Facile Synthesis of Heterocycles via 2-Picolinium Bromide and Antimicrobial Activities of the Products

Elham S. Darwish

Department of Chemistry, Faculty of Science, University of Cairo, Giza, 12613, Egypt; E-mail: elham_darwish@yahoo.com

Received: 3 April 2008; in revised form: 24 April 2008 / Accepted: 24 April 2008 / Published: 1 May 2008

Abstract: The 2-picolinium N-ylide 4, generated in situ from the N-acylmethyl-2-picolinium bromide 3, underwent cycloaddition to N-phenylmaleimide or carbon disulfide to give the corresponding cycloadducts 6 and 8, respectively similar reactions of compound 3 with some electron-deficient alkenes in the presence of MnO2 yielded the products 11 and 12. In addition, reaction of 4 with arylidene cyanothioacetamide and malononitrile derivatives afforded the thiphene and aniline derivatives 15 and 17, respectively. Heating of picolinium bromide 3 with triethylamine in benzene furnished 2-(2-thienyl)indolizine (18). The structures of the isolated products were confirmed by elemental analysis as well as by 1H- and 13C-NMR, IR, and MS data. Both the stereochemistry and the regioselectivity of the studied reactions are discussed. The biological activity of the newly synthesized compounds was examined and showed promising results.

Keywords: Dihydrothiophene, indolizine, aniline derivatives, biological activity

Introduction

Many thiophene derivatives have been reported to exhibit interesting pharmaceutical properties, including antimicrobial [1], anticancer [2], anti-inflammatory [3], bacteriostatic and fungistatic activity [4]. Some other derivatives exhibit strong inhibition of NHE-1 and cardioprotective efficacy [5]. Indolizine derivatives also possess valuable biological activities and have been studied for their
psychotropic, anti-inflammatory, analgesic, antimicrobial, antiexudative and hypoglycemic properties [6-9]. Herein, the synthesis of some new aromatic and heteroaromatic compounds having a thiophene moiety utilizing the highly reactive nitrogen ylide 4 is described. Some of the newly synthesized compounds were tested for their antimicrobial activities.

Results and Discussion

The required starting material 2-bromoacetylthiophene (1) was prepared as previously described [4]. Treatment of 1 with 2-picoline (2) in refluxing dry THF afforded the 2-picolinium salt 3 in 80% yield (Scheme 1). The structure of product 3 was elucidated by its spectroscopic (MS, IR, $^1$H-NMR) and elemental analysis data (see Experimental).

![Scheme 1](image)

Initially, the cycloaddition reactions of the N-ylide 4, generated in situ by base-catalyzed dehydrobromination of 3, with symmetrical dipolarophiles were examined (Scheme 2). Thus, reaction of the salt 3 with N-phenylmaleimide (5) in refluxing benzene in the presence of triethylamine afforded one product, as evidenced by tlc analysis. The structure of the isolated product proved to be 6, as evidenced by its spectroscopic and elemental analysis data. Thus, its $^1$H-NMR spectrum in DMSO-$d_6$ revealed two characteristic doublet signals at $\delta = 4.28$ and 5.72 ppm, with a coupling constant ($J$) of 7.6 Hz, assignable to the 3a and 9b protons. This observed value of the coupling constant indicates that the product 6 has cis-configuration [10-12] and this, in turn, suggests that the cycloaddition of the N-ylide 4 to N-phenylmaleimide 5 is a concerted cycloaddition.

Also, treatment of 3 with carbon disulfide in dimethylformamide in the presence of potassium carbonate at room temperature yielded one product, identified as 8 on the basis of its spectroscopic data ($^1$H-NMR, MS and IR) and elemental analysis. For example, its $^1$H-NMR spectrum showed a D$_2$O-exchangable singlet signal at $\delta = 11.80$ ppm, assignable to the SH proton. This finding suggests that the isolated cycloadduct exists predominantly in the depicted thiol tautomeric form 8.

Next, in order to shed some light on the regiochemistry of the cycloaddition of the N-ylide 4, its reactions in refluxing dry benzene in the presence of triethylamine and manganese dioxide with each of $\omega$-nitrostyrene (9a), benzylideneacetophenone (9b) and ethyl $\alpha$-cyano-4-chlorocinnamate (9c) were investigated (Scheme 2). In each case, only one product was isolated, as evidenced by tlc analysis, indicating that such cycloadditions are regioselective. On the basis of their spectroscopic ($^1$H-NMR, MS and IR) and elemental analysis data (see Experimental) the isolated products were identified as 12a, 12b and 11c, respectively and the other regioisomeric structures 13 were discarded. To account for the formation of the latter products 12a,b and 11a,b it is suggested that the N-ylide 4, generated in

...
situ by dehydrobromination of 3, undergoes cycloaddition to the C=C double bond to form the respective cycloadduct 10, which underwent in situ oxidation to form 11 as end product. The products 11a,b underwent further in situ oxidation and afforded 12a,b, respectively. The regioselectivity in the studied reactions of 4 with 9a-c can be satisfactorily rationalized by the electrostatic attraction during the approach of both reagents. As shown in Scheme 3, the electrostatic interaction will favor bonding of the α- and β-carbon atoms of each of the used dipolarophiles 9 with the ring and exocyclic carbon atoms of the N-ylide 4, respectively. Such approach will lead to the cycloadducts 10, rather than the regioisomers 13 [13].

Scheme 2

\[ \text{Het-} \text{CO-CH}_2 - N^+ \text{Me} \]

\[ \text{Het-} \text{CO-CH-N} \text{Me} \]

\[ \text{Het-} \text{CO-CH-N} \text{Ph} \]

\[ \text{Het-} \text{CO-CH-N} \text{Ph} \]

\[ \text{S=C=S} \]

\[ \text{Ar/} \text{X/W: a, Ph/H/NO}_2; \text{b,Ph/H/PhCO; c, 4-ClC}_6\text{H}_4/\text{CN/EtOOC} \]

Het = 2-thienyl
The reaction of the \( N \)-ylide \( 4 \) with each of arylidenecyanothioacetamides \( 14a,b \) in refluxing ethanol in the presence of \( \text{Et}_3\text{N} \) was found to afford the corresponding trans-4,5-dihydrothiophene derivatives \( 15a,b \), respectively (Scheme 4). The IR spectra of these products showed absorption bands in the \( \nu \) 3360 – 3364, 3192 – 3212, 2215 and 1720 – 1723 cm\(^{-1} \) regions, assignable to NH\(_2\), CN and CO groups, respectively. Their \(^1\)H-NMR in DMSO-d\(_6\) revealed two characteristic doublet signals (\( J = 17 \) Hz) in the \( \delta \) = 4.0 – 5.0 ppm region, due to the 4-CH and 5-CH protons of the dihydrothiophene ring. The suggested pathway for the formation of \( 15 \) from \( 3 \) and \( 14 \) is depicted in Scheme 4. This pathway is analogous to that reported in the literature for the preparation of trans-4,5-dihydrothiophene derivatives from pyridinium salts and benzylidenecyanothioacetamides [14-17].
Reactions of 3 with each of the arylidenemalononitrile derivatives 16a-c in refluxing pyridine were also investigated (Scheme 5). In our hands, these reactions afforded in each case one product, as evidenced by tlc, which proved to be the respective aniline derivatives 17a-c.

Scheme 5

For example, the corresponding IR spectra revealed in each case four characteristic bands due to NH$_2$, CN and CO groups in the $\nu$ 3326 – 3342, 3104 – 3205, 2197 – 2211 and 1658 – 1667 cm$^{-1}$ regions, respectively. Their mass spectra showed the molecular ion peaks at the expected m/z values (see Experimental). To account for the formation of 17, the reaction mechanism outlined in Scheme 5 is suggested.

According to this mechanism, the reaction starts with nucleophilic attack of the N-ylide at the $\beta$-carbon atom of 16 to form the intermediate A, which in turn adds to another arylidenemalononitrile molecule to give the intermediate B. The latter undergoes concurrent cyclization and elimination of picoline to give 17 as end product [18,19].

Finally, when the salt 3 was heated in benzene in the presence of triethylamine, it yielded a product that was identified as 2-(2-thienyl)indolizine (18). A plausible pathway leading to the latter product is shown in Scheme 6. The structure of product 18 was elucidated by its spectroscopic (MS, IR, $^1$H- and $^{13}$C-NMR) and elemental analysis data. Its IR spectrum revealed the absence of the carbonyl group, but showed bands due to C=C stretching at $\nu$ = 1626 cm$^{-1}$. 
The electronic absorption spectra of compounds 3, 6, 8, 11a-b, 12c, 15a-b, 17a-c and 18 in ethanol were recorded and the data are given in Table 1.

### Table 1: Electronic Absorption and Spectral Data of the Compounds 3-18 in ethanol.

| Cpd. no. | \(\lambda_{\max}\) (log \(\varepsilon\)) | Cpd. no. | \(\lambda_{\max}\) (log \(\varepsilon\)) |
| --- | --- | --- | --- |
| 3 | 290 (4.67), 265 (4.93) | 15a | 347 (3.90), 269 (4.30), 224 (4.35) |
| 6 | 296 (4.28), 260 (4.43) | 15b | 345 (4.09), 257 (4.84), 229 (4.83) |
| 8 | 400 (4.49), 354 (4.60), 267 (4.88) | 17a | 351 (4.28), 247 (4.87) |
| 11a | 299 (4.50), 247 (4.83) | 17b | 358 (4.06), 260 (4.60) |
| 12a | 348 (4.38), 295 (4.65), 248 (4.93) | 17c | 295 (4.00), 265 (4.54), 230 (4.64) |
| 12b | 378 (4.06), 303 (4.79), 245 (5.05) | 18 | 310 (3.95), 264 (4.62) |

**Antimicrobial activity.**

Most of the compounds were tested *in vitro* against a Gram negative bacterium [*Escherichia coli* anaerobic (EC)], a Gram positive bacterium [*Staphylococcus albus* (SA)] and for antifungal activity against [*Candida albicans* (CA) and *Aspergillus flavus* (AF)]. The antibiotics ampicillin and tetracycline were used as references to evaluate the potency of the tested compounds under the same conditions. The solvent used was DMSO and the concentration of the sample used is 100 µg/mL. The test results are summarized in Table 2. They reveal that all compounds exhibited moderate activity against the two tested bacteria species and [*Candida albicans*].
Table 2. Antibacterial and Antifungal Activities of the Synthesized Compounds.a.

| Compd. No. | Gram (-) | Gram (+) | Fungi |
|------------|----------|----------|-------|
|            | (EC)     | (SA)     | (AF)  | (CA) |
| 6          | ++       | ++       | ++    | ++   |
| 8          | ++       | ++       | –     | ++   |
| 11c        | ++       | ++       | –     | ++   |
| 12a        | ++       | ++       | –     |   +  |
| 12b        | ++       | ++       | –     |   +  |
| 15a        | ++       | ++       | –     |   +  |
| 17a        | ++       | ++       | –     |   +  |
| 17b        | ++       | ++       | –     |   +  |
| 18         | ++       | ++       | –     |   +  |
| tetracyclin| +++      | +++      |       |+++  |
| ampicillin | +++      | +++      |       |+++  |

a) + = low activity, ++ = moderate activity, +++ = high activity, – = no activity.

Conclusions

The synthesis of new indolizine derivatives by cycloaddition of the 2-picolinium N-ylide 4 with N-phenylmaleimide, carbon disulfide, and electron-deficient alkenes to give the corresponding cycloadducts 6, 8, 11 and 12 is reported. Also, reaction of 4 with arylidene derivatives of cyanothioacetamide and malononitrile afforded the thiophene and aniline derivatives 15 and 17. A novel synthesis of new indolizine derivatives by heating of picolinium bromide 3 with triethylamine in benzene furnished 2-(2-thienyl) indolizine (18). The structures of all new synthesized compounds were established from their spectral data and elemental analysis. Additionally, the antimicrobial activity of selected compounds was examined.

Experimental

General

All melting points were determined on an Electrothermal Gallenkamp apparatus and are uncorrected. The IR spectra (KBr disks, cm⁻¹) were measured on a Pye-Unicam SP300 instrument in potassium bromide discs. The ¹H- and ¹³C-NMR spectra were recorded in DMSO-d₆ on a Varian Mercury VX300 spectrometer operated at 300 and 75.46 MHz, respectively. The mass spectra were recorded on a GCMS-Q1000-EX Shimadzu and GCMS 5988-A HP spectrometers, the ionizing voltage was 70 eV. Electronic absorption spectra were recorded on Perkin-Elmer Lambda 40 spectrophotometer. Elemental analyses were carried out by the Microanalytical Center of Cairo University, Giza, Egypt. The starting materials 2-bromoacetylthiophene (1) [4], N-phenylmaleimide (5) [20], ethyl (4-chlorophenylmethylene)cyanoacetate 9c, benzylidene acetophenone and 2-
substituted 3-aryl-or heteroarylprop-2-ene nitriles 16 and 14, were prepared as previously reported in the literature [21].

1-(Thiophen-2-yl)-1-oxo-ethane-2-picolinium bromide (3)

2-picoline (0.93 g, 10 mmol) was added to a solution of 2-bromoacetylthiophene (1) (2.05 g, 10 mmol) in dry THF (50 mL), the mixture was refluxed for 30 min and then left to cool. The solid was filtered off, washed with ether, and dried to afford the title compound 3 as yellowish white crystals; mp 110-112 °C (from EtOH); yield 80%; IR: ν = 1672 (CO); 1H-NMR: δ (ppm) = 2.41 (s, 3H, CH3), 4.89 (s, 2H), 7.09-7.88 (m, 3H), 7.94-8.02 (m, 3H), 8.61 (d, 1H); MS m/z (%) = 299 (M+1, 10.5), 298 (M+, 22.6), 111 (100.0), 92 (33.7), 83 (31.1), 64 (15.7); Anal. Calcd. for C12H12BrNOS (298.20): C, 48.33; H, 4.06; Br, 26.80; N, 4.70; S, 10.75%. Found: C, 48.25; H, 3.69; Br, 26.22; N, 4.24; S, 10.63.

6-Methyl-2-phenyl-4-(2-thienylcarbonyl)-3a,4,9a,9b-tetrahydro-1H-pyrrolo[3,4-a]indolizine-1,3(2H) dione (6)

To a mixture of 3 (0.298 g, 1 mmol) and the N-phenylmaleimide (5) (0.173 g, 1 mmol) in benzene (30 mL) was added triethylamine (0.15 mL, 1.5 mmol). The mixture was refluxed for 6 hours while stirring, then cooled. The precipitated salt was filtered off and the filtrate was evaporated under vacuum. The residue was treated with methanol and the solid formed was filtered and crystallized from ethanol to give the product 6 as pale yellow crystals; mp 218 °C; yield 80%; IR: ν = 1665, 1709, 1776 (3CO) cm⁻¹; 1H-NMR: δ (ppm) = 2.48 (s, 3H, CH3), 4.28 (d, 1H, J = 7.6 Hz ), 4.85 (d, 1H, J = 3.8 Hz), 5.54 (d, 1H, J = 3.8 Hz ), 5.72 (d, 1H, J = 7.6 Hz), 5.96-6.65 (m, 3H), 6.68 (d, 2H), 6.96 (dd, 2H), 7.72 (dd, 1H), 7.85-8.02 (m, 3H); MS: m/z (%)= 390 (M⁺, 19.4), 388 (10.6), 265 (10.2), 173 (10.7), 158 (22.2), 111 (100.0), 104 (12.5), 91 (35.8), 83 (14.0), 77 (15.4%), 64 (13.8); Anal. Calcd. for C22H18N2O3S (390.46): C, 67.67; H, 4.65; N, 7.17; S, 8.21%. Found: C, 67.11; H, 4.28; N, 7.24; S, 8.11.

2-Mercapto-5-methyl-8aH-[1,3]thiazolo[3,2-a]pyridin-3-yl)(2-thienyl)methanone (8)

To a stirred suspension of 3 (0.59 g, 2 mmol) in dimethylformamide (20 mL) and potassium carbonate (0.28 g), carbon disulphide (7) (4 mL) was added, and the mixture was stirred for 20 hours. The reaction mixture was diluted with water and the so-formed precipitate was filtered off and crystallized from ethanol to afford compound 8 as yellow crystals; mp 160-161 °C; yield 60%; IR: ν = 1662 (CO); 1H-NMR: δ (ppm) = 2.49 (s, 3H, CH3), 5.91 (s, 1H), 6.15-6.58 (m, 3H), 6.95-8.21 (m, 3H), 11.80 (s, 1H, SH); 13C-NMR: δ (ppm) = 19.27, 55.99, 115.51, 118.05, 124.23, 127.18, 130.08, 135.96, 138.48, 141.64, 146.14, 148.28, 188.97 (CO); MS: m/z (%) = 294 (M⁺+1, 10.6), 293 (M⁺, 35.6), 181 (85.4), 111 (100), 92 (23.6), 83 (22.5), 75 (14.2), 63 (11.3); Anal. Calcd. for C13H11NOS3 (293.43): C, 53.21; H, 3.78; N, 4.77; S, 32.78%. Found: C, 53.38; H, 3.65; N, 4.88; S, 32.51.
General procedure for the preparation of the indolizine derivatives 11c and 12a,b

To a mixture of 1-(thiophene-2-yl)-1-oxo-ethane-2-picolinium bromide (3) (0.298 g, 1 mmol) and the nitrostyrene (9a), benzylideneacetophenone (9b) or ethyl (4-chlorobenzylidene) cyanoacetate (9c) (6 mmol) in benzene (30 mL), triethylamine (0.15 mL, 1.5 mmol) and manganese dioxide (0.7 g, 8 mmol) were added. The mixture was refluxed for 6 hours then cooled to room temperature. The precipitate was filtered, and the filtrate was evaporated under vacuum. The residue was treated with methanol and the solid precipitate was filtered off, washed with methanol, and dried. Crystallization from ethanol afforded the corresponding indolizine derivatives 11c and 12a-c respectively. The physical constants and spectroscopic data of the isolated products 11c and 12a-c are given below.

Ethyl 2-(4-chlorophenyl)-1-cyano-5-methyl-3-(2-thienylcarbonyl)-1,8a-dihydroindolizine-1-carboxylate (11c): yellow crystals; mp 200 °C; yield 75%; IR: ν = 2217 (C=N), 1725, 1663 (2CO); ¹H-NMR: δ (ppm) = 1.30 (t, 3H, CH₃, J = 6.9 Hz), 2.48 (s, 3H, CH₃), 4.33 (q, 2H, CH₂, J = 6.9 Hz), 5.42 (s, 1H), 6.21-7.60 (m, 6H, aromatic protons), 7.64 (d, 2H), 8.03 (d, 2H); ¹³C-NMR: δ (ppm) = 17.75, 24.36, 54.85, 60.16, 64.52, 115.23, 117.00, 121.23, 126.46, 130.23, 131.77, 132.65, 133.41, 133.85, 134.61, 135.10, 135.26, 139.21, 140.82, 145.01, 169.08, 180.63; MS: m/z (%) = 452 (M⁺+2, 11.3) 451 (M⁺+1, 10.1), 450 (M⁺, 20.5), 448 (36.2), 323 (55.2), 322 (100.0), 242 (11.3), 212 (52.0), 201 (9.3), 199 (80.8), 154 (16.3), 122 (11.5), 111 (7.9), 105 (18.6), 93 (5.9), 83 (4.8), 77 (20.1), 76 (12.9), 63 (16.1), 60 (20.4); Anal. Calcd. for C₂₄H₁₉ClN₂O₃S (450.93): C, 63.92; H, 4.25; Cl, 7.86; N, 6.21; S, 7.11%. Found: C, 63.98; H, 4.01; Cl, 7.65; N, 6.02; S, 7.51.

(5-Methyl-1-nitro-2-phenylindolizin-3-yl)(2-thienyl)methanone (12a): yellow crystals; mp 179-181 °C; yield 75%; IR: ν = 1670 (CO); ¹H-NMR: δ (ppm) = 2.50 (s, 3H, CH₃), 6.90-8.25 (m, 6H, aromatic protons), 7.52 (dd, 1H), 7.68 (d, 2H), 7.79 (dd, 2H); ¹³C-NMR: δ (ppm) = 17.54, 116.68, 118.92, 120.73, 121.13, 122.63, 125.96, 126.79, 129.37, 130.85, 131.13, 131.60, 132.35, 132.85, 133.33, 133.93, 134.76, 140.57, 178.15; MS: m/z (%) = 362 (M⁺, 31.1), 322 (40.0), 280 (26.7), 263 (31.1), 255 (42.2), 236 (60.0), 149 (100.0), 128 (24.4), 113 (22.2), 104 (31.1), 92 (4.4), 83 (9.5), 77 (75.6), 62 (26.7), 50 (62.2); Anal. Calcd. for C₂₀H₁₄N₂O₃S (362.41): C, 66.28; H, 3.89; N, 7.73; S, 8.85%. Found: C, 66.51; H, 3.62; N, 7.52; S, 8.25.

(1-Benzoyl-5-methyl-2-phenylindolizin-3-yl)(2-thienyl)methanone (12b): pale yellow crystals; mp 210 °C; yield 76%; IR: ν = 1670, 1655 (2CO); ¹H-NMR: δ (ppm) = 2.49 (s, 3H, CH₃), 6.69-8.21 (m, 6H, aromatic protons) 7.45 (d, 2H), 7.54 (dd, 1H), 7.69 (dd, 2H), 7.77 (dd, 2H), 7.83 (dd, 1H), 8.09 (d, 2H); ¹³C-NMR: δ (ppm) = 17.52, 40.73, 114.65, 119.79, 122.64, 125.31, 125.76, 126.17, 126.59, 126.96, 127.32, 129.81, 130.87, 131.13, 131.53, 131.89, 133.95, 135.73, 136.27, 139.31, 140.23, 173.64, 190.56; MS: m/z (%) = 421 (M⁺, 16.8), 407 (13.0), 288 (26.6), 208 (23.1), 207 (24.3), 199 (15.4), 131 (14.8), 111 (49.1), 105 (85.2), 103 (20.7), 78 (33.1), 77 (100.0), 76 (11.8), 63 (9.5); Anal. Calcd. for C₂₇H₁₉NO₃S (421.52): C, 76.94; H, 4.54; N, 3.32; S, 7.61%. Found: C, 76.95; H, 4.21; N, 3.24; S, 7.63.
4,5-Dihydrothiophene-3-carbonitriles 15a,b

A mixture of the picolinium salt 3 (0.59 g, 2 mmol) and the appropriate arylidenecyanothioacetamide 14 (2 mmol) were refluxed in absolute ethanol (30 mL) in the presence of triethylamine (0.15 mL) for 4 hours and then cooled. The reaction mixture was poured onto ice cold water and neutralized with 10% hydrochloric acid. The solid product was collected, washed with water, dried and finally recrystallized from dioxane and ethanol, respectively, to afford the corresponding 4,5-dihydrothiophene derivatives 15a and b.

2-Amino-4-(4-bromophenyl)-5-(2-thienylcarbonyl)-4,5-dihydrothiophene-3-carbonitrile (15a): yellow crystals from ethanol; mp 250 °C; yield 70%; IR: ν = 3364, 3212 (NH2), 2215 (C=N), 1720 (CO); 1H-NMR: δ (ppm) = 3.94 (d, 1H, \( J = 17 \) Hz), 4.89 (d, 1H, \( J = 17 \) Hz), 7.21 (d, 2H), 7.34-7.94 (m, 3H), 7.88 (d, 2H), 8.65 (br. S, 2H, NH2, D2O exchangeable); 13C-NMR: δ (ppm) = 44.09, 53.08, 68.29, 110.73, 118.15, 130.67, 131.42, 131.78, 132.97, 137.93, 139.13, 141.98, 161.27, 186.49; MS: \( m/z (%) \) = 393 (M++2, 6.4), 392 (M++1, 7.4), 391 (M+, 18.3), 279 (16.5), 111 (44.5), 83 (11.8), 80 (100.0), 64 (70.6); Anal. Calcd. for C16H11BrN2OS2 (391.30): C, 49.11; H, 2.83; Br, 20.42; N, 7.16; S, 16.39%. Found: C, 49.22; H, 2.54; Br, 20.32; N, 7.16; S, 16.39%.

2-Amino-4-(4-methoxyphenyl)-5-(2-thienylcarbonyl)-4,5-dihydrothiophene-3-carbonitrile (15b): yellow crystals from ethanol; mp 170-172 °C; yield 60%; IR: ν = 3360, 3192 (NH2), 2215 (C=N), 1723 (CO); 1H-NMR: δ (ppm) = 3.70 (s, 3H, OCH3), 4.02 (d, 1H, \( J = 17 \) Hz), 4.96 (d, 1H, \( J = 17 \) Hz), 6.65 (br. S, 2H, NH2, D2O exchangeable), 7.27-8.31 (m, 3H), 7.45 (d, 2H); 13C-NMR: δ (ppm) = 49.64, 56.90, 57.82, 70.39, 113.58, 116.82, 130.80, 131.35, 131.93, 132.72, 135.89, 144.82, 155.92, 161.59, 184.18; MS: \( m/z (%) \) = 343 (M++1, 11.4), 342 (M+, 26.8), 233 (18.6), 124 (16.7), 111 (86.1), 107 (48.5), 83 (100.0), 80 (18.3), 64 (10.2); Anal. Calcd. for C17H14N2O2S2 (342.43): C, 59.63; H, 4.12; N, 8.18%; Found: C, 59.58; H, 3.99; N, 8.02; S, 18.51.

General procedure for the synthesis of 2-(thiophene-2-yl)carbonyl-3,5-diaryl-4,6-dicyanoaniline derivatives 17a-c:

A mixture of compound 3 (0.59 g, 2 mmol) and the appropriate arylidenemalononitrile 16a-c (4 mmol) was refluxed in pyridine (20 mL) for 6 hours, then cooled. The reaction mixture was poured onto ice cooled water, neutralized with 10% hydrochloric acid. The solid product was collected, washed with water, dried, and finally recrystallized from a mixture of ethanol and dimethylformamide (3:1) to afford the corresponding compounds 17a-c.

2-(Thiophene-2-yl)carbonyl-3,5-diphenyl-4,6-dicyanoaniline (17a): yellow crystals; mp 224 °C; yield 70%; IR: ν = 3335, 3104 (NH2), 2197(CN), 1665(CO); 1H-NMR: δ (ppm) = 6.86-7.80 (m, 3H), 7.45 (dd, 1H), 7.60 (dd, 1H), 7.78 (d, 2H), 7.92 (dd, 2H), 8.00 (d, 2H), 8.10 (dd, 2H), 8.20 (br. s, 2H, NH2, D2O exchangeable); 13C-NMR: δ (ppm) = 92.08, 96.95, 99.15, 120.96, 125.37, 127.59, 129.20, 129.37, 130.65, 130.57, 131.12, 131.93, 134.82, 135.66, 136.94, 137.96, 139.50, 148.36, 151.49, 152.29, 193.42(CO); MS: \( m/z (%) \) = 405 (M++, 18.8), 293 (15.6), 207 (12.6), 180 (18.5), 179 (31.9), 178 (40.2),...
2-(Thiophene-2-yl)carbonyl-3,5-di-2-furyl-4,6-dicyanoaniline (17b): yellow crystals; mp 280 °C; yield 80%; IR: ν = 3342, 3119 (NH₂), 2211 (C=N), 1667 (CO); ¹H-NMR: δ (ppm) = 6.70-7.95 (m, 9H, aromatic protons), 8.20 (br. s, 2H, NH₂, D₂O exchangeable); MS m/z (%) 385 (M⁺, 22.6), 301 (16.6), 111 (100), 83 (20.5), 67 (20.5), 64 (15.1); Anal. Calcd. for C₂₁H₁₁N₃O₃S (385.40): C, 65.45; H, 2.88; N, 10.90; S, 23.04%. Found: C, 65.38; H, 2.65; N, 10.88; S, 23.30.

2-(Thiophene-2-yl)carbonyl-3,5-di-2-thienyl-4,6-dicyanoaniline (17c): yellow crystals; mp 276 °C; yield 65%; IR: ν = 3326, 3205 (NH₂), 2210 (C=N), 1658 (CO); ¹H-NMR: δ (ppm) = 7.00-7.86 (m, 9H, aromatic protons), 8.00 (br. s, 2H, NH₂, D₂O exchangeable); MS: m/z (%) = 417 (M⁺, 11.3), 304 (15.6), 253 (9), 236 (8), 111 (35), 94 (46), 83 (100.0), 66 (10.5), 54 (15.1); Anal. Calcd. for C₂₁H₁₁N₃OS₃ (417.53): C, 60.41; H, 2.66; N, 10.06; S, 23.04%. Found: C, 60.38; H, 2.65; N, 10.88; S, 23.30.

2-(2-Thienyl)indolizine (18)

To a solution of compound 3 (0.59 g, 2 mmol), in benzene (30 mL), triethylamine (0.15 mL, 1.5 mmol) was added and the mixture was refluxed for 6 hours, then cooled to room temperature. The solid salts were removed by filtration, and the filtrate was evaporated under vacuum. The residue was treated with methanol and the solid precipitate was filtered off, washed with methanol, and dried. Recrystallization from ethanol afforded 18 as yellow crystals; mp 170 °C; yield 45%; IR: ν = 3098 (CH, aromatic), 1626 (C=C stretching); ¹H-NMR: δ (ppm) = 6.55-8.21 (m, 4H), 6.73 (s, 1H), 7.07-7.41 (m, 3H), 7.83 (s, 1H); ¹³C-NMR: δ (ppm) = 99.82, 111.73, 114.82, 115.51, 116.25, 124.15, 125.87, 127.18, 127.91, 129.17, 137.20, 141.64; MS: m/z (%) = 200 (M⁺+, 15.3), 199 (M⁺, 100.0), 198 (20.8), 154 (32.2), 141 (18.8), 113 (7.5), 100 (12.3), 93 (9.9), 86 (14.4), 83 (2.5), 82 (9.9), 77 (11.6), 69 (17.5), 64 (10.7), 50 (29.2); Anal. Calcd. for C₁₂H₉NS (199.28): C, 72.33; H, 4.55; N, 7.03; S, 16.09%. Found: C, 72.21; H, 4.36; N, 7.22; S, 16.11.

Biological activity

The antibacterial and antifungal activity assays were carried out in the Microbiology Division of Microanalytical Center of Cairo University using the diffusion plate method [22-24]. A bottomless cylinder containing a measured quantity (1mL, mg/mL) of the sample is placed on a plate (9 cm diameter) containing a solid bacterial medium (nutrient agar broth) or fungal medium which has been heavily seeded with a spore suspension of the test organism. After incubation (24 hours for bacteria and 5 days for fungi), the diameter of the clear zone of inhibition surrounding the sample is taken as measure of the inhibitory power of the sample against the particular test organism.
References and Notes

1. Queiroz, M. R. P.; Ferreira, I. C. F. R.; Gaetano, Y. D.; Kirsch, G.; Calhelha, R. C.; Estevinho, L. M. Synthesis and antimicrobial activity studies of orthochlorodiarylamines and heteroaromatic tetracyclic systems in the benzo[b]thiophene series. *Bioorg. Med. Chem.* **2006**, *14*, 6827-6831.

2. Thomson, P.; Naylor, M. A.; Everett, S. A.; Stratford, M. R. L.; Lewis, G.; Hill, S.; Patel, K. B.; Wardman, P.; Davis, P. Synthesis and biological properties of bioreductively targeted nitrothienyl prodrugs of combretastain A-4. *Mol. Cancer Therapeut.* **2006**, *5*, 2886-2894.

3. Kumar, P. R.; Raju, S.; Goud, P. S.; Sailaja, M.; Sarma, M. R.; Reddy, G. O.; Kumar, M. P.; Krishna Reddy, V. V. R M.; Suresh, T. and Hegde, P. Synthesis and biological evaluation of thiophene [3,2-b] pyrrole derivatives as potential antiinflammatory agents. *Bioorg. Med. Chem.* **2004**, *12*, 1221-1230.

4. Kipnis, F.; Soloway, H.; Ornfelt, J. 2-Acyloxyacetylthiophenes. *J. Am. Chem. Soc.* **1949**, *71*, 10-11.

5. Lee, S.; Lee, H.; Yi, K. Y.; Lee, B. H.; Yoo, S.; Lee, K.; Cho, N. S. 4-substituted (benzo[b]thiophene-2-carbonyl)guanidines as novel Na⁺/H⁺ exchanger isoform-1 (NHE-1) inhibitors. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2998-3001.

6. Fang, X.; Wu, Y.; Deng, J.; Wang, S. Synthesis of monofluorinated indolizines and their derivatives by the 1,3-dipolar reaction of N-ylides with fluorinated vinyl tosylates. *Tetrahedron* **2004**, *60*, 5487-5493.

7. Flitsch, W., Pyrroles with fused six-membered heterocyclic rings: (i) α-fused. In *Comprehensive Heterocyclic Chemistry*. Katritzky, A. R.; Rees, C. W., Eds.; Pergamon: Oxford, **1984**; vol. 4, pp. 443-495.

8. Gubin, J.; Lucchetti, J.; Nisato, D.; Rosseees, G.; Chinet, M.; Polster, P.; Chatelain, P. A novel class of calcium-entry blockers: the 1-[[4-(aminoalkoxy)phenyl]sulfonyl]indolizines *J. Med. Chem.* **1992**, *35*, 981-988.

9. Nugent, R. A.; Murphy, M. The synthesis of indolizines: the reaction of α-halo pyridinium salts with β-dicarbonyl species. *J. Org. Chem.* **1987**, *52*, 2206-2208.

10. Tsuge, O.; Kanemasa, S.; Takenaka, S. Stereochemical study on 1,3-dipolar cycloaddition reactions of heteroaromatic N-Ylides with unsymmetrically substituted olefinic dipolarophiles. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 3320-3336.

11. Kanemasa, S.; Takenaka, S.; Watanabe, H.; Tsuge, O. Tandem 1,3-dipolar cycloadditions of pyridinium or isoquinolinium methylides with olefinic dipolarophiles leading to cycl[3.2.2]azines."Enamine route" as a new generation method of azomethine ylides. *J. Org. Chem.* **1989**, *54*, 420-424.

12. Tsuge, O.; Kanemasa, S.; Takenaka, S. Stereochemical study on 1,3-dipolar cycloaddition reactions of heteroaromatic N-ylides with symmetrically substituted cis and trans olefins. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 3137-3157.

13. Zhang, L.; Liang, F.; Sun, L.; Hu, Y.; Hu, H. A novel and practical synthesis of 3-unsubstituted indolizines. *Synthesis* **2000**, 1733-1737.

14. Samet, A. V.; Shestopalov, A. M.; Nesterov, V. N.; Semenov, V. V. An improved stereoselective synthesis of 5-acyl-2-amino-4-aryl-3-cyano-4,5-dihydrothiophenes. *Synthesis* **1997**, 623-624.
15. Dawood, K. M. An efficient route to trans-4,5-dihydrothiophenes and thiazoles via nitrogen and sulfur ylides. *Synth. Commun.* **2001**, *31*, 1647-1658.

16. Hanedanian, M.; Loreau, O.; Taran, F.; Mioskowski, C. αJ-Addition of activated methylenes to alkynoates. A straightforward synthesis of multifunctional compounds *Tetrahedron Lett.* **2004**, *45*, 7035-7038.

17. Dawood, K. M.; Abdel-Gawad, H.; Ellithey, M.; Mohamed, H. A.; Hegazi, B. Synthesis, anticonvulsant, and anti-inflammatory activities of some new benzofuran-based heterocycles. *Arch. Pharm. Chem. Life Sci.* **2006**, *339*, 133-140.

18. Kajigaeshi, S.; Mori, S.; Fujiski, S.; Kanemasa, S. Exo-selective peripheral cycloaddition reactions of pyrido[2,1-α]isoindole. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 3547-3551.

19. Al-Omran, F.; El-Khair, A. A.; Elnagdi, M. H. A novel synthesis of 2-amino diarylketone derivatives and of polyfunctionally substituted quinolines. *Org. Prep. Proced. Int.* **1998**, *30*, 211-215.

20. Christian, S.; Rondestvedt, J.; Vogl, O. Arylation of unsaturated systems by free radicals. II. Arylation of maleimide by diazonium salts. *J. Am. Chem. Soc.* **1955**, *77*, 2313-2315.

21. a) Brunskill, J. S. A.; De, A.; Ewing, D. F. Dimerization of 3-aryl-2-cyanothioacrylamides, a [2s+4s] cycloaddition to give substituted 3,4-dihydro-2H-thiopyrans. *J. Chem. Soc., Perkin Trans 1*, **1978**, 629-633; b) Freemann, F. Properties and reactions of ylidenemalononitriles. *Chem. Rev.* **1980**, *80*, 329-350; c) Tornetta, B.; Scapini, G.; Guerrera, F.; Bernardini, A. Structure-antibacterial activity relations of arylthioamides.IV. Synthesis, UV spectra, and tuberculostatic activity in vitro of some arylvinylenethioamides. *Boll. Seduta Accad. Gioenia Sci. Nat. Catania* **1970**, *10*, 353-363; [Chem. Abstr. **1973**, *78*, 620n].

22. Muanz, D. N.; Kim, B. W.; Euler, K. L.; William, L. Antibacterial and antifungal activities of nine medicinal plants from zaire. *Int. J. Pharmacog.* **1994**, *32*, 337-345.

23. Grayer, R. J.; Harborne, J. B. A survey of antifungal compounds from higher plants, 1982-1993. *Phytochemistry* **1994**, *37*, 19-42.

24. Irab, O. N.; Young, M. M.; Anderson, W. A. Antimicrobial activity of annatto (Bixa orellana) extract. *Int. J. Pharmacog.* **1996**, *34*, 87-90.

Sample availability: Available from the author.

© 2008 by the author(s); licensee Molecular Diversity Preservation International, Basel, Switzerland. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).