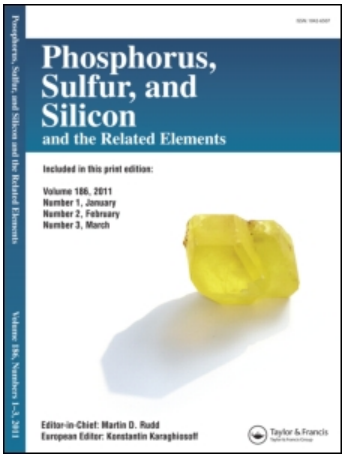

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STUDIES ON THE VILSMEIER-HAACK REACTION: PART XIII. NOVEL HETEROCYCLO-SUBSTITUTED 4,4'-bi-PYRAZOLYL DITHIOCARBAMATE DERIVATIVES

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STUDIES ON THE VILSMEIER-HAACK REACTION: PART XIII. NOVEL HETEROCYCLO-SUBSTITUTED 4,4′-bi-PYRAZOLYL DITHIOCARBAMATE DERIVATIVES

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5-Imino-3-methyl-1-phenyl-2-pyrazoline-4-dithiocarbamic acid (**I**) underwent simultaneous formylation and dimerization reactions with the Vilsmeier reagent giving 4-[5′-imino-3-(1″-formyl-2″-dimethylaminoethenyl)-3′-methyl-1′-phenyl-1′*H*-pyrazolo-4′-dithiocarbamyl-2,4-dihydro-3-imino-5-methyl-2-phenyl-1-pyrazoline]dithiocarbamate (**II**) which hydrolyzed with sodium hydroxide to give 4-[3′-(1″-formyl-2″-hydroxyethenyl)-3′-methyl-1′-phenyl-1′*H*′-pyrazolo-4-dithiocarbamyl-1′-pyrazoline]dithiacarbamate-5,5′-dione (**IV**). Treatment of **II** and/or **IV** with morpholine, piperidine, piperazine, hydroxylamine, hydrazine hydrate, phenylhydrazine) afforded the corresponding dipyrazolo-4,4′-dithiocarbamate derivatives with different heterocyclic systems at the 3-position. The structures of these compounds were confirmed by elemental, IR, and ^{1}H-NMR analyses. All synthesized compounds have been screened in vitro against Gram-positive, Gram-negative bacteria and fungi.

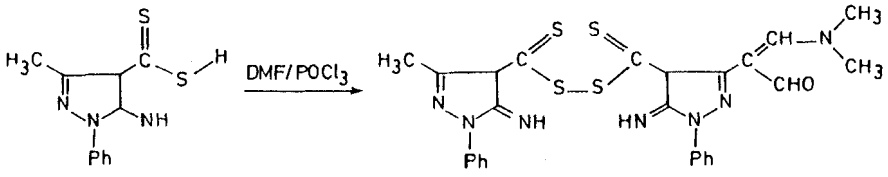
Key words: Imino-piperidine; morpholine; piperazine; isoxazole-bi-(pyrazolyl dithiocarbamate) and biological screening.

INTRODUCTION

It is well known that pyrazolone and thio-pyrazolone derivatives possess antifungal and antibacterial activities.[1–3] Significant importance is being given to pyrazole, isoxazole derivatives due to their wide use in medicinal chemistry.[4–7] The present work describes the application of the Vilsmeier reaction to 5-imino-3-methyl-1-phenyl-2-pyrazoline-4-dithiocarboxylic acid to synthesize novel, hitherto unreported heterocyclic-substituted 4,4′-bipyrazolyl dithiocabamate derivatives with potentially useful pharmacological properties.

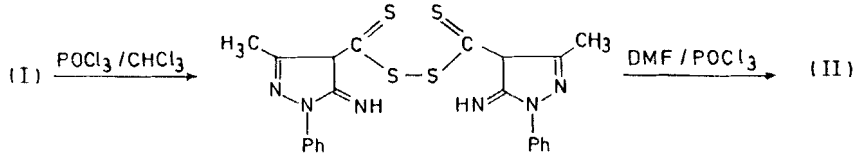
RESULTS AND DISCUSSIONS

The Vilsmeier-Haack reaction is a useful method for synthesizing novel heterocyclic compounds.[9–20] Thus, when 5-imino-3-methyl-1-phenyl-2-pyrazoline-4-dithiocarbamic acid (**I**) reacted with the Vilsmeier reagent with or without heating, the reaction proceeded in a surprising way. A dimerization of monomer **I** and formylation of one of the terminal methyl groups occurred to give 4-[5-imino-3-(1-formyl-2-dimethylaminoethenyl)-3-methyl-1-phenyl-1′*H*-pyrazolo-4-dithiocarbamyl-2,4-dihydro-3-imino-5-methyl-2-phenyl-1-pyrazoline]dithiocarbamate (**II**).



(I) (II)

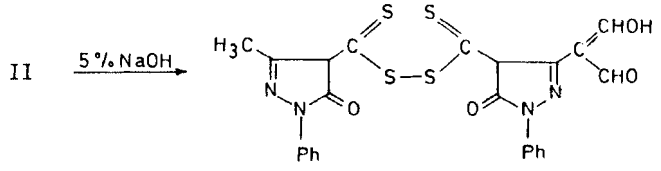
Dimerization of compound **I** in acid medium, also established through an alternative route by the action of $POCl_3$ in the presence of $CHCl_3$ in an equal molar ratio (with or without heating) gave 5,5′-di-imino-3,3′-dimethyl-1,1′-diphenyl-bi-4*H*-pyrazole-4,4′-dithiocarbamate (**III**).



(III)

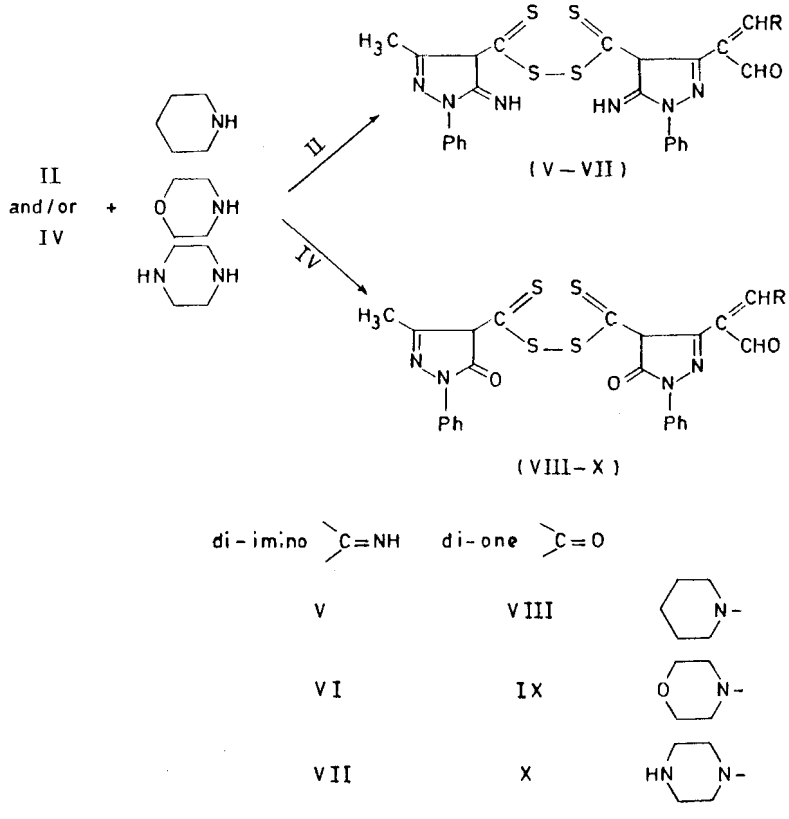
Treatment of compound **III** with Vilsmeier reagent (with or without heating) led to the formylation of one methyl group leaving the other methyl group still intact. Changing the molar ratio to 2:1 of the Vilsmeier reagent to the monomer I gave also the same product II.

Aldehyde II was readily hydrolyzed by stirring with 5% sodium hydroxide solution to give malonaldehyde 4-[3′-(1″-formyl-2″-hydroxyethenyl)-3-methyl-1-phenyl-1′*H*-pyrazol-4′-dithiocarbamyl-1-pyrazoline]dithiocarbamate-5,5′-dione (**IV**). System (**IV**) gave a pale yellowish-brown coloration with ferric chloride due to the formation of enolic malonaldehyde group.[21,22]



(IV)

Condensation of dimers **II** and/or **IV** with selected secondary heterocyclic amines such as piperidine, morpholine and piperazine afforded the expected aminomethylene derivatives **V–VII** and/or **VIII–X**. These structures were confirmed by elemental analyses (Table I). IR spectra were also in agreement with the structures and revealed the presence of a sharp absorption band at 1625 cm^{-1} for the side chain CHO group (Table II). The ^1H-NMR spectrum of compound **V** in F_3CCO_2H (TFA) showed the presence of signals at δ 4.28–2.95 $\left(t, \rangle N—CH_2—\right)$ for the piperidine ring (Table III).



Reaction of imino-aminoacrolein **II** and/or aminoacrolein dione **IV** with hydroxylamine hydrochloride, hydrazine hydrate and phenylhydrazine furnished the corresponding 4′-isoxazolyl, 4′-pyrazolyl and 1′-phenyl-4′-pyrazolyl derivatives at the 3-position of 4,4′-bipyrazolyl dithiocarbamates **XI–XIII** and/or **XI–XVI**. On the other hand, when piperazine was reacted with two moles of imino-aminoacrolein **II** and/or aminoacrolein dione **IV**, there was afforded *N*,*N*′-di[3,3′-bi-(1″-formyl-2″-ethenyl)-pyrazolyl-5,5′-di)-imino-4,4′-dithiocarbamate]piperazine **XVII** and/or *N*,*N*′-di[3,3′-bi-(1″-formyl-2″-ethenyl)-pyrazolyl-5,5′-dione-4,4′-dithiocarbamate]-piperazine **XVIII**. Alkaline hydrolysis of isoxazole compounds **XI** and/or **XIV** with sodium hydroxide 2% (25 ml) gave the cyanoaldehyde dipyrazolyl dithiocarbamate **XIX** as shown by its solubility in alkali and the characteristic strong absorption band at 2220 cm^{-1} ($\nu C{\equiv}N$) and at 1700 cm^{-1} (νCHO) in their IR spectra.

Treatment of cyanoaldehyde derivative **XIX** with hydrazine hydrate and/or phenylhydrazine in acetic acid afforded the corresponding aminopyrazole derivatives **XX** and **XXI**, as shown by their ready solubility in dilute hydrochloric acid and on the bases of the correct elemental analyses. The IR spectra of these compounds were in agreement with the structures indicating the absorption bands at 3495 cm^{-1} (asym. $—NH_2$) and at 3405 cm^{-1} (sym. $—NH_2$ group).

TABLE I

Physico-Chemical Data of Compounds **II–XXI**

Compd. No.	M.P. °C	Yield %	Molecular Formula (M.W.)	Calculated %				Found %			
				C	H	N	S	C	H	N	S
II	260	86	$C_{26}H_{25}N_7OS_4$ (579.791)	53.86	4.34	16.91	22.12	53.72	4.30	16.82	22.20
III	280	85	$C_{22}H_{20}N_6S_4$ (496.700)	53.20	4.05	16.92	25.82	53.12	4.10	17.01	25.88
IV	297	72	$C_{24}H_{18}N_4O_4S_4$ (554.691)	51.96	3.27	10.00	23.12	52.03	3.20	10.02	23.02
V	238	78	$C_{29}H_{29}N_7OS_4$ (619.856)	56.19	4.71	15.81	20.69	56.21	4.67	15.88	20.60
VI	251	81	$C_{28}H_{27}N_7O_2S_4$ (621.829)	54.08	4.37	15.76	20.62	54.10	4.32	15.80	20.60
VII	268	76	$C_{28}H_{28}N_8OS_4$ (620.844)	54.17	4.54	18.05	20.65	54.20	4.60	15.00	20.60
VIII	220	68	$C_{29}H_{27}N_5O_3S_4$ (621.826)	56.02	4.37	11.26	20.62	60.00	4.40	11.20	20.60
IX	231	71	$C_{28}H_{25}N_5O_4S_4$ (623.798)	53.91	4.04	11.22	20.56	54.00	4.00	11.20	20.50
X	280	73	$C_{28}H_{26}N_6O_3S_4$ (622.813)	53.99	4.21	13.49	20.59	53.90	4.07	13.38	20.62

TABLE I (*Continued*)

XI	270	70	$C_{24}H_{19}N_7OS_4$ (549.721)	52.43	3.48	17.83	23.33	52.50	3.42	17.90	23.30
XII	240	74	$C_{24}H_{20}N_8S_4$ (548.736)	52.53	3.67	20.42	23.37	52.50	3.70	20.40	23.40
XIII	262	66	$C_{30}H_{24}N_8S_4$ (624.835)	57.66	3.87	17.93	20.52	57.70	3.80	17.90	20.50
XIV	266	61	$C_{24}H_{17}N_5O_3S_4$ (551.690)	52.25	3.11	12.69	23.24	52.30	3.07	12.60	23.30
XV	248	68	$C_{24}H_{18}N_6O_2S_4$ (550.706)	52.34	3.29	15.26	23.28	52.40	3.30	15.20	23.30
XVI	275	70	$C_{30}H_{22}N_6O_2S_4$ (626.804)	57.48	3.53	13.40	20.46	57.40	3.61	13.35	20.50
XVII	317	73	$C_{52}H_{46}N_{14}O_2S_8$ (1155.551)	54.05	4.01	16.96	22.19	54.00	4.10	17.00	22.10
XVIII	332	67	$C_{52}H_{42}N_{10}O_6S_8$ (1159.489)	53.86	3.65	12.08	22.12	53.80	3.70	12.10	22.10
XIX	268	63	$C_{24}H_{17}N_5O_3S_4$ (551.690)	52.25	3.10	12.69	23.24	52.30	3.05	12.70	23.00
XX	273	60	$C_{24}H_{19}N_7O_2S_4$ (565.720)	50.95	3.38	17.33	22.67	51.00	3.30	17.40	22.60
XXI	281	64	$C_{30}H_{23}N_7O_2S_4$ (641.819)	56.14	3.61	15.27	19.96	56.10	3.65	15.30	20.00

TABLE II

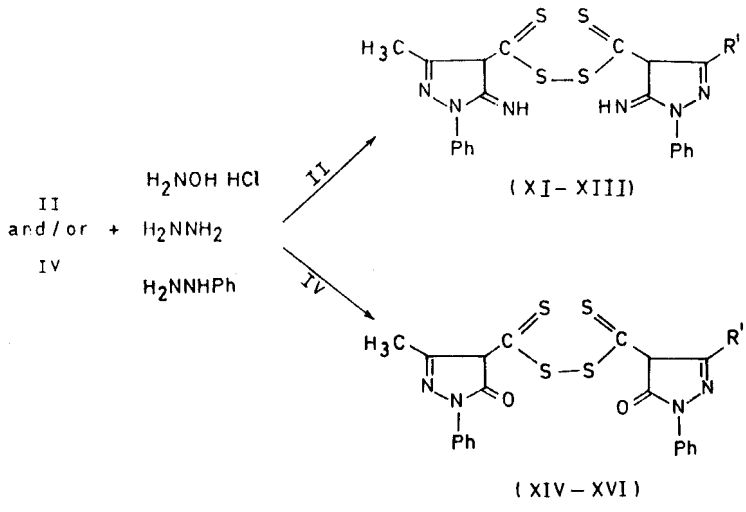
IR Spectra of Some Representative Compounds in cm^{-1}

Assign. / Compd. No.	ν C-CH_3	Imino- ν C=NH	ν C(=S)	α-β-unsaturated νCHO	Enolic- νOH	ν C=O	ν-C≡N	ν NH	νNH_2 asym., sym.	ν C=N	νC-HAr Arom.
II	1395	3350	1315	1625	-	-	-	-	-	1600	755
IV	1390	-	1315	1620	3270	1710	-	-	-	1595	750
VI	1400	3325	1320	1625	-	-	-	-	-	1590	750
VIII	1395	-	1320	1625	-	1715	-	-	-	1600	755
XII	1400	3320	1325	-	-	-	-	3285	-	1600	750
XV	1400	-	1315	-	-	1715	-	3280	-	1595	755
XVI	1395	-	1320	-	-	1710	-	-	-	1600	755
XIX	1393	-	1325	1625	-	1715	2220	-	-	1600	755
XXI	1405	-	1315	-	-	1705	-	-	3495,3405	1595	750

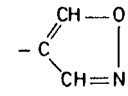
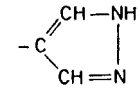
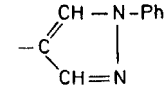
TABLE III

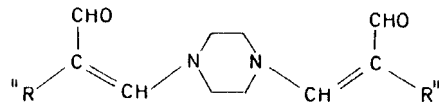
^{1}H-NMR Spectra of Some Synthesized Compounds (Chemical Shifts in δppm)

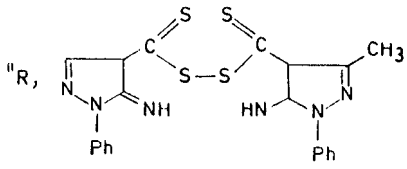
Compd. No.	Aromatic protons (m)	Enolic OH malon-aldehyde (s)	$-N(CH_3)_2$ (s)	Side-chain methine $-C=CH-$ (s)	α-β-Unsat-urated -CHO (s)	>N-H (s)	$-N(CH_2-)_2$ (t)	$-CH_3$ (s)
II	7.15—7.85 (10 H)	-	2.95 (3 H)	8.25 (1 H)	8.75 (1 H)	-	-	2.40(3 H)
IV	7.25- 7.80 (10 H)	3.50	-	8.25 (1 H)	8.80 (1 H)	-	-	2.35(3 H)
V	7.20—7.85 (10 H)	-	-	8.30 (1 H)	8.70 (1 H)	-	-	2.40(3 H)
VIII	7.25—7.85 (10 H)	-	-	8.40 (1 H)	8.85 (1 H)	-	2.70 (t, 4 H, 2 CH_2) 3.60 (t, 4 H, 2 CH_2) 3.75 (t, 4 H, 2 CH_2)	2.40(3 H)
XI	7.20—7.80 (10 H)	-	-	8.30 (1 H)	-	-	-	2.35(3 H)
XVI	7.15—7.90 (15 H)	-	-	8.35 (1 H)	-	-	-	2.40(3 H)
XIX	7.20—7.80 (10 H)	-	-	-	-	-	-	2.35(3 H)



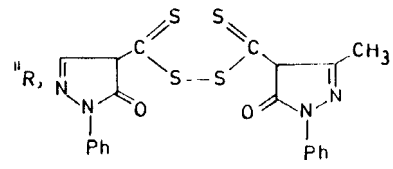
(XI– XIII) (XIV – XVI)

di-imino $\rangle C=NH$	di-one $\rangle C=O$	R'
XI	XIV	
XII	XV	
XIII	XVI	

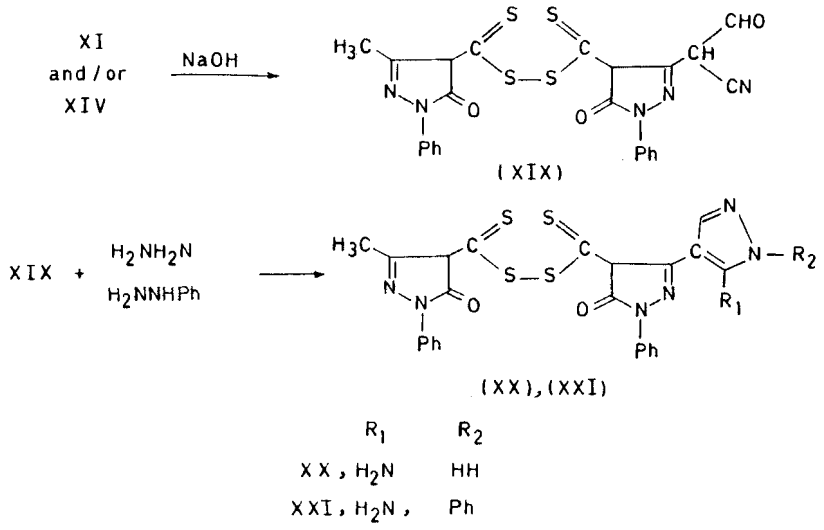




(XVII)



(XVIII)



ANTIMICROBIAL RESULTS

A. Antibacterial activity

The data for inhibition zones of various isolates of bacteria revealed that the synthesized compounds **II–XXI** exhibited variable and pronounced activities against all bacteria isolates used (inhibition zones ranged from 30–220 mm). Compounds **II, IV, VI, X, XII, XIII, XV, XVI** are potent effects against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Micrococcus luteus*. On the other hand, compounds **XVIII–XX** were active against *Pseudomonas aeruginosa* and *Escherichia coli* only and not effective against all the other isolates (Table IV).

B. Antifungal activity

The antifungal results (Table IV) clearly show that compounds **II–IV, VII, XI, XII, XV**, were highly effective against all the isolates of fungi tested (inhibition zones ranged from 80–255 mm). However, all synthesized compounds except **XIX, XX** and **XXI** revealed strong activities (inhibition zones ranged from 40–250 mm). Interestingly, the synthesized compounds showed good and more antifungal than antibacterial activities.

EXPERIMENTAL

Melting points were determined on Kofler melting point apparatus and are uncorrected. Elemental analyses were performed on Perkin-Elmer 240 E Microanalyzer. IR spectra were recorded on a Pye-Unicam SP-200 G infrared spectrophotometer, using KBr wafer technique. ^{1}H-NMR spectra were recorded on a Varian EM-390 MHz instrument in the suitable deuterated solvent (F_3CCO_2H) using TMS as internal reference.

5-Imino-3-methyl-1-phenyl-2-pyrazoline-4-dithiocarbamic acid (**I**): This compound was prepared as reported previously.[8]

TABLE IV

Biological Results of Synthesized **II–XXI** Compounds (Inhibition zones in mm)

Compd. No.	Antibacterial activity					Antifungal activity		
	Staphylococcus aureus DSM-346	Escherichia coli DSM-423	Pseudomonas aeruginosa DSM-1299	Klepsiella pneumoniae DSM-681	Micrococcus luteus DSM-348	Aspergillus ochraceus Wilhelm AUCC-?30	Penicillum chrysogen Thom AUCC-530	Aspergillus flavus Link AUCC-164
II	195	78	65	105	130	95	150	210
III	30	-ve	-ve	90	75	120	138	122
IV	118	128	105	-ve	-ve	55	-ve	40
V	-ve	-ve	35	45	50	60	-ve	48
VI	120	145	98	110	135	210	185	230
VII	-ve	-ve	20	32	41	60	55	81
VIII	28	-ve	35	55	65	-ve	70	-ve
IX	35	48	-ve	41	-ve	-ve	-ve	70
X	180	172	115	158	205	195	225	250
XI	55	-ve	-ve	-ve	30	-ve	40	50

TABLE IV (*Continued*)

XII	215	205	185	178	168	250	195	95
XIII	220	120	165	95	165	98	135	215
XIV	50	30	-ve	10	-ve	68	70	85
XV	200	135	110	155	205	230	185	235
XVI	166	20	-ve	185	198	240	155	178
XVII	65	-ve	-ve	38	-ve	80	-ve	65
XVIII	-ve	85	105	-ve	30	60	85	55
XIX	60	110	115	60	85	-ve	50	-ve
XX	40	95	125	-ve	-ve	-ve	40	-ve
XXI	-ve	38	52	-ve	72	220	240	195

DSM = Deutsche Sammlungvon Microorgamifman (German Collection of Microorganisms).

AUCC = Assiut University Culture Collection.

*4-[5'-Imino-3'-(1"-formyl-2"-dimethylaminoethenyl)-3'-methyl-1'-phenyl-1'*H*-pyrazolo-4'-dithiocarbamyl-2,4-dihydro-3-imino-5-methyl-2-phenyl-1-pyrazoline]dithiocarbamate* (**II**): To dimethylformamide (10 ml), cooled to −3°C, phosphoryl chloride (1.8 ml, 0.04 mol) was added dropwise, and the mixture left to stand for 20 min until the solution became a reddish-yellow colour. To this solution, was added dropwise with stirring the imino-pyrazoline dithiocarbamic acid **I** (0.02 mol) dissolved in *N,N*-dimethylformamide (15 ml). The reaction mixture was allowed to stand for 20 min while stirring and then was heated to 70°C for 6–7 h. The cooled reaction mixture was poured into ice-cold water and treated with 100 ml sodium bicarbonate solution 5% to pH 8. The deep reddish-orange solid that separated was filtered, washed thoroughly with cold water and recrystallized from ethanol. The physical and chemical data are deposited in Table I.

*5,5'-di-Imino-3,3'-dimethyl-1,1'-di-phenyl-bi-4*H*-pyrazole-4,4'-dithiocarbamate* (**III**): To a chloroform (50 ml) solution of iminopyrazoline-dithiocarbamic acid I (0.02 mol), cooled to 15°C, was added dropwise with stirring for 2 h at 50°C phosphoryl chloride (0.1 mol). The orange precipitate that separated was filtered, washed thoroughly with chloroform and recrystallized from ethanol. The physical and chemical data are depicted in Table I.

*4-[3'-(1"-Formyl-2"-hydroxyethenyl)-3'-methyl-1-phenyl-1'*H*-pyrazol-4'-dithiocarbamyl-1-pyrazoline]dithiocarbamate-5,5'-dione* (**IV**): The aminoacrylaldehyde derivative IV (1 g, 0.0016 mol) taken in 8% NaOH (25 ml) was heated to 50–55°C (40 min). It was then filtered off, cooled, and acidified. The yellowish orange solid that separated was filtered, washed well with cold water and recrystallized from ethanol. Analytical data are verified in Table I.

*4-[5'-Imino-3'-(1"-formyl-2'-(piperidinyl, morpholinyl and piperazinyl)-1-phenyl-1'*H*-pyrazolo-4'-dithiocarbamyl-2,4-dihydro-3-imino-5-methyl-2-phenyl-1-pyrazoline]dithiocarbamate* (**V–VII**) *and/or 4-[3'-(1"-formyl-2"(piperidinyl, morpholinyl and piperazinyl)ethenyl)-1-phenyl-3'-methyl)-1'*H*-pyrazolo-4'-dithiocarbamyl-2'-pyrazoline]dithiocarbamate-5,5-dione* (**VIII–X**): To compound **II** and/or **IV** (0.01 mol) taken in ethanol (40 ml) was added (0.01 mol) of the amine, and the mixture was gently heated on a water bath for 40 min. The solid that separated after concentration and pouring into ice-cold water was filtered, washed with cold water and recrystallized from ethanol. The physical and chemical data are recorded in Table I.

*4-[5'-Imino-3'-(4-isoxazolyl, 4-pyrazolyl, and 1-phenyl-4-pyrazolyl)-1'-phenyl-1'*H*-pyrazolo-4'-dithiocarbamyl-2,4'-dihydro-3-imino-5-methyl-2-phenyl-1-pyrazoline]dithiocarbamate* (**XI–XIII**) *and/or 4-[3'-(4-isoxazolyl, 4-pyrazolyl, and 1-phenyl-4-pyrazolyl)-3'-methyl-1'-phenyl-1'*H*-pyrazolo-4'-dithiocarbamyl-2,4-dihydro-1-phenyl-2-pyrazoline]dithiocarbamate-5,5'-dione* (**XIV–XVI**): To a solution of compound **II** and/or **IV** in ethanol (45 ml) was added an equimolar quantity of hydroxylamine hydrochloride, hydrazine hydrate or phenylhydrazine, respectively. The reaction mixture was refluxed for 3 h, cooled, concentrated, and poured into crushed ice. The precipitate solid was filtered, washed with cold water and recrystallized from ethanol. Analytical data are presented in Table I.

Formation of dimers

N,N'-di[3,3'-bi-(1"-formyl-2"-ethenyl)-pyrazolyl-5,5'-di-imino-4,4'-dithiocarbamate]piperazine **XVII** *and/or N,N'-di[3,3'-bi-(1"-formyl-2"-ethynyl)-pyrazolyl-5,5"-dione-4,4'-dithiocarbamate]piperazine* **XVIII**: To compounds **II** and/or **IV** (0.02 mol) taken in ethanol (40 ml) was added (0.01 mol) of piperazine, and the mixture was gently heated on a water bath for 1 h. The solid that separated was filtered, washed with cold water, then with cold ethanol and recrystallized from ethanol. Microanalytical data are listed in Table I.

*4-[3-(1"-Formyl-1"-cyanomethyl)-3'-methyl-1'-phenyl-1'*H*-pyrazolo-4'-dithiocarbamyl-2'-pyrazoline]dithiocarbamate-5,5'-dione* (**XIX**): Isoxazole compounds **XI** and/or **XIV** (1 g, 0.0018 mol) taken in 5% aqueous sodium hydroxide was heated to 45–50°C until a clear solution was obtained (50 min). It was then cooled and acidified with hydrochloric acid. A deep orange solid separated out was filtered, washed thoroughly with water and recrystallized from methanol. The physical and analytical data are listed in Table I.

*4-[3-(5'-Amino-4-pyrazolyl and/or 5"-amino-1-phenyl-4"-pyrazolyl)-3'-methyl-1'-phenyl-1'*H*-pyrazolo-4'-dithiocarbamyl-2'-pyrazoline]dithiocarbamate-5,5'-dione* (**XX, XXI**): A mixture of **XIX** compound (1 g, 0.0018 mol) and hydrazine hydrate (80%, 0.4, 0.0125 mol) and/or phenylhydrazine (0.3, 0.0028 mol) taken in acetic acid (25 ml) was refluxed for 3 h. The reaction mixture was concentrated, cooled and pour into crushed ice. The aminopyrazole **XX** and aminophenyl pyrazole **XXI** compounds obtained as reddish-orange solid separated out were filtered, washed thoroughly with water and recrystallized from ethanol. The microanalytical data are presented in Table I.

Antimicrobial Screening

The disc-diffusion method was used to measure the antibacterial and antifungal activities (Sleigh & Timbury, 1981).[23-25] The tested compounds were dissolved in sterile *N,N*-dimethylformamid and added at a concentration of 0.5 mg/disk (Whatman No. 3 filter paper, 0.5 cm diameter). The antibacterial screening of the novel synthesized compounds was tested against some of Gram-positive and Gram-negative bacteria namely: *Staphylococcus aureus DSM 346, Escherichia coli DSM 423, Pseudomonas aeruginosa DSM 1299, Klebsiella pneumoniae DSM 681* and *Micrococcus luteus DSM 348*. Furthermore, the antifungal screening of these compounds was tested with three species of fungi namely: *Aspergillus ochraceus Wilhelm AUCC-230, Penicillium chrysogenum Thom AUCC-530* and *Aspergillus flavus Link AUCC-164*.

The culture medium for the bacteria tested was nutrient agar (NA) (compound of beef extract, 3 gm, peptone 5 gm, agar, 15 gm/L and adjusted to pH 7 before sterilization at 121°C for 20 min). Glucose-Czapek's agar medium ($NaNO_3$, 2 gm; KH_2PO_4, 1 gm; $HgSO_4$, 0.5 gm; KCl, 0.5 gm glucose, 10 gm; agar, 15 gm/L of distilled water) was used for fungi. The inoculated plates were incubated at 37 ± 1°C for 24 h in the case of bacteria and at 28°C for 7–8 days in the case of fungi. The inhibition zones of microbial growth produced by different compounds were measured.[23-25]

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