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BINUCLEOPHILIC REACTION IN SYNTHESIS OF PYRIDOPYRROLOPYRAZINES AS POTENTIAL ANTIMICROBIAL AGENTS

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ABSTRACT

Synthesized from dichloromaleimides and 5-bromo -2,3-diaminopyridine as binucleophil, novel pyridopyrrolopyrazines have been described. The synthetic approach involved application of reductive nitrosation reaction recently developed in our laboratories.

INTRODUCTION

In a previous study a new reductive nitrosation reaction [1] has been developed for conversion of maleimides having vicinal dichloro groups into 2- (N-nitroso) amino succinimides , a class of compounds which are not easily synthesized by other methods.

This work aimed at checking the versatile applicability of the reaction and its scope in synthesis of new potential antimicrobial pyridopyrrolopyrazines.

5-Bromo-2,3 - diaminopyridine [2] is a binucleophilic reactant which on treatment with N- substituted phenyl -2,3- dichloromaleimides [3,4] in methanolic solution is described. The assignment of the new products were achieved by study of IR , ^{1}H-NMR and microanalysis .

The IR showed just one carbonyl confirming an intramolecular nuclephilic condenstation with imidecarbonyl at position 1.

The 5- bromo- 2,3- diaminopyridine firstly attacks at position 2 via Michael type addition followed by intramolecular nucleophilic cyclization at position 1 rather than position 3 which becomes comparatively less reactive due to the positive electronic effects induced by the intermediate enamine structure.

This binucleophilic reaction is the best way to obtaine pryidopyrrolopyrazines.

However, this reaction was used by the auther and others[5] in previous study to obtain pyrrolobenzoxazines and pyrroloquinoxalines by reacting O. aminophenol and O. phenylenediamine respectively in place of 5-boromo -2,3- diaminopyridine. So, it was encouraging to find out the effect of the presence of the bromopyridine ring in place of benzene ring on the activity against different strains of microorganisms. Reaction of the maleiomides (I, a - g) with 5-bromo -2,3-diaminopyridine in methanolic solution under reflux afforded 6- bromo -3- chloro-2-oxo-1- substituted phenyl -1 1H, 4H - pyrido [2,3-b]-pyrrolo [3,2 -2) pyrazines (II, a-g) in approximately quantitative yields. Attempted reduction of the chlorovinylic structure of compounds (II, a g) to cholorin free pyridopyrrolopyrazines by the usual reducing agents (eg) NaBH4., zinc and acetic or tributyline hydride was unsuccessful. However, reation of the compounds(II a-g)with hydrazine hydrate gave the hydrazino derivatives (III, a-g) with no effect on bromine [6,7].

Theoretically, these compounds are to be present either in the form A or B . The latter was proved by ^{1}H -NMR sepectra . Structures of (III, a-g) were further established by the synthesis of their arylidene derivatives (IV, a-g) through the interaction with anisaldehyde .

Application of the nitrosative reductive elimination reaction [8,9] involved the nitrosation of compounds (III, a-g).

The structure of compounds (IV, a-g) and (V, a-g) was established based on the study of elemental analysis , IR and ^{1}H -NMR spectral data.

EXPERIMENTAL

All melting points were determined using Gallenkamp apparatus and are uncorrected. Microanalyses were carried out at the Microanalytcal Centre, Cairo University. IR Spectra (KBr) were determined on a Perkin Elmer Model -137 infracord. The ^{1}H -NMR spectra were obtained at 90 MHz using TMS as an internal standard.

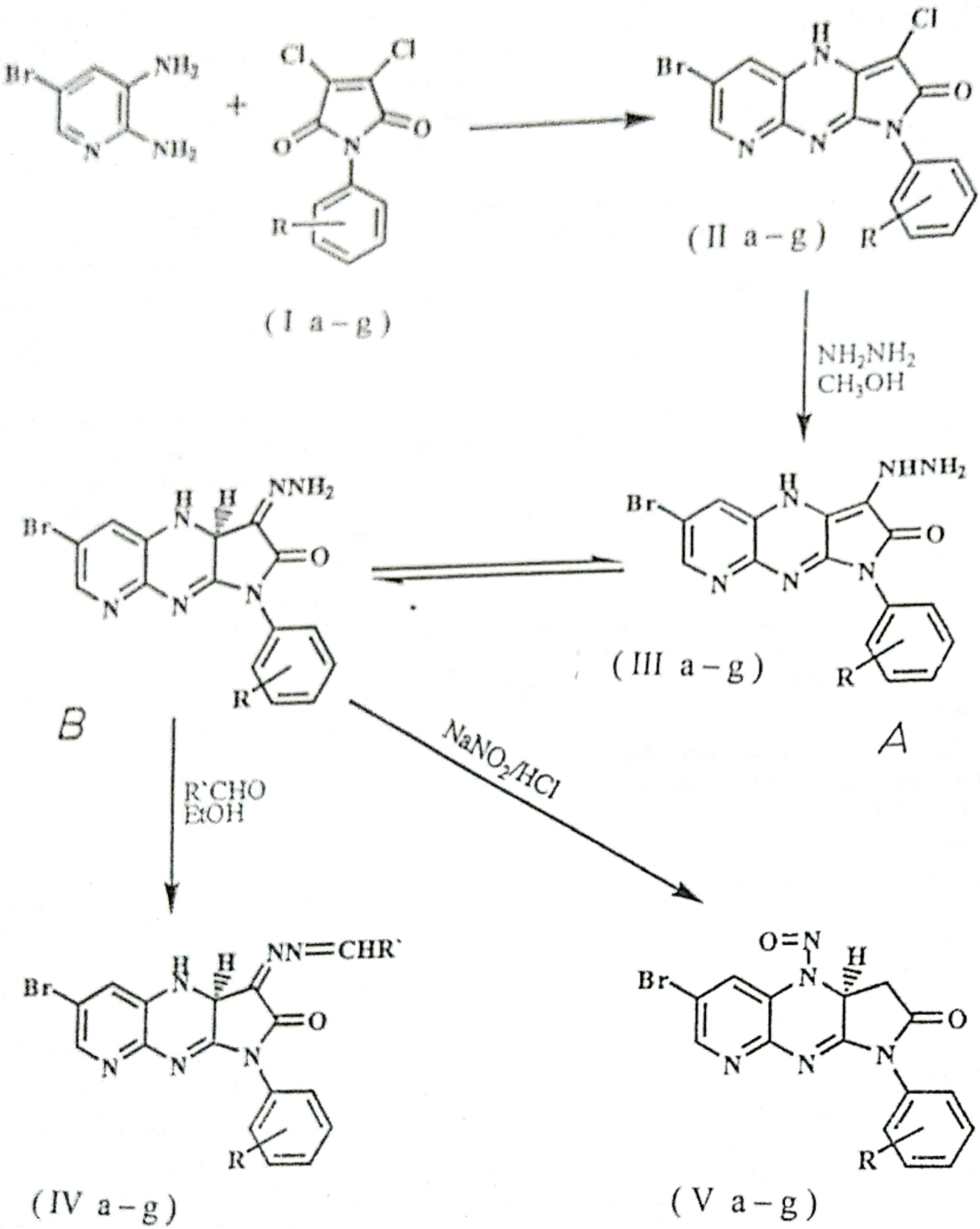
5-Bromo-2,3- diaminopyridine was prepared according to the reported method [2].

2,3-Dichloromaleimides (I, a-g) were prepared according to the reported methods [3,4].

6-Bromo-3- chloro-3-oxo-1-substituted phenyl -1H , 4H - pyrido [2,3-b]- pyrrolo [3,2-e]- pyrazines (II, a-g);

To a solution of (I, a - g)(10 mmol) in Me OH (30 mL), 5- bromo-2,3- diaminopyridine (1.9 g, 10 mmol) was added while stirring under reflux for 1 h. The reaction mixture was cooled, filtered and the separated crystals were crystallized from DMF / H_2O (Table 1).

6-Bromo-3,4- dihydro-3- hydrazino-2-oxo-1-substituted phenyl-1H , 4H- Pyrido [2,3-b) -pyrrolo [3,2-e] - pyrazine (III, a-g) .



R = H, o-CH_3, p-CH_3, p-OCH_3, p-CL, p-Br, and 2,6-$(CH_3)_2$.

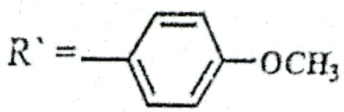
R` = 

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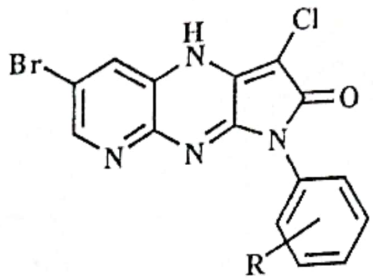
Table I 6-Bromo-3- chloro-2-oxo-1- substited phenyl -1H , 4H pyrido [2,3-b] - pyrrolo [3,2-e) pyrazines.



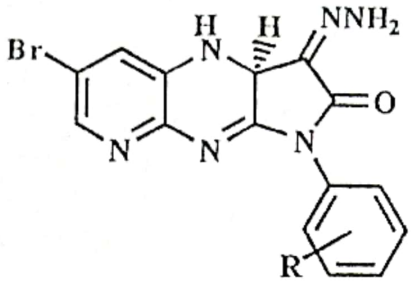
Comp	R	M.P.	Yield %	M. F. & M.Wt.	Microanalysis	
					Calc.	Found
IIa	H	289	84	$C_{15}H_8BrClN_4O$ (375-5)	C= 47.94 H= 2.13 N= 14.91	47.9 2.1 15.0
IIb	o.CH_3	296	82	$C_{16}H_{10}BrClN_4O$ (389.5)	C= 49.29 H= 2.57 N= 14.38	49.3 2.5 14.4
IIc	p.CH_3	298	83	$C_{16}H_{10}BrClN_4O$ (389.5)	C= 49.29 H= 2.57 N= 14.38	49.3 2.6 14.4
IId	p.OCH_3	267	91	$C_{16}H_{10}BrClN_4O_2$ (405.5)	C= 47.35 H= 2.47 N= 13.81	47.4 2.4 13.9
IIe	p.Cl	235	89	$C_{15}H_7BrCl_2N_4O$ (410)	C= 43.90 H= 1.71 N= 13.66	43.9 1.7 13.6
IIf	p.Br	284	79	$C_{15}H_7BrClN_4O$ (454.5)	C= 39.60 H= 1.54 N= 12.32	39.7 1.6 12.4
IIg	2,6 $(CH_3)2$	287	87	$C_{17}H_{12}BrClN_4O$ (403.5)	C= 50.56 H= 2.97 N= 13.88	50.5 2.9 13.9

IR (Cm^{-1}) : 3260-3220 (NH) , 3040 - 2910 (Ar- H and aliph.), 1670 - 1660 (C=O) 1640 - 1630 (C=N), 1590 - 1575 (C=C)

1H NMR (ppm) for compound :

IIc : 2.0 (s, 3H , CH_3) , 4.2 (s, br, 1H, NH) ; 7-7.7 (m, 6 H , aromatic protons).

Table II 6-Bromo-3-4- dihydro -3- hydrazono -2- oxo-1 substituted phenyl- 1H , 4H - pyrido [2,3-b] - pyrrolo [3,2-e) pyrazines.



Comp	R	M.P.	Yield %	M. F. & M.Wt.	Microanalysis	
					Calc.	Found
IIa	H	282	85	$C_{15}H_{11}BrN_6O$ (371)	C= 48.52 H= 2.96 N= 22.64	48.5 2.9 22.7
IIb	o. CH_3	289	89	$C_{16}H_{13}BrN_6O$ (385)	C= 49.87 H= 3.38 N= 21.82	49.9 3.3 21.9
IIc	p.CH_3	291	80	$C_{16}H_{10}BrN_6O$ (385)	C= 49.87 H= 3.38 N= 21.82	49.9 3.4 21.8
IId	p.OCH_3	260	83	$C_{16}H_{13}BrN_6O_2$ (401)	C= 47.88 H= 3.24 N= 20.95	47.9 3.3 21.0
IIe	p.Cl	229	91	$C_{15}H_{10}BrClN_6O$ (405.5)	C= 44.39 H= 2.47 N= 20.72	44.4 2.4 2.9
IIf	p.Br	280	95	$C_{15}H_{10}Br_2N_4O$ (450)	C= 40.00 H= 2.22 N= 18.67	40.2 2.2 18.7
IIg	2,6 $(CH_3)2$	279	79	$C_{17}H_{15}BrN_6O$ (399)	C= 51.13 H= 3.76 N= 21.05	51.3 3.6 21.2

1H NMR (ppm) for compound :

III a : 5.1 (s, br, 3H , NH + NH_2) ; 5.7 (s, 1H, proton at 3a position) ; 6.9 - 7.9 (m, 7H, aromatic protons).

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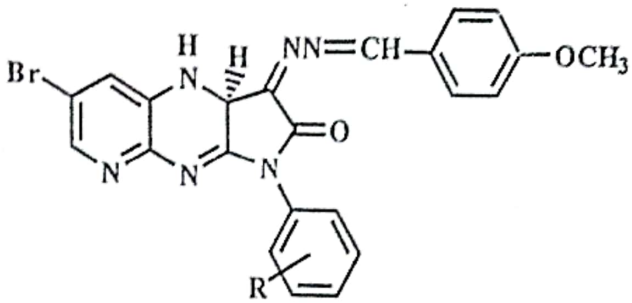
Table III 3-Arylidenchydrazino -6- bromo-2- oxo-1- subsrtituted -phenyl -1H, 4H - pyrido [2,5-b] pyrrolo [3,2-b] - pyrrolo [3,2 - e] pyrazines.



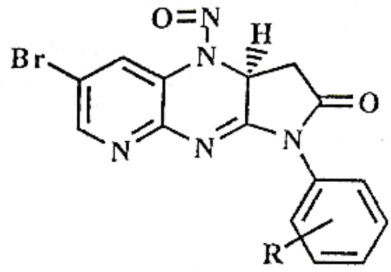
Comp	R	M.P.	Yield %	M. F. & M.Wt.	Microanalysis	
					Calc.	Found
IVa	H	185	82	$C_{22}H_{17}BrN_6O_2$ (489)	C= 56.44 H= 3.48 N= 17.18	56.6 3.3 17.3
IVb	o. CH_3	201	79	$C_{24}H_{19}BrN_6O_2$ (503)	C= 57.26 H= 3.78 N= 16.70	57.4 3.7 16.9
IVc	p.CH_3	205	88	$C_{24}H_{19}BrN_6O_2$ (503)	C= 57.26 H= 3.78 N= 16.70	57.3 3.6 16.9
IVd	p.OCH_3	175	91	$C_{24}H_{19}BrN_6O_3$ (519)	C= 55.49 H= 3.66 N= 16.18	55.6 3.6 16.3
IVe	p.Cl	142	93	$C_{23}H_{16}BrClN_6O_2$ (523.5)	C= 52.72 H= 3.06 N= 16.05	52.9 3.1 16.2
IVf	p.Br	187	83	$C_{22}H_{16}Br_2N_6O_2$ (568)	C= 48.59 H= 2.82 N= 14.79	48.6 2.7 14.8
IVg	2,6 $(CH_3)2$	191	86	$C_{25}H_{21}BrN_6O_2$ (517)	C= 58.03 H= 4.06 N= 16.25	58.2 4.0 16.4

IR (Cm^{-1}) : 3300-3210 (NH) , 3090-3000 (Ar- H and aliph.), 1660 - 1650 (C=O) 1640-1630 (C=N), 1600-1595 (C=C), 600-800 (out of plane bending) .

1H NMR (ppm) for compound :

IV g : 2.2 (s, 6H , 2,6 9 CH_3)2 ; 3.7 (s, 3H, OCH_3) ; 6.0 (s, 1H CH at 3 a apposition) ; 6.8 - 7.5 (m, 9H , aromatic protons) , 8.4 (s, 1H - N= CH- Ar) 10.2 (s, br, NH).

Table IV 6 - Bromo -3,4 - dihydro-4- nitroso-2- oxo-1- substituted phenyl -1H, 4H - pyrido [2,3-b] - pyrrolo [3,2 - e] pyrazines.



Comp	R	M.P.	Yield %	M. F. & M.Wt.	Microanalysis	
					Calc.	Found
Va	H	285	83	$C_{15}H_{10}BrN_5O_2$ (372)	C= 48.39 H= 2.67 N= 18.82	48.5 2.4 18.7
Vb	o. CH_3	292	81	$C_{16}H_{12}BrN_5O_2$ (386)	C= 49.74 H= 3.11 N= 18.13	49.9 3.0 18.3
Vc	p.CH_3	296	79	$C_{16}H_{12}BrN_5O_2$ (386)	C= 49.74 H= 3.11 N= 18.13	49.9 3.1 18.3
Vd	p.OCH_3	265	88	$C_{16}H_{12}BrN_5O_3$ (402)	C= 47.76 H= 2.99 N= 17.41	47.9 2.9 17.6
Ve	p.Cl	235	92	$C_{15}H_9BrClN_5O_2$ (406.5)	C= 44.28 H= 2.21 N= 17.22	44.3 2.1 17.4
Vf	p.Br	287	87	$C_{15}H_9Br_2N_5O_2$ (451)	C= 39.91 H= 1.96 N= 15.52	39.8 1.8 15.7
Vg	2,6 $(CH_3)2$	282	85	$C_{17}H_{14}BrN_5O_2$ (400)	C= 51.00 H= 3.50 N= 17.50	51.3 3.4 17.6

IR (Cm^{-1}) : 3050-2910 (Ar- H and aliph.), 1700-1670 (C=O) 1640-1620 (C=N) 1600-1590 (C=C), 650-850 (out of plane bending) .

1H NMR (ppm) for compound :

Vc : 2 (s, 3H , CH_3) ; 2.7 - 2.72 (d, 2H , CH_2 at 3 position) ; 5.97 - 6.0 (t, 1H , CH at 3 a position) ; 6.9 - 8.4 (m, 6H, aromatic protons).

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To a solution of the appropriate (II, a-g) (10 mmol) in Me OH on cold (40 mL), hydrzine hydrate (0.62 g. 20 mmol) was added while stiring . The reaction mixture was refluxed for 1h. cooled, diluted with H_2O and filtered. The separated product was washed with ammonia solution and H_2O then crystallized from DMF / H_2O) (Table II).

3-Aryidenehydrazono -6- bromo-2- oxo-1-substituted phenyl -1H , 4H - Pyrido [2,3 -b] pyrrolo [3,2-e] pyrazine (IV, a - g).

To a mixture of the appropriate III a-g (10 mmol) in Et OH (30 mL), anisaldehyde (1. 4g. 10 mmol) was added while stirring under reflux for 2h. The mixture was filtered while hot and the filtrate was concentrated and diluted with pet. ether (60 mL). The separated product was filtered and crystallized from aq. EtOH (Table III) .

6-Bromo-3,4- dihydro-4- nitroso -2- oxo-1-substituted phenyl-1H, 4H -pyrido [2,3-b] pyrrolo[3,2-e] pyrazine (V, a-g).

To a solution of the appropriate (III, a-g) (10 mmol) in dil Hcl (30 mL) , a solution of $NaNO_2$ (1g, 15 mmol) in ice cold H_2O (10mL) , was added portionwise while stirring for 1h. The separated product was filered, washed with H_2O and crystallized from aq. dioxane (Table IV).

ANTIMICROBIAL ACTIVITY

Eight of the new, compounds (IId, IIg, IIId, IVe, IVf, Vc and Vg were evaluated for their antimicrobial activity employing the disc plate agar diffusion method[10] against seven strains of microorganisms representing Gram positive and Gram negative as well as yeast . These microorganisms are ; *Staphylococcus aureus, Sarcina lutea, Bacillus subtilis, Neisseria sp., Escherichia coli, Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* (yeast). The compound were dissolved in DMF (1 mg/mL). The a filter paper discs were saturated with the solution , dried the air then applied to the surface of the agar plates seeded with the microorganisms.

Most of the investigated compounds showed variable degree of antimicrobial activity against all of the these microorganisms (Table V) .

Compounds IId and IIg showed the most pronounced antimicrobial activity against all of the tested microorganisms, except *Pseudomonas aeruginosa* . It could be concluded that these activities were due to the presence of the free chlorine atom at position 3 of the heterocyclic ring, which play an important role in the antimicrobial activity of the reported compounds. Replacement of the chlorine atom with hydrogen, hydrazono or arylidino group decreased the antimicrobial activity .

The minimum inhibitory concentration (MIC, ugmL) for the present compounds showed that these new compounds are more active against all the tested microorganisms than the previously studied pyrrolobenzoxazines and pyrroloquinoxalines.

Table (V) Microbiologicial activities of selected compounds

Microorganism	MIC (μg/mL)	
	Compound IId	Compound IIg
Staphylococcus aureus	390	83
Sarcina lutea	364	180
Bacillus subtilis	290	66
Neisseria sp.	402	310
Escherichia coli	510	75
Pseudomonas aeruginosa	660	550
Saccharomyces cerevisia	390	150

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استخدام تفاعل محبات الأنوية الثنائية فى تحضير بيريدوبيرولوبيرازين كمضادات للميكروبات

السيد محمد منصور لاشين
قسم الكيمياء الطبية - كلية الصيدلة - جامعة الزقازيق

فى هذا البحث تم تشيد بعض مركبات بيريدوبيرولوبيرازين باستخدام داى كلورماليدات كمواد أولية بتفاعلها مع ٥ - برومو-٢ر٣-١١ امينوبيريدين كمحب ثنائى للانوية . ويعتمد هذا التفاعل على النترزة الأختزالية وقد تم إثبات التركيب الكيميائى لهذه المركبات باستخدام الأشعة الحمراء والرنين النووى المغناطيسى وكذلك التحليل الدقيق لعناصره.

وقد تم عمل إختبارات بيولوجية لبعض هذه المركبات كمضادات للمكيروبات على عينات مختارة .