Synthesis of Some New 5-Chloro-7-mercapto-1-methyl/phenyl-1,2,4-triazolo[4,5-*b*]pyrazin-2(1*H*)-ones and 5-Chloro-3-thiopyrazin-2(1*H*)-one Derivatives as Possible Antibacterial and Antifungal Agents

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Several new 5-chloro-7-mercapto-1-methyl/phenyl-1,2,4-triazolo[4,5-*b*]pyrazin-2(1*H*)-ones V and their disulphides VI, 5-chloro-3-mercapto-2(1*H*)-pyrazinononones III, 5-chloro-3-(*N*-aryl-*N*-acetylthioureido)-1-methyl/-phenyl-2(1*H*)-pyrazinones VII, 5-chloro-1-methyl/phenyl-3-sulphonamido-2(1*H*)-pyrazinones X and 5-chloro-2-methyl/phenyl-(3-methyl)-3-thio-2(1*H*)-pyrazinones XI were synthesized starting from 3,5-dichloro-1-methyl/phenylpyrazin-2(1*H*)-ones I. Fifteen of these compounds were screened for their antibacterial and antifungal activity against two bacteria *B. subtilis* and *S. aureus*, and two fungi *A. niger* and *H. oryzae*. A possible structure activity relationship is given.

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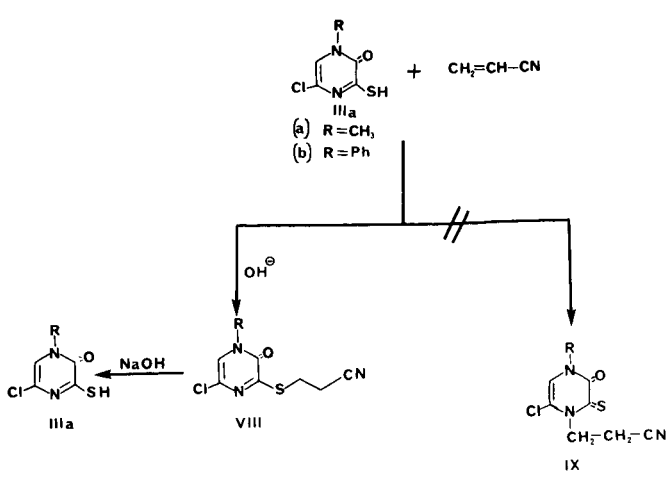
Introduction.

Pyrazines and pyrazinones showed a wide range of antibacterial and antileukemia activity [2,3]. The 1-hydroxy-2-pyrazinone residue is found in natural antibiotics of the aspergillic acid type. Some other derivatives of pyrazine display a wide range of nematocidal, insecticidal and germicidal activities [4-8]. A number of heteroaryl disulphides have been reported as useful bactericides, fungicides and herbicides [9-13]. Furthermore, the compounds having >N-(C<)-S- moiety have significant fungicidal, bactericidal and herbicidal activities, the toxophoric importance of which has been well stressed [14-16]. Considering these facts in view, it is worthwhile to synthesize some new 5-chloro-2(1*H*)-pyrazinones by exotic combination of sulfur containing active moieties having >N-(C<)-S- toxophoric group with the hope that they might be of promising antibacterial and antifungal agents.

The precursor 3,5-dichloro-1-methyl/phenylpyrazin-2(1*H*)-ones I were synthesized following the literature procedure by cyclization of methyl/phenylaminoacetonitrile hydrochloride with oxalyl chloride in ODCB [17]. The 3-chlorine of this compound can be easily replaced as the C-3 is susceptible to nucleophilic attack [17]. Thus, on treatment with dry hydrazine in dry 1,4-dioxane at 20°, it gives 5-chloro-3-hydrazino-1-methyl/phenyl-2(1*H*)-pyrazinones IV [18]. This 3-hydrazinopyrazinone IV proved to be a useful precursor in the synthesis of fused nitrogen bridged triazolo pyrazinones V. The 3-hydrazinopyrazinones IV on refluxing with carbon disulphide/potassium hydroxide gave 5-chloro-7-mercapto-1-methyl/phenyl-1,2,4-triazolo[4,5-*b*]pyrazin-2(1*H*)-ones V which gave corresponding disulphides VI on treatment with bromide in absolute ethanol.

The 3,5-dichloro-1-methyl/phenyl-2(1*H*)-pyrazinone (I) on treatment with thiourea in absolute ethanol gave thiouronium salt II in 85-90% of yield (Rf, 0.35, R = CH_3, chloroform:methanol, 3:1) which on hydrolysis with 2.5 *N* sodium hydroxide followed by acidification with hydrochloric acid (*p*H 2), gave 5-chloro-3-mercapto-1-methyl/-phenyl-2(1*H*)-pyrazinones III in a 70-75% of yield. One of the very interesting observations in this synthesis of compound III, is the reaction of thiourea with 3,5-dichloro-1-methyl/phenyl-2(1*H*)-pyrazinones I which did not give III directly, but went *via* the formation of thiouronium salt II which was isolated as a light yellow solid amorphous compound. On the other hand, Ismail *et al.* [19] did not isolate any intermediate thiouronium salt in the synthesis of 3-mercapto-6-methylpyridazine from 4-aryl/methyl-3-chloropyridazines with thiourea reaction.

The 5-chloro-3-mercapto-1-methyl/phenyl-2(1*H*)-pyrazinones III exists as a thiol structure IIIa not as a thione structure IIIb which is confirmed by its conversion into 5-chloro-2-(2-cyanoethylthio)-1-methyl/phenyl-2(1*H*)-pyrazinones VIII with acrylonitrile. The 3-mercapto-2(1*H*)-pyrazinones III react with acrylonitrile in absolute ethanol under basic conditions and gives *S*-(2-cyanoethyl)-2(1*H*)-pyrazinones VIII instead of *N*-cyanoethyl-2(1*H*)-pyrazinones IX, which firmly confirms the existence of III as a thiol IIIa structure not as a thione IIIb structure.



The structure of VIII was confirmed by ir, the presence of a sharp band in the range between 2245-2250 cm^{-1} clearly confirms the characteristic C=N stretching frequencies. There is no C=S band observed in ir spectrum in the compounds VIII. Further, attempted hydrolysis of VIII with 30% of aqueous sodium hydroxide solution, did not give expected acid but underwent in the cleavage of the cyanoethyl group to give 5-chloro-3-mercapto-1-methyl/phenyl-2(1*H*)-pyrazinones IIIa. This observation clearly confirms that the reaction of III with acrylonitrile results in the formation of *S*-(2-cyanoethyl) derivative VIII not the *N*-cyanoethyl derivative IX, since the compounds of this type undergo hydrolysis to give the corresponding acid [19]. This further confirms the existence of the structure of III is as thiol IIIa not as a thione IIIb.

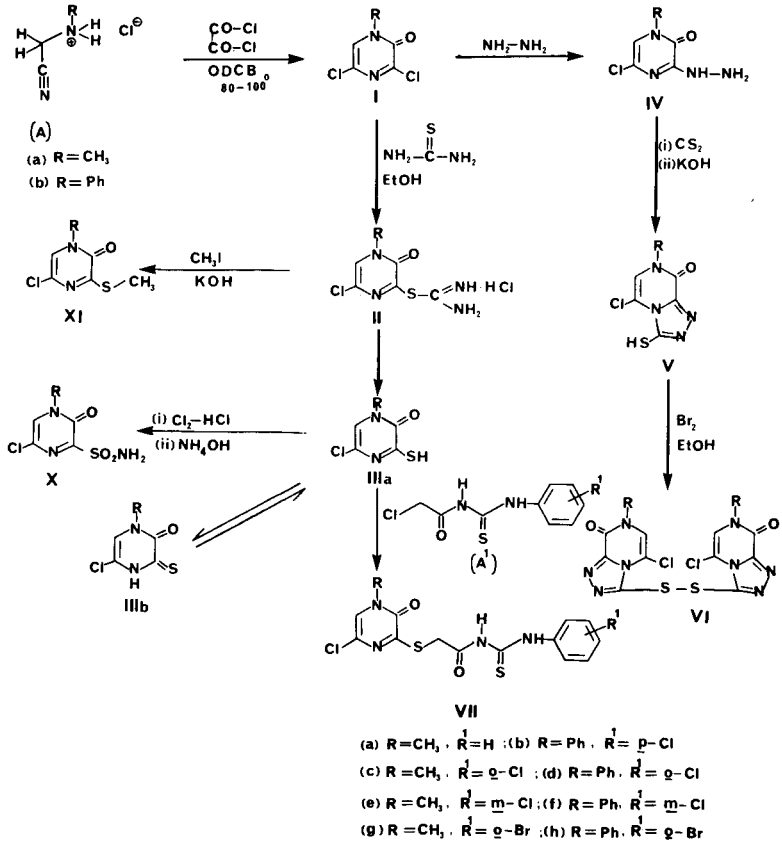
It has been found that the treatment of thiouronium salt II with methyl iodide under basic condition (0.5 *N* potassium hydroxide), gave 3-*S*-methyl-2(1*H*)-pyrazinones XI in a good yield which clearly indicates that the thiouronium salt II which is an intermediate of III, is a very stable intermediate and a very good precursor in the synthesis of 3-*S*-methyl-2(1*H*)-pyrazinones XI.

The 5-chloro-3-mercapto-1-methyl/phenyl-2(1*H*)-pyrazinones are very useful starting material in the synthesis of variety of 3-*S*-substituted pyrazinone derivatives. Compound IIIa on treatment with chloroacetylthiourea (A^1) in the presence of anhydrous potassium carbonate led to the formation of 5-chloro-3-(*N*-aryl-*N'*-acetylthioureido)-1-me-

Table I

Bactericidal Screening

Compound No.	Bacteria	
	B. subtilis	*S. aureus*
Va	+	−
Vb	+	−
VIa	−	−
VIb	−	+
VIIa	+ +	+
VIIb	+	+ +
VIIc	+ +	+ +
VIId	+ +	+ +
VIIe	+	+ +
VIIf	+	+ +
VIIg	+	+ + +
VIIIh	+ +	+ + +
Xa	+ +	+ + +
Xb	−	+
XIa	+	+
Inhibition zones	+ = 5.2 mm + + = 10-14 mm + + + = 16-18 mm	



Scheme I

thyl/phenyl-2(1*H*)-pyrazinones VII. On the other hand, oxidation and amidation of 5-chloro-3-mercapto-2(1*H*)-pyrazinones IIIa with chlorine at 20°, gave 5-chloro-1-methyl/-phenyl-3-sulphonamido-2(1*H*)-pyrazinones X in a 50-55% of yield.

The synthesis of the title compounds has been depicted in Scheme 1.

Fifteen of these new compounds have been screened for their bactericidal and fungicidal activities against two bacteria *B. subtilis* and *S. aureus*, and two fungi *A. niger* and *H. oryzae* respectively and a possible structure activity relationship is given.

EXPERIMENTAL

The melting points were recorded on a Fisher-Johns and Mettler apparatus model R-52241 and are uncorrected. The ir spectra were recorded on a Perkin-Elmer spectrophotometer model 257 and ^{1}H-nmr were recorded on a Perkin-Elmer Hitachi R-600, 60 MHz, and Varian XL-100 spectrophotometer using TMS as the internal reference. The mass spectra were taken on an AEI-MS-12 (ionization energy 70 eV) apparatus. All compounds were dried *in vacuo* at 80° for 15 hours before elemental analyses. The elemental analyses were performed on a Perkin-Elmer 240 elemental analyzer. The uv spectra have been taken on a Perkin-Elmer 402 spectrophotometer.

2-Methylaminoacetonitrile Hydrochloride (A, R = CH_3).

This compound was commercially available and used directly without any further purification.

2-Phenylaminoacetonitrile Hydrochloride (A, R = C_6H_5).

This compound was prepared following the literature procedure [17, 18].

3,5-Dichloro-1-methylpyrazin-2(1*H*)-one (I, R = CH_3).

This compound was synthesized following the reported literature procedure [17,18].

3,5-Dichloro-1-phenylpyrazin-2(1*H*)-one (I, R = C_6H_5).

This compound was also synthesized following the literature procedure by cyclization of 2-phenylaminoacetonitrile hydrochloride in ODCB with oxalyl chloride at 100° [17,18].

5-Chloro-3-hydrazino-1-methyl/phenylpyrazin-2(1*H*)-ones IVa-b.

These were synthesized according to our previous method [18].

5-Chloro-7-mercapto-1-methyl/phenyl-1,2,4-triazolo[4,5-*b*]pyrazin-2(1*H*)-ones Va-b.

In a general procedure [20], 5-chloro-3-hydrazino-1-substituted-pyrazin-2(1*H*)-one (IV) (1.28 g, 6.8 mmoles) in dry 1,4-dioxane (50 ml) was refluxed with carbon disulfide (1.5 g, 19.74 mmoles) and powdered potassium hydroxide (500 g) for 8 hours under continuous stirring. The solution was concentrated to a small volume and poured into ice cold distilled water. On acidification with 3*N* hydrochloric acid, a precipitate was obtained which was filtered, washed several times with water and recrystallized from ethanol. The analytical data of compounds Va-b are summarized below.

Compound Va.

This compound was obtained in a yield of 70%, mp 145°; ir (potassium bromide): ν cm^{-1}, 1641 (C=O), 1630 (C=N), 2590 (SH), 885 (=C-H); ^{1}H-nmr (DMSO-d_6): δ 3.45 (s, 3H, N-CH_3), 7.48 (s, 1H, H-6), 3.3 (s, 1H, S-H); ms: m/z 216.

Anal. Calcd. for $C_6H_5ClN_4OS$: C, 33.33; H, 2.31; N, 25.93; S, 14.81. Found: C, 33.41; H, 2.35; N, 25.98; S, 14.78.

Compound Vb.

This compound was obtained in a yield of 65%, mp 156°; ir (potassium bromide): ν cm^{-1}, 1640 (C=O), 1625 (C=N), 2592 (SH); ^{1}H-nmr (DMSO-d_6): δ 7.42 (s, 1H, H-6), 7.58 (m, 5H, aryl-H), 3.35 (s, 1H, S-H); ms: m/z 278.

Anal. Calcd. for $C_{11}H_7ClN_4OS$: C, 47.48; H, 2.52; N, 20.14; S, 11.51. Found: C, 47.55; H, 2.56; N, 20.32; S, 11.48.

Bis[5-chloro-1-methyl/aryl-1(1,2,4-triazolo][4,5-*b*]pyrazin-2(1*H*)-one] Disulfides VIa-b.

The 5-chloro-7-mercapto-1-methyl/phenyl-1,2,4-triazolo[4,5-*b*]pyrazin-2(1*H*)-one (V), (1.46 g, 6.75 mmoles) was suspended in dry ethanol (45 ml) and stirred with bromine (3.5 mmoles) at 20° overnight. The ethanol was removed under reduced pressure in a rotavapor and the concentrated reaction mixture was cooled and ice cold water was added. A light yellow product was obtained which on recrystallization from ethanol/-water gave pure product. The analytical data of compounds VIa-b are summarized below.

Compound VIa.

This compound was obtained in a yield of 86%, mp 180-181°; ir (potassium bromide): ν cm^{-1}, 1672 (lactam, C=N), 1640 (C=O), 886 (=C-H); ^{1}H-nmr (DMSO-d_6): δ 3.56 (s, 3H, N-CH_3), 7.45 (s, 1H, H-6); ms: m/z 430.

Anal. Calcd. for $C_{12}H_8Cl_2N_8O_2S_2$: C, 33.41; H, 1.86; N, 25.98; S, 14.85. Found: C, 33.48; H, 1.96; N, 25.87; S, 14.81.

Compound VIb.

This compound was obtained in a yield of 60%, mp 181°; ir (potassium bromide): ν cm^{-1}, 1640 (C=O), 1628 (C=N), 885 (=C-H); ^{1}H-nmr (DMSO-d_6): δ 7.43 (s, 1H, H-6), 7.55 (m, 5H, aryl-H); ms: m/z 554.

Anal. Calcd. for $C_{22}H_{12}Cl_2N_8O_2S_2$: C, 47.57; H, 2.16; N, 20.18; S, 11.46. Found: C, 47.58; H, 2.22; N, 20.14; S, 11.46.

5-Chloro-1-methyl/phenyl-3-isothiouronium-2(1*H*)-pyrazinone Hydrochloride (II).

Thiourea (1.14 g, 15 mmoles) was added to a solution of 3,5-dichloro-2(1*H*)-pyrazinone (I) (1.78 g, 10 mmoles) in 25 ml of absolute ethanol and the reaction mixture was stirred at 20° for 3 hours. The light yellow deposit thus obtained was filtered and treated with acetonitrile. The residue was filtered and recrystallized quickly from methanol. Care must be taken because decomposition takes place on heating.

The analytical data of compounds IIa-b are summarized below.

Compound IIa.

This compound was obtained in a yield of 71%, mp 191-193°; Rf, 0.36 (chloroform:methanol, 3:1); ir (potassium bromide): ν cm^{-1}, 1670 (lactam, C=N), 1590 (C=C), 3320 (=NH-), 1645 (C=O), 880 (=C-H); ^{1}H-nmr (DMSO-d_6): δ 3.45 (s, 3H, N-CH_3), 8.22 (s, 1H, H-6), 9.75 (b, s, 2H, NH_2); ^{13}C-nmr (DMSO-d_6): δ 164.8 (C-2), 153.6 (C-3), 130.8 (C-6), 124.9 (C-5), 37.6 (N-C, N-CH_3), 148.5 (-S-C=N-); ms: m/z 255.

Anal. Calcd. for $C_6H_6Cl_2N_4OS$: C, 28.23; H, 3.14; N, 21.96; S, 12.55. Found: C, 28.31; H, 3.24; N, 21.86; S, 12.48.

Compound IIb.

This compound was obtained in a yield of 68%, mp 202°; Rf, 0.35 (chloroform:methanol, 3:1); ir (potassium bromide): ν cm^{-1}, 1675 (lactam, C=N), 1585 (C=C), 882 (=C-H), 1640 (C=O), 3322 (=NH-); ^{1}H-nmr (DMSO-d_6): δ 8.12 (s, 1H, H-6), 9.66 (b, s, 2H, NH_2), 7.54 (m, 5H, aryl-H); ^{13}C-nmr (DMSO-d_6): δ 165.7 (C-2), 154.8 (C-3), 132.6 (C-6), 123.8 (C-5), 126.7, 128.8 and 137.7 (aryl-C, N-C_6H_5); ms: m/z 317.

Anal. Calcd. for $C_{11}H_{10}Cl_2N_4OS$: C, 41.64; H, 3.15; N, 17.67; S, 10.09. Found: C, 41.48; H, 3.25; N, 17.71; S, 10.11.

5-Chloro-3-mercapto-1-methyl/phenyl-2(1*H*)-pyrazinone (IIIa).

The 3-isothiouronium salt of 2(1*H*)-pyrazinone II, (2.28 g, 9.0 mmoles)

was refluxed with 2.5 *M* sodium hydroxide (14 ml) for 1 hour. The reaction mixture was concentration to half of its volume and cooled in an ice bath. The reaction mixture was acidified with hydrochloric acid (*p*H 2). The deep yellow product thus obtained was filtered and recrystallized from ethanol/water.

The analytical data of compounds IIIa-i and IIIa-ii are summarized below.

Compound IIIa-i.

This compound was obtained in a yield of 75%, mp 160-162°; ir (potassium bromide): ν cm^{-1}, 1665 (lactam, C=N), 1580 (C=C), 1640 (C=O), 2560 (SH); ^{1}H-nmr (DMSO-d_6): δ 3.5 (s, N-CH_3), 3.25 (s, 1H, S-H), 7.78 (s, 1H, H-6); ms: 176 (M^+, 100), 148 (M^+, -CO, 25).

Anal. Calcd. for $C_5H_5ClN_2OS$: C, 34.09; H, 2.48; N, 15.91; S, 18.18; Cl, 19.89. Found: C, 34.19; H, 2.81; N, 15.89; S, 18.28; Cl, 19.88.

Compound IIIb-ii.

This compound was obtained in a yield of 70%, mp 175°; ir (potassium bromide): ν cm^{-1}, 1672 (lactam, C=N), 1578 (C=C), 1645 (C=O), 885 (=C-H), 2540 (SH); ^{1}H-nmr (DMSO-d_6): δ 3.32 (s, 1H, S-H), 7.54 (m, aryl-H, 5H), 7.82 (s, 1H, H-6); ms: 238 (M^+, 100), 210 (M^+-CO, 35).

Anal. Calcd. for $C_{10}H_7ClN_2OS$: C, 50.42; H, 2.94; N, 11.76; S, 13.45; Cl, 14.71. Found: C, 50.38; H, 2.91; N, 11.78; S, 13.43; Cl, 14.78.

5-Chloro-2-methyl/phenyl-(3-methyl)thio-2(1*H*)-pyrazinone (XI).

These were synthesized from thiouronium salt II. A solution of thiouronium salt II (8.00 mmoles) in aqueous potassium hydroxide (0.5 *M*, 10 ml) was stirred with methyl iodide (8.00 mmoles) for 3 hours at 20°. The light yellow deposit thus obtained was extracted with dichloromethane. The dichloromethane was removed in rotavapor and the residue was crystallized from dichloromethane/ether.

The analytical data of compounds XIa-b are summarized below.

Compound XIa.

This compound was obtained in a yield of 75%, mp 145°; Rf, 0.45 (chloroform:methanol, 9:1); ir (potassium bromide): ν cm^{-1}, 1670 (lactam, C=N), 1640 (C=O), 882 (=C-H); ^{1}H-nmr (deuteriochloroform): δ 3.66 (s, 3H, N-CH_3), 3.52 (s, 3H, S-CH_3), 7.38 (s, 1H, H-6); ^{13}C-nmr (deuteriochloroform): δ 160.5 (C-2), 153.7 (C-3), 126.8 (C-5), 36.9 (N-CH_3), 13.1 (S-CH_3); uv (ethanol): λ max, 344 nm.

Anal. Calcd. for $C_6H_7ClN_2OS$: C, 37.53; H, 3.71; N, 14.60; S, 16.50; Cl, 18.42. Found: C, 37.80; H, 3.70; N, 14.69 S, 16.82; Cl, 18.60.

Compound XIb.

This compound was obtained in a yield of 72%, mp 145°; Rf, 0.46 (chloroform:acetonitrile, 9:1); ir (potassium bromide): ν cm^{-1}, 1675 (lactam, C=N), 1641 (C=O), 883 (=C-H); ^{1}H-nmr (deuteriochloroform): δ 7.33 (s, 1H, H-6), 7.52 (m, 5H, aryl-H), 3.55 (s, 3H, S-CH_3); ^{13}C-nmr (deuteriochloroform): δ 162.25 (C-2), 152.78 (C-3), 125.7 (C-5), 13.25 (S-CH}), 126.5, 129.8 and 152.2 (aryl-C); ms: 252 (M^+, 90), 224 (M^+-CO, 45); uv (ethanol): λ max 345 nm.

Anal. Calcd. for $C_{11}H_9ClN_2OS$: C, 52.38; H, 3.57; N, 12.70. Found: C, 52.42; H, 3.61; S, 12.72.

5-Chloro-1-methyl/phenyl-3-sulphonamido-2(1*H*)-pyrazinone (X).

In a general method, 3-mercaptopyrazinone IIIa (11.36 mmoles) in a concentrated hydrochloric acid (10 ml) and water (2.5 ml), were cooled to -10 to $-15°$ and to this solution, chlorine was passed in such a rate that the temperature should not go up and should be maintained between -15 to $-20°$. After 1 hour, a concentrated solution of ammonium hydroxide was added to the reaction mixture at $-20°$. The reaction mixture was kept under stirring at 20° for 6 hours and extracted with ethyl acetate. The ethyl acetate was removed *in vacuo* on a rotavapor. The syrup residue thus obtained was chromatographed over silica gel-60 (grain size 0.063-0.2, Merck) and were eluted with acetonitrile and dichloromethane (9:1). The product thus obtained was recrystallized from benzene/*n*-pentane.

The analytical data of compounds Xa-b are summarized below.

Compound Xa.

This compound was obtained in a yield of 60%, mp 175°; ir (potassium bromide): ν cm^{-1}, 1677 (lactam, C=N), 1590 (C=C), 885 (=C-H), 1350 (SO_2), 1541 (NH_2); ^{1}H-nmr (DMSO-d_6): δ 3.38 (s, 3H, N-CH_3), 6.78 (s, 2H, NH_2), 7.34 (s, 1H, H-6); ms: m/z 223.

Anal. Calcd. for $C_5H_6ClN_3O_3S$: C, 26.9; H, 2.69; N, 18.83; S, 14.35. Found: C, 26.95; H, 2.58; N, 18.85; S, 14.45.

Compound Xb.

This compound was obtained in a yield of 55%, mp 185°; ir (potassium bromide): ν cm^{-1}, 1675 (lactam, C=N), 1560 (C=C), 880 (=C-H), 1340 (SO_2), 1540 (NH_2); ^{1}H-nmr (DMSO-d_6): δ 7.31 (s, 1H, H-6), 7.52 (m, 5H, N-C_6H_5), 6.76 (s, 2H, NH_2); ms: m/z 285.

Anal. Calcd. for $C_{10}H_8ClN_3O_3S$: C, 42.11; H, 2.81; N, 14.74; S, 11.23. Found: C, 42.32; H, 2.88; N, 14.81; S, 11.26.

5-Chloro-3-(*N*-aryl-*N'*-acetylthioureido)-1-methyl/phenyl-2(1*H*)-pyrazinone (VII).

In a typical method, 0.06 mole of 5-chloro-3-mercapto-1-methyl/phenyl-2(1*H*)-pyrazinone (IIIa) and 0.06 mole of chloroacetylthiourea (A^1) [21], were refluxed in absolute ethanol in the presence of anhydrous potassium carbonate (0.04 mole) for 4 hours. The reaction mixture was poured in to ice cold water. The compound thus obtained was filtered and recrystallized from ethanol/ether.

The analytical data of compounds VIIa-h are summarized below.

Compound VIIa.

This compound was obtained in a yield of 70%, mp 235°; ir (potassium bromide): ν cm^{-1}, 3350 (NH), 1185 (C=S), 1670 (lactam, C=N), 1640 (C=O); ^{1}H-nmr (DMSO-d_6): δ 3.55 (s, 3H, N-CH_3), 7.58 (m, 4H, aryl-H), 2.42 (s, 2H, CH_2), 9.86-9.35 (s, br, NH); ms: m/z 403.

Anal. Calcd. for $C_{14}H_{12}Cl_2N_4O_2S_2$: C, 41.69; H, 2.98; N, 13.89; S, 15.88. Found: C, 41.72; H, 2.85; N, 13.99; S, 15.86.

Compound VIIb.

This compound was obtained in a yield of 65%, mp 245°; ir (potassium bromide): ν cm^{-1}, 3310 (NH), 1690 (lactam, C=N), 1200 (C=S), 1642 (C=O); ^{1}H-nmr (DMSO-d_6): δ 7.52 (m, 5H, N-C_6H_5), 7.65 (m, 4H, aryl-H), 7.43 (s, 1H, H-6), 2.75 (s, 2H, CH_2), 9.8 (s, br, NH); ms: m/z 465.

Anal. Calcd. for $C_{19}H_{14}Cl_2N_4O_2S_2$: C, 49.03; H, 3.01; N, 12.04; S, 13.76. Found: C, 49.22; H, 3.12; N, 12.21; S, 13.65.

Compound VIIc.

This compound was obtained in a yield of 72%, mp 265°; ir (potassium bromide): ν cm^{-1}, 3340 (NH), 1690 (CO-NH), 1180 (C=S), 1672 (lactam, C=N), 1640 (C=O); ^{1}H-nmr (DMSO-d_6): δ 3.56 (s, 3H, N-CH_3), 7.58 (m, 4H, aryl-H), 2.45 (s, 2H, CH_2), 9.86 (s, br, NH); ms: m/z 402.

Anal. Calcd. for $C_{14}H_{12}Cl_2N_4O_2S_2$: C, 41.69; H, 2.98; N, 13.89; S, 15.88. Found: C, 41.65; H, 3.15; N, 13.88; S, 15.84.

Compound VIId.

This compound was obtained in a yield of 68%, mp 238°; ir (potassium bromide): ν cm^{-1}, 3320 (NH), 1695 (CO-NH), 1190 (C=S), 1675 (lactam, C=N), 1642 (C=O); ^{1}H-nmr (DMSO-d_6): δ 7.55 (m, 5H, N-C_6H_5), 7.42 (s, 1H, H-6), 7.64 (m, 4H, aryl-H), 2.85 (s, 2H, CH_2), 9.9 (s, br, NH); ms: m/z 465.

Anal. Calcd. for $C_{19}H_{14}Cl_2N_4O_2S_2$: C, 49.03; H, 3.01; N, 12.04; S, 13.76. Found: C, 49.21; H, 3.10; N, 12.11; S, 13.55.

Compound VIIe.

This compound was obtained in a yield of 62%, mp 248°; ir (potassium bromide): ν^{-1}, 3360 (NH), 1680 (CO-NH), 1188 (C=S), 1670 (lactam, C=N), 1640 (C=O); ^{1}H-nmr (DMSO-d_6): δ 3.48 (s, 3H, N-CH_3), 7.56 (m, 4H, aryl-H), 2.45 (s, 2H, CH_2), 9.86 (s, br, NH), 7.43 (s, 1H, H-6); ms: m/z 403.

Anal. Calcd. for $C_{14}H_{12}Cl_2N_4O_2S_2$: C, 41.69; H, 2.98; N, 13.89; S, 15.88. Found: C, 41.71; H, 3.08; N, 13.84; S, 15.84.

Compound VIIf.

This compound was obtained in a yield of 70%, mp 242°; ir (potassium bromide): ν cm^{-1}, 3330 (NH), 1680 (CO-NH), 1185 (C=S), 1670 (lactam, C=N), 1640 (C=O); ^{1}H-nmr (DMSO-d$_6$): δ 7.56 (m, 5H, N-C$_6$H$_5$), 7.42 (s, 1H, H-6), 2.95 (s, 2H, CH$_2$), 9.98 (s, br, NH); ms: m/z 464.

Anal. Calcd. for $C_{19}H_{14}Cl_2N_4O_2S_2$: C, 49.03; H, 3.01; N, 12.04; S, 13.76. Found: C, 49.21; H, 3.11; N, 12.44; S, 13.81.

Compound VIIg.

This compound was obtained in a yield of 75%, mp 265°; ir (potassium bromide): ν cm^{-1}, 3335 (NH), 1685 (CO-NH), 1180 (C=S), 1670 (lactam, C=N), 1644 (C=O); ^{1}H-nmr (DMSO-d$_6$): δ 7.45 (m, 4H, aryl-H), 3.55 (s, 3H, N-CH$_3$), 7.44 (s, 1H, H-6), 2.94 (s, 2H, CH$_2$), 9.88 (s, br, NH); ms: m/z 447.

Anal. Calcd. for $C_{14}H_{12}ClBrN_4O_2S_2$: C, 37.58; H, 2.68; N, 12.53; S, 14.31. Found: C, 37.68; H, 2.75; N, 12.68; S, 14.68.

Compound VIIh.

This compound was obtained in a yield of 65%, mp 275°; ir (potassium bromide): ν cm^{-1}, 3330 (NH), 1680 (CO-NH), 1185 (C=S), 1670 (lactam, C=N), 1654 (C=O); ^{1}H-nmr (DMSO-d$_6$): δ 7.65 (m, 5H, N-C$_6$H$_5$), 7.45 (s, 1H, H-6), 2.89 (s, 2H, CH$_2$), 9.96 (s, br, NH); ms: m/z 510.

Anal. Calcd. for $C_{19}H_{14}ClBrN_4O_2S_2$: C, 44.71; H, 2.74; N, 10.98; S, 12.55. Found: C, 44.95; H, 2.78; N, 10.88; S, 12.65.

5-Chloro-1-methyl/phenyl-3-(2-cyanoethylthio)-2(1*H*)-pyrazinone (VIII).

A mixture of IIIa (10 mmoles), vinyl cyanide (acrylonitrile) (15 mmoles) in ethanol (25 ml) and 12% of sodium hydroxide solution (5 ml) was refluxed for 24 hours [19]. The light yellow solid thus formed after cooling was filtered and washed several times with ether and recrystallized from benzene/hexane.

The analytical data of compounds VIIIa-b are summarized below.

Compound VIIIa.

This compound was obtained in a yield of 55%, mp 190°; ir (potassium bromide): ν cm^{-1}, 2245 (C≡N), 1670 (lactam, C=N), 1640 (C=O); ^{1}H-nmr (DMSO-d$_6$): δ 3.45 (s, 3H, N-CH$_3$), 7.42 (s, 1H, H-6), 3.25 (t, -CH$_2$CN), 2.95 (t, S-CH$_2$-, 2H); ms: m/z 229.

Anal. Calcd. for $C_8H_8ClN_3OS$: C, 41.92; H, 3.49; N, 18.34; S, 13.97. Found: C, 41.85; H, 3.55; N, 18.66; S, 13.95.

Compound VIIIb.

This compound was obtained in a yield of 60%, mp 185°; ir (potassium bromide): ν cm^{-1}, 2250 (C≡N), 1675 (lactam, C=N), 1645 (C=O); ^{1}H-nmr (DMSO-d$_6$): δ 7.65 (m, 5H, N-C$_6$H$_5$), 7.45 (s, 1H, H-6), 3.15 (t, -CH$_2$), 2.89 (t, S-CH$_2$); ms: m/z 303.

Anal. Calcd. for $C_{14}H_{10}ClN_3OS$: C, 55.45; H, 3.30; N, 13.86; S, 10.56. Found: C, 55.55; H, 3.42; N, 13.92; S, 10.66.

5-Chloro-3-mercapto-1-methyl/phenyl-2(1*H*)-pyrazinone (IIIa) from VIII.

A suspension of VIII (12 mmoles) in 25% of aqueous sodium hydroxide solution (20 ml) is heated under reflux for 16 hours, a clear solution was obtained. The solution was cooled in an ice bath to −5° and to this solution, concentrated hydrochloric acid was added just to get acidic. A solid was obtained, filtered, washed several times with water and recrystallized from ethanol/water. The mp, ir and nmr data of this compound was compared with the authentic samples (the compound obtained from the hydrolysis of thiouronium salt II) and was found to be identical.

Biological Activity.

Fifteen compounds have been tested for their antibacterial activity by cup-plate agar diffusion method [22] against two bacteria *B. subtilis* and *S. aureus* at two different concentrations of 500 μg/ml and 250 μg/ml and the discussion is based on the results with former concentration. The antifungal activity was evaluated by agar plate technique [22,23], against two fungi, *A. niger* and *H. oryzae* at three different concentrations *vig*, 1000 ppm, 100 ppm and 10 ppm respectively. The number of replication in each case was three. A systematic fungicide *Benomyl* was also tested under the similar condition with a view to comparing the results.

It is evident from the screening data (Table 1) that the majority of the tested compounds inhibited the growth of *B. subtilis* to varying extent while the compound Nos. VIIg, VIIh and Xa were found most active against *S. aureus*. The replacement of the *o*-chlorine atom from the phenyl ring of the compounds VII by an *o*-bromo atom the activity increases against *S. aureus*. Further, it has been observed that the halogen substitution in the phenyl ring of of the compounds VII cause an overall increase in activity against *S. aureus*.

From the fungicidal screening data, it was observed that most of the compounds had significant toxicity at 1000 ppm against both the test fungi *vig.*, *A. niger* and *H. oryzae*, but their activity decreased markedly on dilution. The compound Nos. VIa and VIb have greater activity than their corresponding mercapto compounds Va and Vb against both the test fungi. This could be expected from their structure which provide condition for -S-S- bond fission apparently analogues to that of thiram [25]. In general the compound Nos. Xa, XIa, VIIc, VIIe and VIIg were most active against both the test fungi and inhibit the growth >50% comparable with that of *Benomyl* at 10 ppm.

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