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Synthesis of Thienopyridines and Isoquinolines from 6-Amino-1,2-dihydro-4-methyl-2-thioxopyridine-3,5-dicarbonitrile and of Phthalazines from Thieno[3,4-*c*]pyridazines

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Thienopyridine **1** and thienopyridazine **13** have been readily reacted with electron-poor olefins to give isoquinolines and phthalazines, respectively. Some of the obtained compounds were tested for their antimicrobial activity.

(Keywords: Diels-Alder reaction, Thienopyridines, Isoquinolines, Thienopyridazines, Phthalazines).

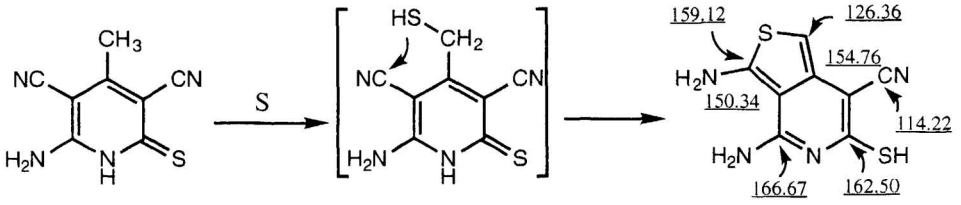
Introduction

One of the major characteristics of alkyl π-deficient compounds is their ability to form carbanions under mild conditions in contrast to alkyl aromatic hydrocarbons.[1,2] As a part of our program[3–6] we paid attention towards developing synthetic approaches to polyfunctionally substituted condensed heterocycles of potential biological activities. We wish to synthesize thienopyridine, isoquinazoline and phthalazine derivatives, also to discuss [4+2] cycloaddition reaction, beside the investigation of the antimicrobial activity of some newly synthesized products.

Results and Discussion

a) Chemistry

It has been found that pyridinethione **1**[4,7] readily reacts with elemental sulfur to yield the thieno[3,4-c]pyridine **3** *via* Gewald[8] like intermediate **2** (Scheme-1).



1 **2** **3**

Scheme-1

Structure **3** was established based on the spectroscopic (see Scheme-1, Experimental part) and the chemical evidence. Thus, Compound **3** reacted with acrylonitrile to yield isoquinoline **4**. It was believed that the latter resulted from a [4+2] cycloaddition of acrylonitrile at the thiophene moiety of **3** followed by hydrogen sulfide elimination[9,10] and a second addition of acrylonitrile at the thiol function. Compound **4** on boiling under reflux in dry benzene in the presence of sodium hydride afforded the thieno-isoquinoline **5** *via* [4+2] cycloaddition reaction. Compound **5** was also obtained *via* another independent route by reacting thienopyridine **1** with acylonitrile to afford thienopyridine **6**. Compound **6** was boiled under reflux in dry benzene in the presence of sodium hydride to furnish thienopyridine **7**. The structure of **7** was confirmed by IR, ^{1}H NMR and ^{13}C NMR (see Fig.-1).

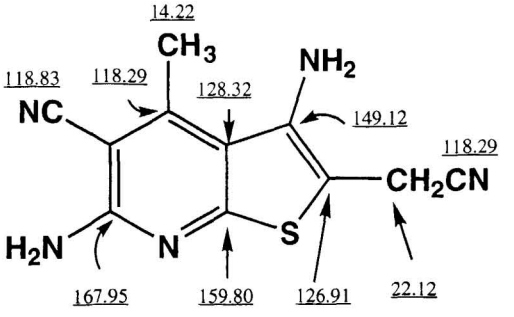
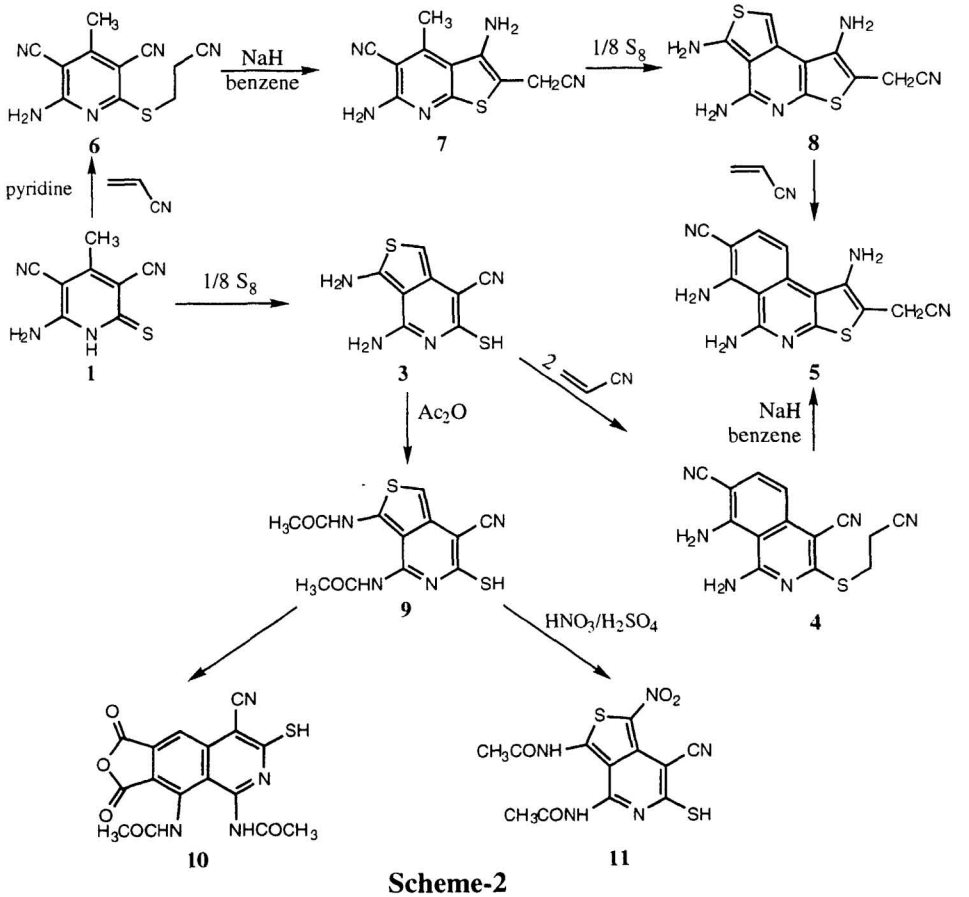


Fig. 1: ^{13}C NMR of compound **7**

Thienopyridine **7** reacted with elemental sulfur[8] to give thienopyridine **8**. This compound was transformed to the final isolable product



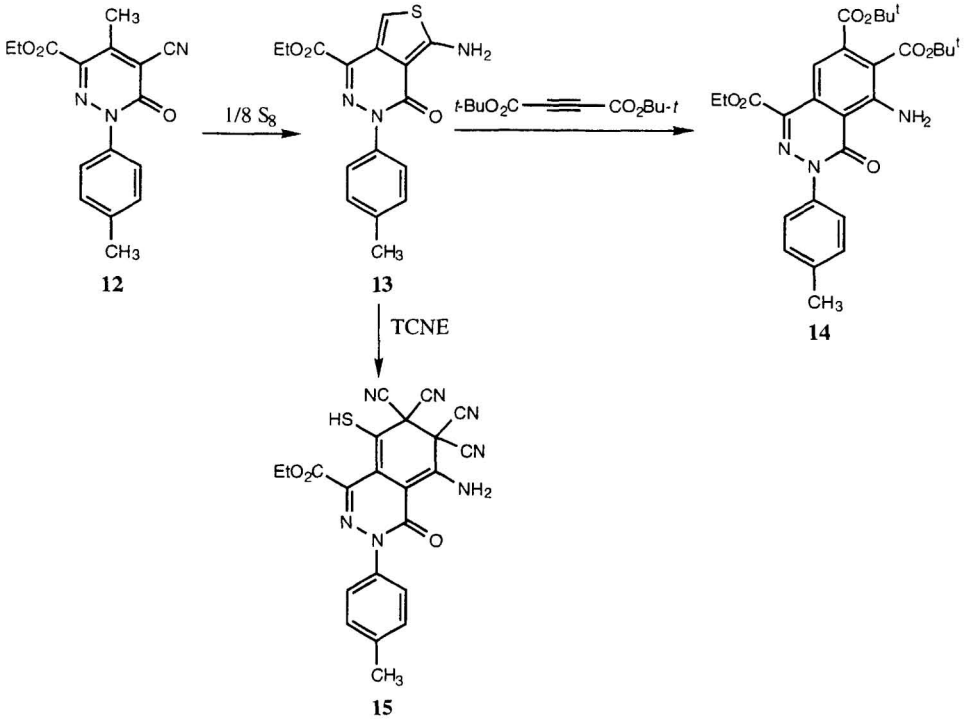
Scheme-2

5 by refluxing with acrylonitrile in the presence of acetic acid. The latter compound in this synthetic route was found to be identical to the one obtained from **4**. The comparison included tlc, mp, IR (Scheme-2).

Acylation of thieno[3,4-*c*]pyridine **3** with acetic anhydride afforded the diacetyl derivative **9**. The latter reacted with maleic anhydride to yield isoquinoline **10** *via* [4+2] cycloaddition with H_2S elimination. Compound **9** was converted into the nitro derivative **11** upon nitration. Attempts to force compound **11** to react with dienophiles such as tetracyanoethylene or acetylene-dicarboxylates under a variety of conditions was failed. We believed that the double bonds in the thieno system became inert as a diene in the presence of strong electron withdrawing nitro group.

Pyridazinone **12**[7] reacted readily with elemental sulfur[8] to yield thienopyridazine **13** which in turn underwent [4+2] cycloaddition reaction. Thus, compound **13** reacted with di-*t*-butylacetylene dicarboxylate to yield phthazinedicarboxylate **14**.

The structure of **14** could be established *via* inspection of its spectral data (*cf.* Experimental part). Its mass spectrum revealed a molecular formula of $C_{28}H_{32}N_3O_7$ (*m/z* 523) corresponding to addition and aromatization by loss of sulfur. Thienopyridazine **13** also reacted at room temperature with tetracyanoethylene to yield tetracyanophthalazinone **15** (Scheme-3).



Scheme-3

b) Antimicrobial activity

The table shows the antimicrobial activity of some of the obtained compounds against a variety of bacteria. Also, in the same table there is a comparison between the activity of these compounds and some known antimicrobial agents. The antimicrobial activity of the compounds considered were tested on the Gram negative bacteria *Salmonella typhimurium* CAIM 1350, *Pseudomonas spp.* CAIM 13, *Shigella, Brodetella*; the Gram positive bacteria *Bacillus subtilis* CAIM 1007, *Staphylococcus aureus* CAIM 1352,

Table 1: Antimicrobial activity (diameter of inhibition zones in mm).

Compd.	*Salm.*	*Pseudo.*	*Staph.*	*Bacil.*
3	8	7	12	23
4	10	12	–	11
7	11	9	13	16
8	12	–	11	7
13	8	7	7	–
14	–	12	12	12
15	8	10	9	10
Tetracycline	25	30	25	27
Gentamycine	25	40	24	26
Chloramphenicol	–	–	7	10
Ampicillin sodium	23	12	12	15
Ampicillin anhydrous	20	10	15	15
Amoxacillin trihydrate	25	15	10	15

(–) means no inhibition.

c) Conclusion

It has been observed from the results obtained that all tested compounds behaved the same with respect to their antimicrobial against the tested microorganism. The most toxic compounds were **7** and **3**, whereas, the other tested compounds were less active in terms of toxicity. When the tested compounds were compared with the tested reference once, with respect to their antimicrobial activity, the last three reference compounds (as listed in table 1) showed a similar level of toxicity against the tested microorganism. Nevertheless, only Gram negative bacteria (*Salm.*) was more sensitive towards these compounds.

Experimental

All melting points are uncorrected. IR spectra were recorded (KBr) on a Pye Unicam SP-1000 spectrophotometer. ^{1}H and ^{13}C NMR spectra

were obtained on Varian Gemini 200 MHz and 50 MHz spectrometer using TMS as internal reference. Chemical shifts are expressed as δ (ppm). Mass spectra were recorded on a GCMS-QP 1000 EX mass spectrometer operating at 70 eV. The elemental analyses were performed by the Microanalytical Unit, Cairo University.

6-Amino-1,2-dihydro-4-methyl-2-thioxopyridine-3,5-dicarbo-nitrile (1)[4]

A solution of cyanothioacetamide (3.0 g, 0.03 mol), malononitrile (1.98 g, 0.03 mol) and acetaldehyde (1.35 g, 0.01 mol) in EtOH (20 ml) with few drops of piperidine was refluxed for 30 min. The solid product formed on cooling, was collected by filtration and crystallized as orange crystals, lit[4] : 280°C.

1,7-Diamino-5-mercaptothieno[3,4-*c*]pyridine-4-carbonitrile (3)

To a solution of **1** (1.98 g, 0.01 mol) in dioxane (30 ml), sulfur (0.32 g, 0.01 mol) and a catalytic amount of triethylamine were added. The reaction mixture was heated under reflux for 3h. The solid product which formed on dilution with water was collected by filtration and crystallized from dioxane/EtOH to give brown crystals, **yield**: 84%; **mp**: > 300°C; **IR**: 3400-3300 (NH_2), 2245 (SH), 2224 (C≡N) cm^{-1}; **^{1}H NMR**: δ = 3.80 (br, 4H, $2NH_2$), 6,53 (s, 1H, H-3), 8.21 (s, 1H, SH); **^{13}C NMR**: see Scheme-1); **Found**: C, 42.90; H, 2.50; N, 24.9; S, 28.60. $C_8H_8N_4S_2$ **Requires**: C, 43.23; H, 2.72; N, 25.21; S, 28.85%.

1-Cyanoethylsulfide-6,7-diaminoisoquinoline-2,5-dicarbonitrile (4)

To a solution of **3** (2.22 g, 0.01 mol) in AcOH (20 ml), acrylonitrile (2.12 g, 0.04 mol) was added. The reaction mixture was heated under

reflux for 4 h. The solid product which formed upon evaporation of AcOH, was washed with hot EtOH and crystallized from dioxane/DMF (3:1) to give brown crystals, **yield**: 55%; **mp**: 282-4°C; **IR**: 3450-3200 (NH_2), 2222-2218 (C≡N) cm^{-1}; **^{1}H NMR**: δ = 2.71 (t, 2H, CH_2), 2.90 (t, 2H, CH_2), 3.71 (br, 4H, $2NH_2$), 7.89-8.0 (m, 2H, aromatic protons); **Found**: C, 57.00; H, 3.20; N, 28.60; S, 10.70. $C_{14}H_{10}N_6S$ **Requires**: C, 57.13; H, 3.42; N, 28.55; S, 10.89%.

6-Cyano-3,7,8-triaminothieno[2,3-*b*]isoquinoline-2-acetonitrile (5)

Method A

To a solution of **8** (2.75 g, 0.01 mol) in dioxane (20 ml), acrylonitrile (2.12 g, 0.04 mol) was added with drops of AcOH. The reaction mixture was heated under reflux for 14h. The solid product which formed upon evaporation, washed with hot EtOH and crystallized from AcOH to give brown crystals, 45%, **mp** > 300°C.

Method B

To a solution of NaH (0.96 g, 0.04 mol) in dry benzene, compound **4** (2.70 g, 0.01 mol) was added. The reaction mixture was refluxed for 10h. The solid product formed on evaporation of benzene was neutralized with dilute HCl to give 35% yield of **5**. **IR**: 3450-3200 (NH_2), 2221-2218 (CN) cm^{-1}; **^{1}H NMR**: δ = 3.78 (4H, $2NH_2$), 4.0 (s, 2H, CH_2), 7.0 (br, 2H, NH_2), 7.91-8.21 (m, 2H, aromatic protons); **Found**: C, 56.90; H, 3.60; N, 28.40; S, 10.60. $C_{14}H_{10}N_6S$. **Requires**: C, 57.13; H, 3.42; N, 28.55; S, 10.89%.

6-Amino-2-cyanoethylsulfide-4-methylpyridine-3,5-dicarbonitrile (6)

To a solution of **1** (1.90 g, 0.01 mol) in pyridine (20 ml), acrylonitrile (2.12 g, 0.04 mol) was added. The reaction mixture was heated under reflux for 30 min. The solid product, formed upon dilution with water, was collected by filtration and crystallized from EtOH to give yellow crystals, **yield**: 75%; **mp**: 210-1°C; **IR**: 3400-3220 (NH_2), 2224-2218 (C≡N) cm^{-1}; **^{1}H NMR**: δ = 2.38 (s, 3H, CH_3), 2.71 (t, 2H, CH_2), 2.90 (t, 2H, CH_2), 3.52 (br, 2H, NH_2); **MS**: m/z 243 (M^+); **Found**: C, 54.20; H, 3.60; 28.80; S, 13.00. $C_{11}H_9N_5S$ **Requires**: C, 54.37; H, 3.73; N, 28.79; S, 13.18%.

3,6-Diamino-5-cyano-4-methylthieno[2,3-*b*]pyridine-2-acetonitrile (7)

To a solution of NaH (0.96 g, 0.04 mol) in dry benzene (30 ml), compound **6** (2.43 g, 0.01 mol) was added. The reaction mixture was refluxed for 5h. The solid product formed on evaporation was neutralized with dilute HCl, then collected by filtration, crystallized from dioxane to give brown crystals, **yield**: 45%; **mp**: 230-1°C; **IR**: 3450-3200 (NH_2), 2222-2218 (C≡N) cm^{-1}; δ = 2.39 (s, 3H, CH_3), 3.50 (br, 2H, NH_2), 4.05 (s, 2H, CH_2), 7.04 (br, 2H, NH_2); **^{13}C NMR**: (see Fig.-2); **Found**: C, 54.10; H, 3.60; N, 28.90; S, 13.00. $C_{11}H_9N_5S$ **Requires**: C, 54.31; H, 3.73; N, 28.79; S, 13.18%.

Reaction of 7 with elemental sulfur (8)

To a solution of **7** (2.43 g, 0.01 mol) in dioxane (30 ml), sulfur (0.32 g, 0.01 mol) and a catalytic amount of triethylamine were added. The reaction mixture was heated under reflux for 5h. The solid product

which formed on evaporation was crystallized from dioxane/DMF (3:1) to give compound **8** to give brown crystals, **yield**: 80%; **mp**: > 300°C; **IR**: 3450-3180 (NH_2), 2218 (C≡N) cm^{-1}; **^{1}H NMR**: δ = 3.80 (br, 4H, $2NH_2$), 4.05 (s, 2H, CH_2), 6.52 (s, 1H, thiene H-4), 7.04 (br, 2H, NH_2); **Found**: C, 47.70; H, 3.10; N, 25.20; S, 23.10. $C_{11}H_9N_5S_2$ **Requires**: C, 47.98; H, 3.29; N, 25.43; S, 23.29%.

1,7-Diacetyl-5-mercaptothieno[3,4-*c*]pyridine-4-carbonitrile (9)

A solution of **3** (2.22 g, 0.01 mol) in Ac_2O (10 ml) was refluxed for 1h. The solid product was collected by filtration and crystallized from AcOH to give brown crystals, **yield**: 90%; **mp**: > 300°C; **IR**: 3400, 3370 (NH), 2255 (SH), 2220 (C≡N), 1670 (CO) cm^{-1}; **^{1}H NMR**: δ = 3.41 (s, 3H, CH_3), 3.44 (s, 3H, CH_3), 5.16 (s, 1H, SH), 6,72 (s, 1H, H-3), 12.01 (br, 1H, NH), 12.41 (br, 1H, NH); **Found**: C, 46.80; H, 3.00; N, 17.90; S, 20.80. $C_{12}H_{10}N_4S_2O_2$ **Requires**: C, 47.05; H, 3.29; N, 18.29; S, 20.93%.

4,5-Bis(acetylamino-1,3-dioxo-7-mercapto-6,8-dihydrofuro[3,4-*c*]-isoquinoline-8-carbonitrile (10)

An equimolar amounts of **9** and maleic anhydride (0.98 g, 0.01 mol) were heated at 160°C (oil bath) for 20 min. The resulting cold product was washed several times with water and crystallized from dioxane. **yield**: 65%; **mp**: > 300°C; **IR**: 3270 (NH), 2252 (SH), 2220 (C≡N), 1810–1782 (C=O) cm^{-1}; **^{1}H NMR**: δ = 3.45 (s, 3H, CH_3), 3.49 (s, 3H, CH_3), 5.16 (s, 1H, SH), 7.12 (s, 1H, H-9), 12.12 (br, 1H, NH), 12.48 (br, 1H, NH); **MS**: m/z 370 (M^+); **Found**: C, 51.60; H, 2.60; N, 14.90; S, 8.80. $C_{16}H_{10}N_4O_5S$ **Requires**: C, 51.89; H, 2.72; N, 15.13; S, 8.66%.

1,7-Bis(acetylamino)-5-mercapto-3-nitrothieno[3,4-*c*]pyridine-4-carbonitrile (11)

A solution of **9** (9.18 g, 0.03 mol) in a concentrated mixture of HNO_3/H_2SO_4 (3:1) was heated in a water bath for 15 min. The solid product formed on dilution with ice, was collected by filtration and crystallized from AcOH to give yellow crystals, **yield**: 63%; **mp**: 222-3°C; **IR**: 3400-3350 (NH), 2250 (SH), 2221 (C≡N), 1680 (CO) cm^{-1}; **^{1}H NMR**: δ = 3.33 (s, 3H, CH_3), 3.42 (s, 3H, CH_3), 8.22 (s, 1H, SH), 12.01 (br, 2H, 2NH); **Found**: C, 40.90; H, 2.30; N, 19.70; S, 18.10. $C_{12}H_9N_5S_2O_4$ **Requires**: C, 41.02; H, 2.58; N, 19.93; 18.25%.

Ethyl di-*t*-butyl(5-amino-3,4-dihydro-3-*p*-tolyl-4-oxophthalazine)-1,6,7-tricarboxylate (14)

An equimolar amounts of **13**[7] and di-*t*-butylacetylene dicarboxylate (2.26 g, 0.01 mol) in dioxane (20 ml) and drops of AcOH were refluxed for 20 min. The reaction mixture poured onto ice. The solid product which formed was collected by filtration and crystallized from EtOH to give orange crystals. **yield**: 60%; **mp**: 135-6°C; **IR**: 3400-3330 (NH_2), 1730, 1655 (C=O) cm^{-1}; **MS**: m/z 523 (M^+); **Found**: C, 64.40; H, 6.00; N, 7.90. $C_{28}H_{32}N_3O_7$ **Requires**: C, 64.36; H, 6.17; N, 8.04%.

Ethyl (5-amino-3,4-dihydro-8-mercapto-4-oxo-3-*p*-tolyl-6,7-tetracyanophthalazine)-1-carboxylate (15)

A mixture of equimolar amounts of **13**[7] and tetracyano-ethylene (1.28 g, 0.01 mol) in dioxane (20 ml) and drops of AcOH was stirred overnight. The solid product which formed was collected by filtration and crystallized from dioxane to give **15** as violet crystals. **yield**: 72%;

mp: 285-6°C; **IR**: 3400-3330 (NH_2), 2245 (SH), 2220-2226 (C≡N), 1720, 1652 (C=O) cm^{-1}; **^{1}H NMR**: δ = 1.22 (t, 3H, CH_3), 2.20 (s, 3H, CH3), 3.21 (br, 2H, NH_2), 4.20 (q, 2H, CH_2), 6.82-7.50 (m, 4H, aromatic protons), 9.21 (s, 1H, SH); **Found**: C, 57.80; H, 3.10; N, 21.60; S, 6.90. $C_{22}H_{15}N_7O_3S$ **Requires**: C, 57.76; H, 3.30; N, 21.43; S, 7.01%.

Antimicrobial Assay

The antimicrobial activity of the tested compounds were carried out by disc diffusion technique as described in The British Pharmacopoeia. Nutrient agar was melted at 45°C and inoculated by the spore suspension (1 ml/100 ml) bacteria. The flask was shaken well and poured into a *Petri*-dish (8 cm in diameter). Four discs (0.5 cm diameter each) were dipped in a solution of *propels* extract (at different concentrations) after solidification of medium, the 4 discs were aseptically transferred and left for evaporation of the solvent and then placed on the upper surface of the inoculated plates, which kept in a refrigerator for 1h to permit diffusion of antimicrobial substances. The plates were incubated at 37°C for 24h. The zone of inhibition was measured in mm. The experiments were performed three times and then the mean values of the inhibition zone were taken.

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