Synthesis and antimicrobial activity of new polyfunctionally substituted pyridazines and their fused derivatives

Yehya M. Elkholy

Chemistry Department, Faculty of Science, Helwan University, Ain Helwan ,
Cairo, Egypt .E-mail: y_elkholy@Yahoo.com

Abstract: Aminoacetonitrilehydrogensulfate was reacted with aldehydes to give the schiff bases **3**. The latter were reacted with dimethyl acetylenedicarboxylate to afford the pyridine derivative **4,** which reacted with hydrazine hydrate and aromatic amine respectively to give pyridopyridazine and pyrrolopyridine. The antimicrobial activity of the prepared compounds was tested.

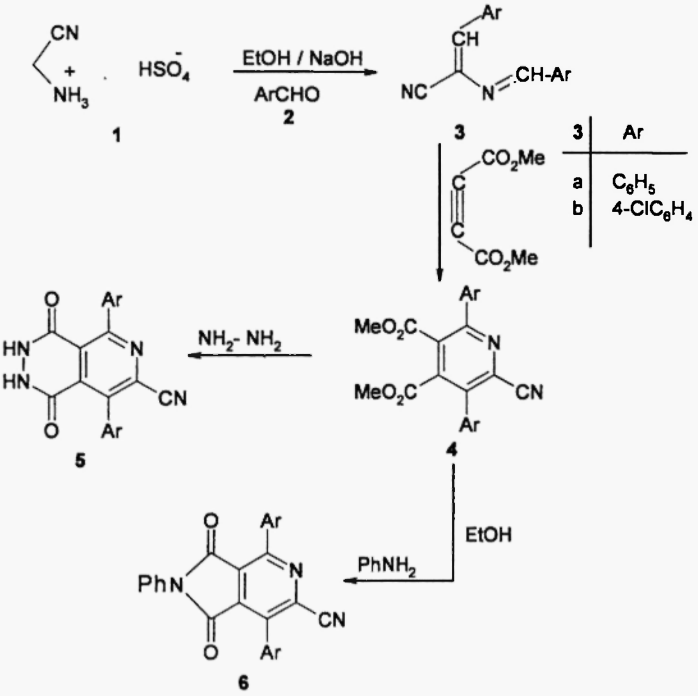
Key words: Aminoacetonitrilehydrogensulfate, schiff bases, [4+2] cycloaddition, active methylene, benzylidenemalononitrile .

Introduction

Pyridazine derivatives comprise a very interesting class of compounds because of their significant biological and pharmaceutical activities [1-5]. As part of our studies aimed at the development of simple and efficient syntheses of polyfunctional heteraromatics from readily obtained starting materials [6-8], we report herein the synthesis of heterocyclic compounds containing both fused pyridine and pyrrole, through the use of aminoacetonitrilehydrogensulfate as the starting material.

Results and discussions:

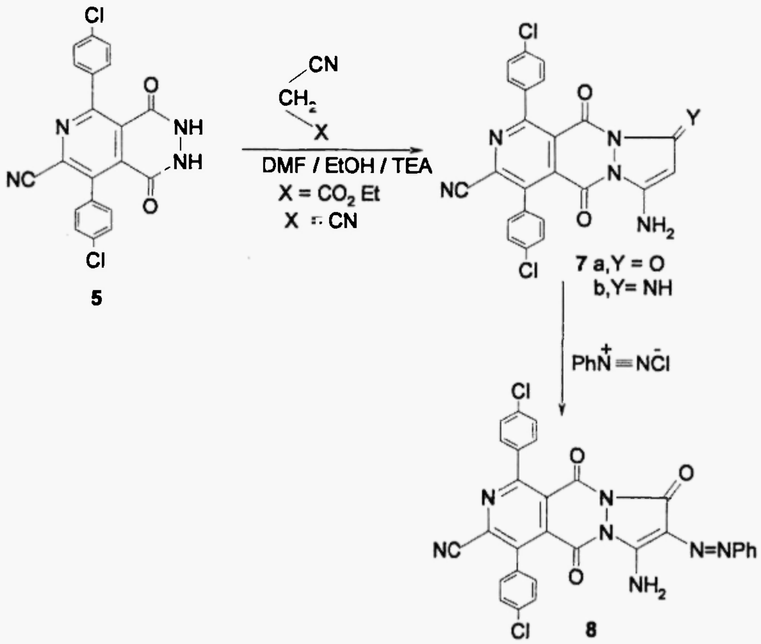
Thus, aminoacetonitrilehydrogensulfate **1** was reacted readily in basic medium with aromatic aldehydes , in 1:2 molar ratio, to give the Schiff bases **3**. The reaction products were assumed to proceed through addition of nucleophilic centers in aminoacetonitrile to the electrophilic carbonyl carbon in aldehyde followed by loss of two molecules of water to furnish the final isolated products **3a,b**. Structure **3b** was established based on MS which shows m/z 300 (M^+, 20%), 302 (M+2, 14%); 304 (M+4, 2.4%) in addition to other elemental and spectral analysis. Schiff base chemistry is probably the most commonly adopted synthetic approach to π-deficient heteroaromatic. Thus, compound **3** reacted undergoes [4 + 2] cycloaddition with dimethyl acetylenedicarboxylate to give the pyridine derivative 4 (scheme 1). The latter product reacted with hydrazine hydrate in ethanol to yield the pyridopyridazine derivative **5** through elimination of two molecules of methanol. Structure **5** was established based on elemental and spectral data. For example IR reveals the presence of amide carbonyl at 1700 and 1680 cm^{-1}, in addition, ^1H-NMR revealed the presence of NH group at δ 11.03 ppm. Reactivity of compound **4** toward aromatic amine, afford the pyrrolopyridine derivative. Structure **6** was established based on IR which revealed the presence of two carbonyl groups at 1700 and 1680 cm^{-1}. (Scheme-1).



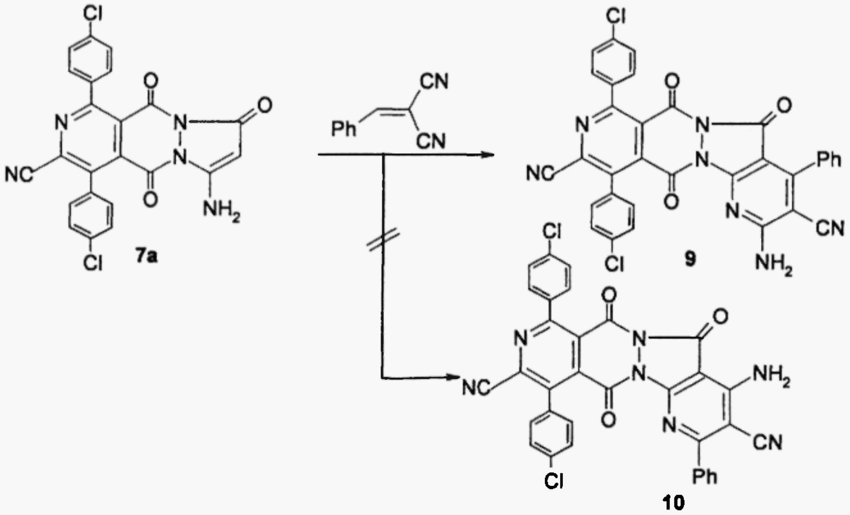
Scheme-1

Furthermore, pyridopyridazine **5** reacts with ethylcyanoacetate in 1:1 a mixture of *N,N*-dimethylformamid (DMF) and ethanol mixture in the presence of a catalytic amount of triethylamine to yield the products **7a,b**. Structure **7a** is suggested for this substance on the basis of elemental analysis and spectral data. For example IR spectra reveals the presence of the amino group at 3400-3350 cm^{-1} and the carbonyl group at 1700 and 1680 cm^{-1}. The ^{1}H NMR spectrum shows a singlet for the amino protons at δ 6.2 ppm (D_2O-exchangeable), a singlet for the CH-pyrazole at 6.71 ppm in addition to the aromatic protons at δ 7.70-7.78 ppm. Similarly, the amino–imine **7b** could be prepared by heating under reflux of **5** with malononitrile in a DMF/ethanol mixture and a catalytic amount of triethylamine. Compound **7a** coupled readily with benzenediazonium salt in DMF sodium acetate to yield the corresponding product **8**.

The reaction of **7a** with benzylidenemalononitrile in DMF/ethanol mixture in basic medium afforded 1:1 adducts which can be formulated as **9** or isomeric **10**. Structure **9** was established for the product based on ^{1}H-NMR spectrum which revealed the presence of phenyl ring protons at high field. This is consistent with structure **9** in which these protons are shielded by the carbonyl group. If the reaction product were **10** one would expect these same protons to be deshielded as a result at C=N anisotropy[9] (scheme-3).



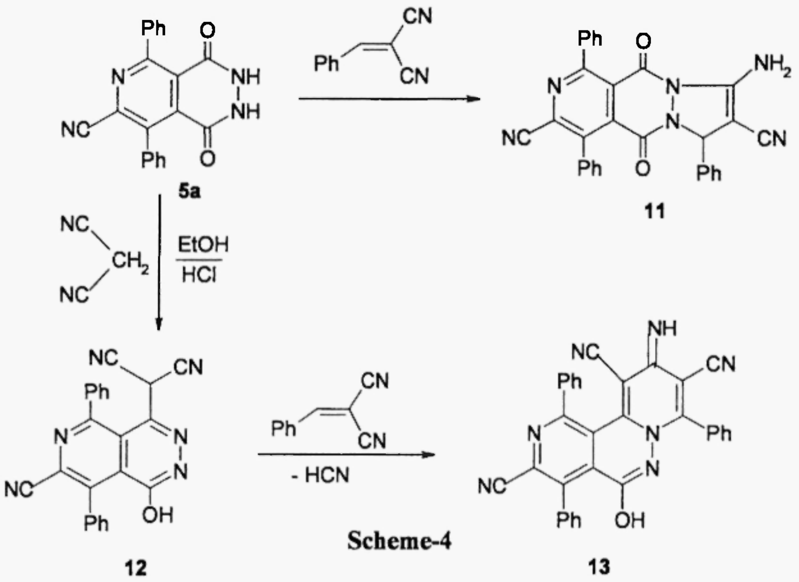
Scheme-2



Scheme-3

Moreover, pyridopyridazine **5** reacted with benzylidenemalononitrile in a mixture of DMF / ethanol (1:1) and a catalytic amount of triethylamine to give the isolated product **11**. Structure **11** was established based on elemental and spectral data. IR revealed the presence of NH_2 group at 3450-3300 cm^{-1} and two CN groups at 2210, 2218 cm^{-1}, in addition ^{1}H-NMR revealed the presence of NH_2 group at δ 5.31ppm. H-pyrazole at δ 5.60 ppm and aromatic at δ 7.70-7.20 ppm.

Compound **5** reacts with malononitrile in ethanolic HCl to yield product **12,** compound **12** reacted with benzylidenemalononitrile to afford product **13**. Structure **13** is suggested for the reaction product based on elemental analysis and spectral data (Scheme 4). ^{1}H-NMR revealed the presence of OH group at δ10.1 ppm, NH group at δ11.35ppm and aromatic protons at δ7.80-7.50 ppm.



Scheme-4

Antimicrobial activity: The various biological activities of pyridazine derivatives Promoted our attention to study the antimicrobial activities of some newly synthesized products. The bactericidal and fungicidal activities were studied. Thus, compounds **5, 7a** and **12** have shown activities against *S.aureus*, *B.subtilis* and *B.cereus*. Antifungal study reveals that compound **13** is the most active against *A.niger* and *C.albicans*, while compound **8** have shown activity against *P.italicum* and *C. albicans* . The data in Table I show that most of the tested compounds are effective..

Experimental

All mps were measured on a Buchi apparatus and are uncorrected. IR spectra were recorded (KBr) on a Pye Unicam sp-1000 spectrophotometer. ^{1}H NMR spectra were obtained on a Varian EM-390 MHz (390) spectrometer in DMSO-d_6 using Me_4Si as internal standared and chemical shifts are expressed as δ(ppm). Mass spectra were recorded on GCMS- Qp 1000 EX mass spectrometer operating at 70 eV. Microanalytical data were performed by the Microanalytical Unit, Cairo University.

2,3-Dibenzalaminoacetonitrile (3a)

2,3-Di(4-chlorobenzal)aminoacetonitrile (3b)

To a solution of aminoaectonitrilehydrogensulfate **1** (0.01 mol) in water (60 mL) containing sodium hydroxide (0.01 mol), a solution of benzaldehyde or 4-chlorobenzaldehyde (0.02 mol) in

EtOH (50 ml) was added portion wise. The reaction mixture was stirred for 3h and set overnight at room temperature. The solid product that separated was collected by filtration and crystallized from ethanol.

3a: Yield 68%, m.p 136 °C. IR : 2218 cm^{-1} (CN). ^{1}H-NMR δ, ppm: 5.81 (s, 1H, CH=C); 6.33(s, 1H, CH=N), 6.78-7.52 (m, 10 H, aromatic-H). (Found: C, 82.72; H, 5.17; N, 12.06 $C_{16}H_{12}N_2$ (232.30) requires C, 82.73; H, 5.21; N, 12.06).

3b: Yield 65% m.p 141 °C. IR : 2210 cm^{-1} (CN). ^{1}H NMR δ, ppm: 5.80, 6.32 (s, 2H, C=CH-, N=CH-); 7.10-7.51(m, 8H, aromatic-H). (Found: C, 63.78; H, 3.32; N, 9.30 $C_{16}H_{10}Cl_2N_2$ (301.18) requires C, 63.80; H, 3.35; N, 9.30). MS: m/z 300 (M$^+$, 20%), 302 (M+2, 14%), 304 (M+4, 2.4%)

Dimethyl-2,5-di(4-chlorophenyl)-6-cyanopyridine-3,4-dicarboxylate (4)

To a solution of **3b** (0.01 mol) in dioxane (30 mL),was added dimethyl acetylenedicarboxylate (0.01 mol) was added. The reaction mixture was heated under reflux for 3h, then. cooled to room temperature triturated with water, where by the resulted solid product was collected by filteration, dried and crystallized from ethanol.

Yield 63 % m.p 115 °C. IR : 2218 (CN), 1735 cm^1 (CO). ^{1}H NMR δ, ppm: 2.33(s, 6H, 2CH$_3$); 6.78-7.31(m, 8H, aromatic-H). (Found: C, 59.86; H, 3.17; N, 6.34 $C_{22}H_{14}$ $Cl_2N_2O_4$ (441.28) requires C, 59.87; H, 3.20; N, 6.34.

5,8-Di(4-chlorophenyl)-1,4-dioxo-1,2,3,4-tetrahydropyrido[3,4-*d*]pyridazine-3-carbonitrile (5)

To a solution of **4** (0.01 mol) in ethanol (20 mL) hydrazine hydrate (0.01 mol) was added. The reaction mixture was heated under reflux for 3h. The reaction mixture was cooled to room temperature and then triturated with water, the solid product so formed was collected by filteration and crystallized from ethanol.

Yield 71% . m.p 210 °C. IR : 2210 (CN), 1700, 1680 cm^{-1} (CO) ^{1}H-NMR δ, ppm: 11.03 (s, 2H, 2 NH), 7.10-7.53 (m, 8H, aromatic-H). (Found: C, 58.67; H, 2.44; N, 13.69 $C_{20}H_{10}Cl_2N_4O_2$ (409.24) requires C, 58.69; H, 2.46; N, 13.69).

4,7-Di(4-chlorophenyl)-1,3-dioxo-2-phenyl-1,3-dihydropyrrolo-[3,4-*c*]pyridine5-carbonitrile (6)

A solution of pyridine derivative **4** (0.01 mol) in ethanol (20 mL), aniline (0.01 mol) was added. The reaction mixture was heated under reflux for 3h, left to cool at room temperature poured onto ice-cold water. The solid product separated was filtered off, dired and recrystallized from acetic acid .

Yield 63% m.p 180 °C. IR : 2218 (CN), 1700-1680 cm^{-1} (CO) ^{1}H NMR δ, ppm: 7.13-7.56 (m, 13H, aromatic-H). (Found: C, 66.38; H, 2.76; N, 8.93 $C_{26}H_{13}Cl_2N_3O_2$ (470.32) requires C, 66.39; H, 2.79; N, 8.93).

7-Amino-1,4-bis(4-chlorophenyl)-5,6,8,9,10,11-hexahydro-5,9,11-trioxopyrazolo- [1,2-*a*]pyrido[3,4-*d*]pyridazine-3-carbonitrile (7a)

7-Amino-1,4-bis(4-chlorophenyl)-5,6,8,9,10,11-hexahydro-5,11-dioxo—11-imino-pyrazolo[1,2-*a*]pyrido[3,4-*d*]pyridazine-3-carbonitrile (7b)

To a solution of **5** (0.01 mol) in a mixture of dimethylfromamide (DMF) and ethanol (40 mL; 1:1) containing triethylamine (0.5 mL); malononitrile or ethylcyanoacetate respectively (0.01 mol) was added. The reaction mixture was heated under reflux for 3h, the solid product formed upon pouring the solution onto ice-cold water was collected by filtration and recrystallized from ethanol.

7a: Yield 73%. m.p 210 °C. IR: 3400-3350 (NH_2), 2218 (CN), 1700-1680 cm^{-1}(CO). ^{1}H NMRδ, ppm: 7.70-7.78 (m, 8H, aromatic-H); 6.71(s, 1H, pyrazole-H), 6.20 (s, 2H, NH_2), (Found: C, 57.98; H, 2.31; N, 14.70 $C_{23}H_{11}Cl_2N_5O_3$ (476.29) requires C, 57.99; H, 2.33; N, 14.70).

7b: Yield 64%. m.p 234 °C. IR : 3450-3300 (NH, NH_2), 2210 (CN), 1700 cm^{-1} (CO). ^{1}H NMRδ, ppm: 7.10-7.50 (m, 8H, aromatic -H); 5.60 (s, 1H, pyrazole-H), 6.10 (s, 2H, NH_2), 5.91 (s, 1H, NH). (Found: C, 58.10; H, 2.52; N, 17.68 $C_{23}H_{12}Cl_2N_6O_2$ (475.31) requires C, 58.11; H, 2.54; N, 17.68).

7-Amino-1,4-di(4-chlorophenyl)5,6,9,10,11-pentohydro-5,9,11-trioxo-8-phenylazopyrazolo[1,2-*a*]pyrido[3,4-*d*]pyridazine-3-carbonitrile (8)

To a solution of **7a** (0.01 mol) in DMF(20 mL) / sodium acetate (3gm) in an ice bath, benzenediazonium chloride, (prepared from 0.01 mol aniline and the appropriate quantities of hydrochloric acid and sodium nitrite), was added drop wise with stirring. The solid product, so formed was collected by filtration and recrystallized from dioxane.

Yield 59%. m.p 194 °C. IR : 3450-3400 (NH_2), 2218 (CN), 1700-1650 cm^{-1}(CO). ^{1}H NMR δ, ppm: 7.80-7.30 (m, 13H, aromatic-H); 8.20 (s, 2H, NH_2), (Found: C, 60.0; H, 2.58; N, 16.89 $C_{29}H_{15}Cl_2N_7O_3$ (580.41) requires C, 60.00; H, 2.61; N, 16.89.

8-Amino-5,6,12,13,10a,11-hexahydro-5,11,13-trioxo-1,4,10-triphenylpyrazolo- [2,1-*c*]pyrido[1,2-*a*]pyrido[3,4-*d*]pyridazine-3,4-dicaronitrile (9)

Equimolar amounts of **7a** (0.01 mol) and benzylidene malononitrile (0.01mol) in DMF/ethanol (40 mL; 1:1) were treated with a few drops of triethylamine. The reaction mixture was refluxed for 3h. The solid product, so formed, was collected by filtration.

Yield 69%. m.p 222 °C. IR : 3400-3200 (NH_2), 2210 ,2218(CN), 1700 ,1690 cm^{-1} (CO). ^{1}H NMR δ, ppm: 7.10-6.90 (m, 13H, aromatic-H); 5.60 (s, 2H, NH_2), (Found: C, 63.94; H, 2.38; N, 15.60 $C_{33}H_{15}Cl_2N_7O_3$ (628.45) requires C, 63.07; H, 2.41; N, 15.60.

9-Amino-5,11-dioxo-5,6,10,11-tetrahydro-1,4,7-triphenylpyrazolo-[1,2-*a*]pyrido- [3,4-*d*]pyridazine-3,8-dicarbonitrile (11)

To a solution of **5a** (0.01mol) in dimethylformamide DMF/ ethanol (20 mL, 1:1) containing triethylamine (0.5 mL), benzylidenemalononitrile (0.01 mol) was added. The reaction mixture was heated under reflux for 3h. The solid product formed upon pouring the solution into ice-cold water was collected by filtration and recrystallized from ethanol.

Yield 68%. m.p 263 °C. IR : 3450-3300 (NH_2), 2210, 2218 (CN), 1700, 1680 cm^{-1} (CO). ^1H NMR δ, ppm: 7.70-7.20 (m, 15 H, aromatic-H); 5.60 (s, 1H, pyrazole-H), 5.31 (s, 2 H, NH_2), (Found: C, 73.02; H, 3.44; N, 17.03 $C_{30}H_{18}N_6O_2$ (494.54) requires C, 72.85; H, 3.67; N, 16.99).

1,4-Diphenyl-5-hydroxy-8-malononitrilopyrido[3,4-*d*]pyridazine-3-carbonitrile (12).

To a solution of **5a** (0.01 mol) in ethanol (20 mL) containing 5 drops of HCl, malononitrile (0,01 mol) was added. The reaction mixture was heated under reflux for 3h.. The solid product, so formed, was collected by filtration and recrystallized from dioxane.

Yield 75%. m.p 241 °C. IR : 3450 (OH), 2220, 2218, 2210 cm^{-1}(CN), ^1H NMR δ, ppm: 4.56 (s, 1H, CH); 7.70-7.30 (m, 10H, aromatic-H); 10.11 (s, 1H, OH), (Found: C, 71.31; H, 2.84; N, 21.70 $C_{23}H_{12}N_6O$ (388.41) requires C, 71.11; H, 3.12; N, 21.64).

10-Imino-5-hydroxy-1,4,8-triphenylpyrido[1,2-*a*]pyrido[3,4-*d*]pyridazine-3,9,11-tricarbonitrile (13).

To a solution of **12** (0.01 mol) in dimethylfomramide DMF/ ethanol (20 mL; 1:1) containing triethylamine (0.5 mL), benzylidenemalononitrile (0.01 mol) was added. The reaction mixture was heated under reflux for 3h. The solid product, formed upon pouring the solution into ice/cold water was collected by filtration and recrystallized from ethanol.

Yield 66% .m.p 218 °C. IR : 3450 (OH), 3440 (NH), 2220, 2218, 2210 cm^{-1} (CN), ^1H NMR δ, ppm: 11.35 (s, 1H, NH), 10.10 (s, 1H, OH), 7.80-7.50 (m, 15H, aromatic-H); (Found: C, 74.80; H, 3.57; N, 19.44 $C_{32}H_{17}N_7O$ (515.56) requires C, 74.54; H, 3.33; N, 19.02).

Biological Activity:-

All the compounds prepared were screened for their activity against Gram-positive, Gram-negative bacteria and fungi. The results are given in Table-1.

Biological Tests

Standard drugs (amoxicillin for bacteria and mycostatin for fungi) were used at a concentration of 1000 ppm for comparisons. The biological activity of these compounds have been evaluated by filter paper disc method [10] after dissolving in *N,N*-dimethylformamide to obtain a 1 mg /mL solution (1000 ppm). The inhibition zones of microbial growth surrounding the filter paper disc (5 mm) were measured in millimeters at the end of an incubation period of 3 days at 37°C for bacteria and fungi. *N,N*-dimethylformamide alone showed no inhibition zone.

Table-1: ***In vitro*** **bactericidal and fungicidal activity of some of the synthesized compounds**

Compound No.	Staphylo-coccus aureus	Bocillus subtilis	Bocillus cereus	Aspergills niger	Penicillium italicum	Candeda albicans
4	+	+	-	+	+	-
5	++	-	++	-	++	+
6	+	+	+	++	-	++
7a	-	++	-	+	-	+
7b	+	-	+	+	-	+
8	+	+	+	+	++	++
9	-	+	+	++	+	+
11	+	-	-	++	-	+
12	++	++	++	+	+	++
13	+	+	-	+++	+	+++

Slight effect = +, moderate effect = ++ , severe effect = +++, complete effect = ++++. **Rating percent control**: no effect = o; slight effect = 10, 20,30; moderate effect = 40,50,60; severe effect = 70,80,90; complete effect = 100

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