- (thiophenyl-2-yl) -3,4-dihydrothieno [2,3-d] pyrimidine-2(1H)-one (3a)*

IR (KBr): υ 3390, 3092 (2NH), 1663 (C=O) $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 6.50 - 7.48 (m , 9 H , 4 thiophene-H , 5 Ar-H) , 9.40 (s , 1 H , NH $D_2O$ exchangeable ) , 10.50 (s , 1H , NH $D_2O$ exchangeable). MS *m/z* (%):325 ($M^+$, 14) and 77(100, base peak).

*4-Imino-3-phenyl-5- (thiophenyl-2-yl) -3,4-dihydrothieno [2,3-d] pyri-midine-2(1H)-thione (3b)*

IR (KBr): υ 3415, 3309 (2NH), 1575, 1520 (C=N, C=C) 1222 (C=S) $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$)**:** δ 6.60–7.50 (m, 9H, 4 thiophene-H and 5 Ar-H), 9.50 (s, 1H, NH $D_2O$ exchangeable), 10.20 (s,1H, NH, $D_2O$ exchangeable.). MS *m/z* (%): 341 ($M^+$, 28) and 77 (100, base peak).

*Synthesis of 5-(thiophene-2-yl) thieno [2, 3-d] pyrimidine-4-yl amine (4)*

A mixture of 1 (0.2 g, 1 mmol) and formamide (30 ml) was heated on oil-bath (210-220 $^o$ C) for 4 h. After cooling, the resulting solution was diluted with water and the obtained solid precipitated was filtered off, dried and recrystallizaed from ethyl acetate to afford (75 %) of compound 4.

IR (KBr): υ 3208, 3055 ($NH_2$), 1607, 1562 (C=N, C=C) $cm^{-1}$. $^1H$ NMR (DMSO-d6): δ 5.08 (s, 2H, $NH_2$, $D_2O$ exchangeable), 7.00-7.80 (m, 4 thiophene-H), 8.65 (s, 1H, $C_2$-H). $^{13}C$ NMR (DMSO-$d_6$)**:** δ $C_2$- (166.74), $C_4$- (158.26), $C_5$-

(112.89), $C_6$- (153.93), $C_{-2}$\ (120.72), $C_3$-\ (126.49), $C_4$-\ (135.88). MS *m/z* (%) 233 ($M^+$, 25) and 83 (100, base peak).

*Synthesis of 1-(substituted)-3-[5-(thiophene-2-yl) thieno [2, 3-d] pyrimidin-4-yl] urea or thiourea (5a-c)*

*General procedure*

A mixture of compound 4 (0.23 g, 1 mmol), and the appropriate isocyanate or isothiocyanate (1.2 mmol) in dry benzene (25 ml) containing drops of triethylamine, was heated under reflux for 15 hr. The solid product which separated after cooling was filtered off, dried and crystallized from the proper solvent to give 5a-c.

*1-Ethyl-3-(5-(thiophenyl-2-yl) thieno [2, 3-d] pyrimidin-4-yl) urea, (5a)*

IR (KBr): υ 3377, 3224 (2 NH), 1670 (C=O) $cm^{-1}$. $^1$H NMR (DMSO-d6): δ 1.05-1.08 (s, 3H, $CH_3$ ethyl), 3.19-3.37 (q, 2 H, $CH_2$ ethyl), 5.40 (b.s, 1H, NH $D_2O$ exchangeable), 7.25-7.88 (m, 4 thiophene-H), 8.20 (s,1H, NH $D_2O$ exchangeable), 8.90 (s, 1H, $C_{-2}$ H). MS *m/z* (%): 304 ($M^+$, 23) and 88 (100, base peak).

*1-Methyl-3-(5-(thiophenyl-2-yl) thieno [2, 3-d] pyrimidin-4-yl) thiourea (5b)*

IR (KBr): υ 3294, 3091 (NH, NH) $cm^{-1}$. $^1$H NMR (DMSO-$d_6$): δ 3.20-3.40 (s, 3H, $CH_3$) 7.19-7.68 (m, 4 thiophene-H, NH $D_2O$ exchangeable), 8.31 (s, 1H, $C_{-2}$H), 8.60, 9.10 (b. s, 2H, 2NH, $D_2O$ exchangeable),. MS *m/z* (%): 306 ($M^+$, 35) and 83 (100, base peak).

*1-Phenyl-3-(5-(thiophenyl-2-yl) thieno [2, 3-d] pyrimidin-4-yl) thiourea (5c)*

$^1$H NMR (DMSO-$d_6$): 7.19-7.68 (m, 10H, 4 thiophene-H, 5 Ar-H, NH), 8.30 (s, 1H, $C_{-2}$ H) δ 9.00, 9.30 (s, 1H, NH $D_2O$ exchangeable). MS *m/z* (%): 368 ($M^+$, 26) and 77 (100, base peak).

*N-[5-(2-thienyl-3-ethoxycarbonyl) thieno [2.3-d] pyrimidin-4-ylidene] carbamate (6)*

A mixture of 4 (0.23 g, 1 mmol) and excess ethyl chloroformate (10 ml) was heated under reflux until a clear solution was obtained (3 hr). The reaction mixture was then evaporated till dryness and the obtained solid product was filtered off. Recrystallization from n-hexane afforded (75 %) of *N*-[5-(2-thienyl)-3-ethoxycarbonyl) thieno [2.3-*d*] pyrimidin-4-ylidene] carbamate (6).

IR (KBr): υ 1760, 1725 (C=O) $cm^{-1}$. $^1$H NMR (DMSO-$d_6$): δ 1.15-1.30 (t, 6H, $2CH_3$-ethyl), 4.10-4.30 (m, 4H, $2CH_2$), 6-70-7.60 (m, 4H thiophene- H), 8.65 (s, 1H, $C_2$-H). MS *m/z* (%):377 ($M^+$, 36) and 83(100, base peak).

*Reaction of 5-(thiophene-2-yl) thieno [2, 3-d] pyrimidine-4-ylamine 4 with alkyl halides*

*General procedure*

Compound 4 (0.23 g, 1 mmol) and the desired alkyl iodide (methyl or ethyl) (1 mmol) were dissolved in dimethyl formamide (20 ml) and heated under reflux for 3 hr. Excess solvent was evaporated till dryness under pressure. The remained residue was triturated with drops of dry acetone. The solid product obtained was filtered off, dried and crystallized from ethanol to give 8a, b.

*3-Methyl-5-(thiophenyl-2-yl) thieno [2, 3-d] pyrimidinium iodide (8a)*

IR (KBr): υ 3385, 3100 ($NH_2$) $cm^{-1}$. $^1H$ NMR (DMSO-d6): δ 3.90 (s, 3H, $CH_3$), 5.20 (b, 2H, $NH_2$ $D_2O$ exchangeable), 6.90-7.50 (m, 4H, thiophene-H), 8.20 (s, 1H, C-$_2$ H). MS *m/z* (%): 247 ($M^+$-HI) and 88(100, base peak).

*3-Ethyl-5-(thiophenyl-2-yl) thieno [2, 3-d] pyrimidinium iodide (8b)*

IR (KBr): υ 3429, 3130 ($NH_2$), 1628, 1544 (C=N, C=C).$cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 1.20-1.40 (t, 3H, $CH_3$), 3.60-3.80 (q, 2H, $CH_2$), 5.55 (b, 2H, $NH_2$ $D_2O$ exchangeable), 6.80-7.60 (m, 4H thiophene-H), 8.55 (s, 1H, C-$_2$H). MS *m/z* (%): 261 ($M^+$-HI) and 88 (100, base peak).

*Rearrangement of thieno pyrimidinium iodides salts (8a-b)*

To a solution of thieno pyrimidinium iodides salts (8a, b) (10 mmol) in dilute ethanol (50 %, 50 ml), sodium hydroxide solution (4%, 2 ml) was added. The reaction mixture was refluxed on water bath for 2 hr and then left to cool. The obtained solid product was filtered off, dried and crystallized from ethanol to give compounds 9a, b.

*N-methyl-5-(thiophen-2-yl) thieno [2,3-d]pyrimidin-4-amine (9a)*

IR (KBr): υ 3429, 3130 ($NH_2$) $cm^{-1}$; $^1H$ NMR (DMSO-d6): δ 3.65 (s, 3H, $NCH_3$), 6.70-7.8.5 (m, 4H thiophene-H), 8.65 (s, 1H,C-$_2$H), 9.20 (br, 1H, NH $D_2O$ exchangeable) MS *m/z* (%):247 ($M^+$, 33) and 88(100, base peak).

*N-ethyl-5-(thiophen-2-yl) thieno [2,3-d]pyrimidin-4-amine (9b)*

IR (KBr): υ 3429, 3130 ($NH_2$) $cm^{-1}$. $^1H$ NMR (DMSO-d6): δ 1.30 (s, 3H, $CH_3$), 4.30 (q, 2H, $CH_2$); 6.70-7.80 (m, 4H, thiophene-H), 8.70 (s, 1H,C-$_2$H), 10.2 (br, 1H, NH $D_2O$ exchangeable). MS *m/z* (%): 261 ($M^+$, 19) and 88 (100, base peak).

*Synthesis of ethyl –N-4-cyano-2,3 -bithophene-5-yl- formimidate (10)*

A mixture compound 1 (0.2 g, 1 mmol), triethylortho-formate (10 ml) and trifluroacetic acid (0.5 ml) in THF (5 ml) was heated under reflux for 6 hr. The solvent was removed by distillation under reduced pressure, the reminder was left to cool and titrated with ethanol. The formed solid product was collected by filtration, washed with ethanol and recrystallized from ethanol to afford 60 % of compound 10.

IR (KBr): υ 2214 (C≡N) $cm^{-1}$; $^1H$ NMR (DMSO-d6): δ1.45-1.58 (t, 3 H, $CH_3$ ethyl), 4.39-4.42 (q, 2 H, $CH_2$ ethyl), 6.90-7.50 (m, 4 H, thiophene-H), 8.00 (s, H, N=CH). MS *m/z* (%): 262 ($M^+$, 29) and 190 (100, base peak).

*Synthesis of (11 a –c)*

*General procedure*

A mixture of compound 10 (0.26 g, 1 mmol) and hydrazine hydrate (0.5 ml, 80%) or alkyl amine (methyl, ethyl amine) in absolute ethanol (10 ml) was stirred at room temperature for 20 hr. The solid product was filtered off, dried and crystallized from ethanol to give 11a-c.

*N'-(4'cyano-2,3'-bithiophen-5'-yl)formimidohydrazide (11a)*

IR (KBr): υ 3317, 3193, 3103 ($NH_2$, NH), 2200 (C≡N) $cm^{-1}$; $^1H$ NMR (DMSO-d6): δ 5.30-5.80 (b, 2H, $NH_2$ $D_2O$ exchangeable.), 6.80-7.80 (m, 4H thiophene-H) 10 8.60(s,1H, NH $D_2O$ exchangeable) (s, 1H, N=CH),). MS *m/z* (%): 248 ($M^+$, 29) and 88(100, base peak).

*N'-(4'-cyano-2, 3-bithiophen-5'-yl)-N-methyl formimidohydrazide (11b)*

IR (KBr): υ 3316 (NH), 2198 (C≡N), (1613) (C=N) $cm^{-1}$; $^1H$ NMR ($CDCL_3$): δ 2.80 (s, 3H, $CH_3$), 7.10-7.60 (m, 4H, thiophene-H), 8.10 (s, 1H, N=CH), 8.40 (s, 1H, NH $D_2O$ exchangeable). MS *m/z* (%): 247 ($M^+$, 19) and 88 (100, base peak).

*N'-(4'-cyano-2.3'-bithiophen-5'-yl)-N'-ethyl formimidohydrazide (11c)*

IR (KBr): υ 3321 (NH), υ 2199 (C≡N) $cm^{-1}$; $^1H$ NMR (DMSO-d6): δ 1.30-1.50 (t, 3H, $CH_3$), 4.10-4.30 (q, 2H, $CH_2$), 7.00-7.50 (m, 4H, thiophene-H), 8.20 (s, 1H, N=CH), 8.30 (s, 1H, NH, exchangeable with $D_2O$). MS *m/z* (%):261 ($M^+$, 15) and 88(100, base peak).

## Materials and Method

*Biological activity*

Antimicrobial activities of the newly synthesized compounds were tested by measuring the inhibitory effects of such compounds against Gram-negative, Gram-positive bacteria and fungi using agar diffusion technique. The fungi (*Aspergillus niger* and *Penicillium sp.*), the yeast (*Rhodotorula ingenious)*, the gram positive bacteria (Bacillus *subtilis* , *Streptococcus lactis*) and the gram negative bacteria (*E. coli*, *Pseudomonas sp*.) and *Streptomycin sp.* were obtained from Biotechnology Department at the National Research Center (Egypt). Streptomycin and fusidic acid were used as references and were also obtained from the same source.

Concentrations of the compounds under test were used according to modified Kirby-Bauer's disk diffusion method[25]. The sterile discs were impregnated with 10µg/disc with the tested compound. Each tested compound was performed in triplicate. The solvent DMSO was used as a negative control, streptomycin and fusidic acid were used as stander. Calculated average diameters (for triplicates) of the zone of inhibition (in mm) for tested samples with that produced by the stander drugs[26].

On the other hand, we used 5 different concentrations (10, 20, 30, 40 and 50 µg) of the compounds that exhibited a moderate antimicrobial activity such as (1, 2d, 3a and 11c), all the compounds exhibit significant antibacterial and antifungal activity at higher concentration (Table 2).

The following media were used:

1–PDA medium: this medium was used for fungi cultivation. It consists of 4 g dextrose /L Potatoes extract.

2-Czapek Dox medium: It consists of 10 g glucose, 2 g $KNO_3$, 1 g $K_2HPO_4$, 0.5 g KCl, and 0.5 g $MgSO_4$, and 0.05 g ferrous sulphate / L distilled water. This medium is specialized for bacteria cultivation.

3-Medium 3: It consists of 10 g glucose, 5g peptone, 3 yeast extract, and 3 Malt extract. It is used for yeast cultivation.

The gram positive, gram negative bacteria and fungi were found to be more sensitive to the compounds 2b, 3b and 5c compared to the corresponding references. The growth of all strains under study was inhibited when high concentration of compounds 1, 2d, 3a and 11c were used.

On the other hand when we used different concentrations of the compounds that exhibited a moderate antimicrobial activity (1, 2d, 3a and 11c), all the compounds exhibit significant antibacterial and antifungal activity at higher concentration as depicted in Table 3 and Fig. 1 & 2.

**TABLE 3. Effect of different concentrations of compounds that have a moderate effect on the growth.**

| Compound conc.(µg) | | Fungi | | | | Bacteria | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ***R.i*** | ***C.alb*** | ***P.sp*** | ***A.n*** | **Gram-ve** | | **Gram +ve** | |
| | | | | | | ***P.s*** | ***E.c*** | ***S.l*** | ***B.s*** |
| **1** | 10 | 14 | 15 | 14 | 19 | 17 | 20 | 18 | 19 |
| | 20 | 18 | 17 | 18 | 20 | 18 | 21 | 21 | 20 |
| | 30 | 21 | 19 | 21 | 22 | 20 | 23 | 23 | 22 |
| | 40 | 21 | 20 | 21 | 25 | 24 | 23 | 24 | 25 |
| | 50 | 21 | 20 | 21 | 25 | 24 | 24 | 24 | 25 |
| **2d** | 10 | 16 | 16 | 16 | 19 | 21 | 19 | 20 | 19 |
| | 20 | 19 | 20 | 19 | 22 | 24 | 22 | 23 | 22 |
| | 30 | 22 | 22 | 22 | 25 | 24 | 24 | 25 | 25 |
| | 40 | 22 | 22 | 22 | 25 | 24 | 24 | 25 | 25 |
| | 50 | 22 | 22 | 22 | 25 | 24 | 24 | 25 | 25 |
| **3a** | 10 | 16 | 17 | 16 | 17 | 18 | 17 | 18 | 17 |
| | 20 | 19 | 20 | 19 | 20 | 21 | 20 | 20 | 20 |
| | 30 | 20 | 21 | 20 | 23 | 24 | 23 | 23 | 23 |
| | 40 | 20 | 21 | 20 | 23 | 24 | 23 | 23 | 23 |
| | 50 | 20 | 21 | 20 | 23 | 24 | 23 | 23 | 23 |
| **11c** | 10 | 15 | 17 | 15 | 19 | 20 | 21 | 20 | 19 |
| | 20 | 17 | 18 | 17 | 21 | 23 | 23 | 23 | 21 |
| | 30 | 19 | 20 | 19 | 24 | 25 | 24 | 24 | 24 |
| | 40 | 19 | 20 | 19 | 24 | 25 | 24 | 24 | 24 |
| | 50 | 19 | 20 | 19 | 24 | 25 | 24 | 24 | 24 |
| **control** | | 17 | 18 | 18 | 17 | 21 | 22 | 21 | 22 |

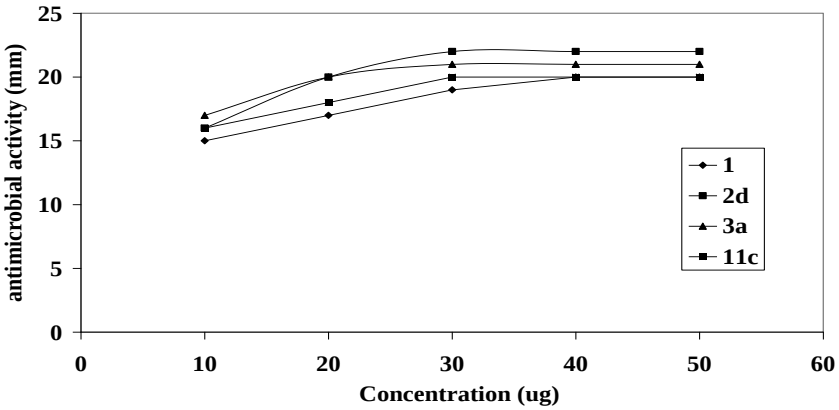



**Fig. 1. Effect of different concentrations of compounds 1,2d,3a and 11c on *Candida albicans.***

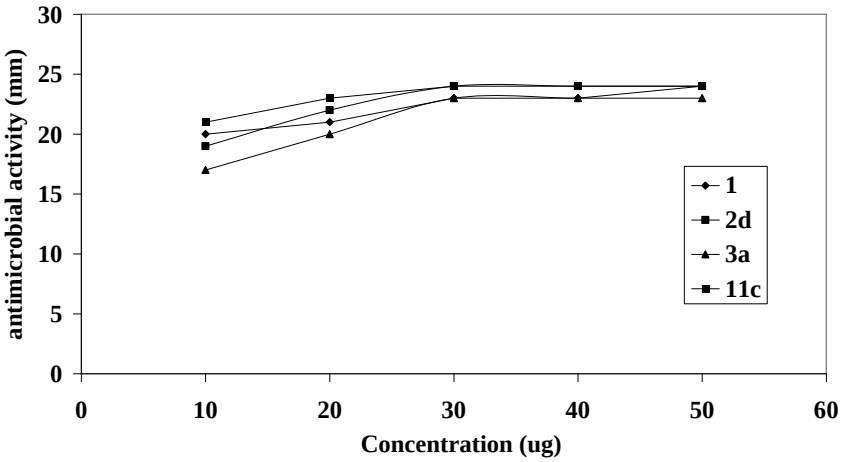



**Fig. 2. Effect of different concentrations of compounds 1,2d,3a on *Echerichia coli.***

*Structure–activity relationship (SAR)*

From the results obtained, we can conclude that the thiophen-thioureido moiety is essential for the activity against true fungi (moulds) and Bacteria. These results agree with previously reported results[1-8]. Amongst the dozens of synthesized bithiophens found in recent few years[1,8], compounds 2b, 3b and 5c showed good observed activities against pathogenic bacteria and fungi such as *Pseudomonas sp* (*Ps*), *Escherichia coli* (E.c) *Streptococcus lactis (*S.l), *Bacillus subtilis (*B.s) and *Candida albican (C.a)* .

So, these compounds can be considered as a lead compound in this field. Further studies are in progress on the same compounds to increase their activities

and understand their QSAR. The overall results of the present study can be considered very promising in the perspective of new drugs discovery, with respect to the medical importance of the tested microorganisms.

**References**


1. **Guetschow, M.,** *Sulfur Lett.* **16**, 71 (1993).

2. **El-Kashef, H.S., Geies, A.A., El-Dean, A.M. K. and Abdel-Hafez, A.A.,** *J. Chem. Technol. Biotechnol*. **57**, 15 (1993).

3. **Abdelrazek, F.M. and Ead, H.A.,** *J. Prakt. Chem*. **330**, 585 (1988).

4. **Gewald, K., Jesckke, T. and Gruner, M.,** *J. Prakt. Chem.* **333**, 229 (1991).

5. **Testa, M.G., Perrini, G., Chiacchio, M. and Corsaro, A.,** *J. Chem. Res. Synop*, **302**, (1993).

6. **Hosni, H.M., Basyouni, W.M. and Nahas, H.A.,** *J. Chem. Res.* (M), 2775-2794 (1999).

7. **Hosni, H.M., Basyouni, W.M. and El-Bayouki**, **KH.A.M.,** *Acta Pol. Pharm. Drug Res.* **56**, 49 (1999).

8. **Basyouni, W.M., Bayouki, Kh.A.M. El-Sayed, M.M. and Hosni, H.M.,** *J. Chem. Res.* (6) **127** (M), 801 (1996).

9. **Solian, T.Z. and Arzneim, F.,** *Drg. Res.* **16**, 870 (1966).

10. **Jark , I., Plantanida Suman, L., Zinic, M., Pavelic, K. and arminskizanola, G.,** *Bioorg. Med. Chem.* **14**, 2859 (2006).

11. **Peters, D., Hornfeldt, A.B. and Gronowitz**, **S.J.,** *Heterocycl. Chem*. **27**, 2165 (1990).

12. **Kukolja, S., Draheim, S.E., Graves, B.J., Hunden, D.C., Pfeil, J.L., Cooper, R.D.G., Ott, J.L. and Coute**r, **F.T.,** *J. Med. Chem*. **28**, 1896 (1985).

13. (a) **Manetti, F., Santucci, A., Locatelli, G.A., Maga, G., Spreafico, A., Serchi, T., Orlandini, M., Bernardini, G., Caradonna, N.P., Spallarossa, A., Brullo, C., Schenone, S., Bruno, O., Ranise, A., Bondavalli, F., Hoffmann, O., Bologna, M., Angelucci, A. and Botta**, **M.,** *J. Med. Chem.* **50**, 5579 (2007). (b) **Naya, S., Ohtoshi, H. and Nitta**, **M.,** *J. Org. Chem*. **71**, 176 (2006). (c) **Wendt, J.A., Deeter, S.D., Bove, S.E., Brooker, C.S., Knauer, R.M., Augelli-Szafran, E.C., Schwarz, R.D., Kinsora, J.J. and Kilgore**, **K.S.,** *Bioorg. Med. Chem. Lett***. 17**, 396 **(**2007).

14. (a) **Wang, W., Lagniton, L.M., Constantine, R.N. and Burger**, **M.T.,** *WO Patent* 2004111058, *Chem. Abstr*. (2004), **142**, 74600 (2004). (b) **Walte**r, **H.,** *WO Patent* 9914202, (1999); *Chem. Abstr*. **130**, 252368k (1999). (c) **Chambhare, R.V., Khadse, B.G., Bobde, A.S. and Bahekar**, **R.H.,** *Eur. J. Med. Chem.* **38**, 89 (2003).

15. **Dai, Y., Guo, Y., Frey, R.R., Ji, Z., Curtin, M.L., Ahmed, A.A., Albert, D.H., Arnold, L., Arries, S.S., Barlozzari, T., Bauch, J.L., Bouska, J.J., Bousquet, P.F., Cunha, A.G., Glaser, K.B., Guo, J., Li, J., Marcotte, P.A., Marsh, K.C., Moskey, M.D., Pease, L.J., Stewart, K.D., Stoll, V.S., Tapang, P., Wishart, N., Davidsen, S.K. and Michaelides**, **M.R.,** *J. Med. Chem.* **48**, 6066 (2005).

16. **Duval, E., Case, A., Stein, R.L. and Cuny, G.D.,** *Bioorg. Med. Chem. Lett.* **15**, 1885 (2005).

17. **Munchhof, M.J., Beebe, J.S., Casavant, J.M., Cooper, B.A., Doty, J.L., CHigdon, R., Hillerman, S.M., Soderstrom, C.I., Knauth, E.A., Marx, M.A., Rossi, A.M.K. and Sobolov, Sun**, **S.B.,** *J. Bioorg. Med. Chem. Lett*. **14**, 21 (2004).

18. **El-Bayouki, K.A., Hammed, M.M., Osman, S. and Basyouni**, **W.M.,** *Oriental J. Chem.* **2**(1), 45 (1986).

19. **Ali, K.A., Elkholy, Y.M. and Farag**, **A.M.,** *Letters in Organic Chemistry*, **3**, 195 (2006).

20. **Amr, A.E., Ali, K.A. and Abdalla, M.M.,** *Eur. J. Med. Chem*. **44**, 901-907 (2009).

21. **Ali, K.A., Elkholy, Y.M. and Farag**, **A.M.,** *J. Heterocycl. Chem*. **45**, 279-283 (2008).

22. **Barsoum, F.F., Hosni, H.M. and Girigs**, **A.S.,** *Bioorg. Med. Chem*. **14**(11), 3929-3937 (2006).

23. **Girigs, A.S., Hosni, H.M. and Barsoum, F.F.,** *Bioorg. Med. Chem*. **14**(13), 4466-4476 (2006).

24. **Rajiv, D.,** *Sci .Pharm* **76**, 217–239 (2008).

25. **Furtada, G.L. and Medeiros, A.A.** *J. Clin. Microbiol.* 1980. October, **12**(4), 550-553 (1980).

26. **Drew, W.L.,Barry, A.L., Toole, R. and C Sherri's, J.,** *App. Environ. Microbiol* **24**(2), 240-247 (1972).

(*Received* 12/4/2010;
*accepted* 3/5/2010)

# تحضير مشتقات جديدة من ثنائى الثيوفين وكذلك ثيينو (٢،٣-د) بيريميدين ذات الفاعلية كمضادات للميكروبات

**هناء محمد حسنى ، قرنى على* والسيد مصطفى****
قسم مبيدات الآفات ، *قسم الكيمياء العضوية التطبيقية و**قسم الكيمياء الحيوية – المركز القومى للبحوث – الجيزة – مصر .

فى هذا البحث تم استخدام ٢-( امينو )-٤-(ثيوفين-٢-ايل) ثيوفين-٣-كربونيتريل (١) – كمادة بادئة لتحضير سلسلة من مشتقات ثنائى الثيوفين وكذلك مشتقات من ثينو(٢،٣-د)بيريميدين . عند مفاعلة المركب رقم (١) مع بعض مشتقات الايزوسيانات والايزوثيوسيانات تم الحصول على المشتقات (١٢- ه ). بعض هذه المشتقات (١٢،ب) تم تحويلها الى المشتقات (١٣،ب) وذلك بمعالجتها بالقلوى.تم ايضا حلوقة المركب (١) عن طريق غليانة فى الفورماميد للحصول المركب (٤) الذى تم استخدامه فى تحضير العديد من مشتقات الثينوبيريميدينات.

مثلا عند مفاعلة المركب (٤) مع مشتقات من الايزوسيانات والايزوثيوسيانات تم الحصول على المشتقات (١٥- جـ).

ايضا عند غليان المركب (٤) مع كلوروفورمات الايثيل- نتج عن ذلك الحصول على مركب البيريميدين كربااميت(٦). ايضا عن مفاعلة المركب (٤) مع يوديدات الالكيل (ميثيل وايثيل) تم الحصول على الاملاح التربيعية -٣-(ميثيل أو ايثيل) ثيينو (٢،٣-ر) بيريميدين ايوديد (١٨،ب) . عند معاملة المشتقات الغير تربيعية (١٩،ب).

ايضا عند مفاعلة المركب (١) مع ترای ايثيل اورثوفورمات نتج المركب (١٠) والذى بدورة تمت مفاعلتة مع الهيدرازين المائى وميثيل واثيل امين للحصول على المشتقات (١١- جـ) .

تم عمل مسح بيولوجى لهذة المركبات الجديدة على انواع من البكتريا سالبة وموجبة الجرام وكذلك بعض الفطريات وقد ثبت لعدد من هذه المركبات فاعلية كبيرة مقارنة بالمادة المرجعية المستخدمة فى المقارنة.