Studies on the Vilsmeier-Haack Reaction. Part XI. Novel Heterocyclo-Substituted Thioquinoxalinone Dyes

Ibrahim M. A. AWAD
Chemistry Department, Faculty of Science, Assiut University, Assiut, Egypt.
(Received June 1, 1992)

Novel azo sulfa drugs of 3-methyl-1-[4-(substituted sulfamoyl)phenylazo]-2(1*H*)-quinoxalinethiones were synthesized and found to undergo diformylation reaction with Vilsmeier reagent giving the 3-(3-aminoacrylaldehyde-substituted)-1-(4-sulfamoylphenylazo)-2(1*H*)-quinoxalinethione dyes **4**—**6**. Reaction of **4**, **5**, and/or **6** with proper reagents afforded the corresponding morpholinyl, piperidinyl, 4-piperazinyl-1*H*-pyrazol-4-yl, 1-phenyl-1*H*-pyrazol-4-yl, 4-isoxazolyl, and 5-amino-1*H*-pyrazol-4-yl substituted 2(1*H*)-quinoxalinethione derivatives at the 3-position of the quinoxaline moiety. The structures of these novel compounds were confirmed by elemental analysis, IR and ^{1}H NMR spectroscopy and screened in vitro for antibacterial (Gram-positive and Gram-negative bacteria) and antifungal activites.

Sulfa drug compounds containing azo group are of great value due to their usefulness as models for biological systems (antifungal, antibacterial),[1] good chelating agents for occupational poisoning by metals,[2,3] excellent azo dye compounds.[4—9] Moreover, the azo compounds occupy a scientific position as analytical reagents.[10—12] It is also reported that the replacement of the carbonyl oxygen by sulfur atom enhances the fungicidal and bactericidal activity.[13]

In continuation of our interest in azo-substituted sulfonamide derivaties, we report the preparation of novel azo dye sulfa drugs based on quinoxalinethione moiety and application of Vilsmeier reagent to synthesize novel sulfa drugs containing a different heterocyclic moieties at the 3-position of 2(1*H*)-quinoxalinethione moiety.

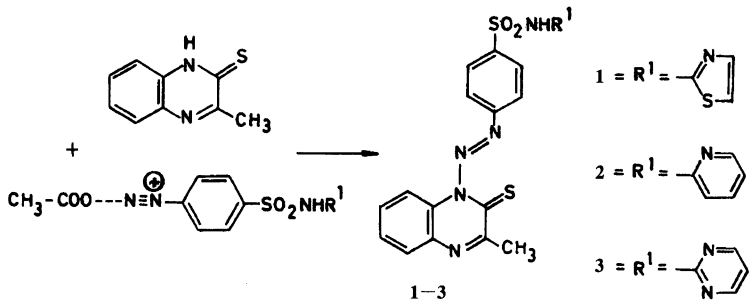
Results and Discussion

Recently, considerable study has been directed in our laboratory toward the application of Vilsmeier reaction to 2(1*H*)-quinoxalinethione derivatives.[14] In spite of many reports on the synthesis and biological activity of some organic compounds, none of these reports applied the Vilsmeier reaction[15—21] on the 3-methyl group of new azo sulfa 2(1*H*)-quinoxalinethione derivatives. Thus, the present paper describes the synthesis and application of the Vilsmeier reaction to novel azo dye sulfa drug derivatives which synthesized by diazotization of *p*-aminobenzenesulfonamide derivatives and coupling them with 3-methyl-2(1*H*)quinoxalinethione to give the corresponding azo dye derivatives, 3-methyl-1-[4-(2-thiazolylsulfamoyl)phenylazo]-2(1*H*)-quinoxalinethione (**1**), 3-methyl-1-[4-(2-pyridylsulfamoyl)phenylazo]-2(1*H*)-quinoxalinethione (**2**), and 3-methyl-1-[4-(2-pyrimidinylsulfamoyl)phenylazo]-2(1*H*)-quinoxalinethione (**3**). The structures of **1**—**3** azo dye sulfa drugs were established and confirmed by the correct elemental analyses data and IR spectroscopic evidence showing bands at 3300 cm^{-1} ν-N–H, 1370 cm^{-1} ν-SO_2NH, 1570 and 1260 cm^{-1} ν-C=S and at 1530—1545 cm^{-1} ν-N=N. The ^{1}H NMR spectrum of compound **2** in $CDCl_3$ revealed some signals, at δ=2.10 (s, 3H, CH_3), 8.50 (s, 1H, SO_2NH) and at 8.20—6.68 (m, 11H, aromatic

Table 1. Yields and Melting Points of Compounds **1**—**36**

Compd No.	Formula[a]	Yield %	Mp °C
1	$C_{18}H_{14}N_6O_2S_3$	94	240
2	$C_{20}H_{16}N_6O_2S_2$	78	215
3	$C_{19}H_{15}N_7O_2S_2$	88	190
4	$C_{22}H_{19}N_7O_3S_3$	78	295—297
5	$C_{24}H_{21}N_7O_3S_2$	68	255—256
6	$C_{23}H_{20}N_8O_3S_2$	72	278—280
7	$C_{20}H_{14}N_6O_4S_3$	73	270
8	$C_{28}H_{16}N_6O_4S_3$	60	252—253
9	$C_{21}H_{15}N_7O_4S_2$	67	258—260
10	$C_{20}H_{13}N_7O_3S_3$	68	208—209
11	$C_{20}H_{14}N_8O_2S_3$	61	185
12	$C_{26}H_{18}N_8O_2S_3$	65	178—180
13	$C_{22}H_{15}N_7O_3S_2$	71	235—238
14	$C_{22}H_{16}N_8O_2S_2$	60	210—212
15	$C_{28}H_{20}N_8O_2S_2$	60	258
16	$C_{21}H_{14}N_8O_3S_2$	69	258—259
17	$C_{21}H_{15}N_9O_2S_2$	70	270
18	$C_{27}H_{19}N_9O_2S_2$	68	242—244
19	$C_{29}H_{21}N_7O_4S_3$	70	189—190
20	$C_{25}H_{23}N_7O_3S_2$	73	196—199
21	$C_{24}H_{22}N_8O_3S_2$	68	201—202
22	$C_{26}H_{23}N_7O_4S_2$	70	218
23	$C_{27}H_{25}N_7O_3S_2$	70	228—230
24	$C_{26}H_{24}N_8O_3S_2$	66	236
25	$C_{25}H_{22}N_8O_4S_2$	76	243—244
26	$C_{26}H_{24}N_8O_3S_2$	78	320—322
27	$C_{25}H_{23}N_9O_3S_2$	70	327
28	$C_{20}H_{13}N_7O_3S_3$	68	260—262
29	$C_{22}H_{15}N_7O_3S_2$	62	281—283
30	$C_{21}H_{14}N_8O_3S_2$	72	290—291
31	$C_{20}H_{15}N_9O_2S_3$	63	238—240
32	$C_{22}H_{17}N_9O_2S_2$	65	280
33	$C_{21}H_{16}N_{10}O_2S_2$	70	286
34	$C_{26}H_{19}N_9O_2S_3$	72	310
35	$C_{28}H_{21}N_9O_2S_2$	69	318
36	$C_{27}H_{20}N_{10}O_2S_2$	65	322

a) Elemental analysis of C, H, N, and S gave results equal to those calculated within experimental error.



Scheme 1.

Table 2. IR Spectra of Some Representative Compounds in cm^{-1}

Assignment NO.	ν=C=S		νC=N	νN=N	Acrolein-CHO	νSO_2NH	νOH	νNH	νSO_2-asym.	νSO_2-sym.	νCN	νNH_2
1	1565	1235	1605	1535	—	1375	—	—	1335	1155	—	—
2	1560	1250	1605	1530	—	1370	—	—	1335	1155	—	—
5	1555	1250	1600	1535	1625	1365	—	—	1340	1160	—	—
8	1560	1245	1605	1530	1630	1370	3260	—	1340	1160	—	—
9	1655	1255	1610	1535	1625	1370	3265	—	1335	1165	—	—
14	1560	1250	1605	1540	—	1365	—	3295	1335	1150	—	—
19	1565	1245	1610	1530	1620	1365	—	—	1340	1155	—	—
23	1550	1255	1600	1535	1630	1370	—	—	1345	1160	—	—
26	1550	1245	1600	1535	1625	1365	—	—	1335	1165	—	—
24	1560	1250	1600	1545	1630	1370	—	3295	1340	1155	—	—
27	1555	1245	1605	1535	1625	1365	—	3290	1335	1160	—	—
29	1550	1250	1600	1550	1640	1365	—	—	1335	1155	2228	—
32	1560	1245	1600	1545	—	1365	—	3290	1340	1155	—	3455
35	1555	1245	1600	1540	—	1370	—	—	1340	1160	—	3460

protons). The signal at δ=8.50 disappeared by D_2O treatment (Scheme 1).

Application of the Vilsmeier reaction[15—21] to 3-methyl group of compounds **1**—**3** gave the expected products of 3-dimethylaminoacrylaldehyde derivatives (**4**—**6**) in good yield (Table 1). The IR spectra showed one carbonyl band at 1615 cm^{-1} (CHO, vinylogous amide) and bands at 1520 cm^{-1} ν-N=N, 1370 cm^{-1} ν-SO_2NH, 1340 cm^{-1} ν_{asym}-SO_2, 1155 cm^{-1} ν_{sym}-SO_2, 3250 cm^{-1} ν-NH and at 1565 and 1265 cm^{-1} ν-C=O group (Table 2). ^{1}H NMR spectra of **3** and/or **4** compounds in $CDCl_3$ showed signals at δ=3.75—3.60 (s, 6H, $-N(CH_3)_2$), 9.55 (d, 1H, CHO, J=3 Hz, allylic coupling), 8.30 (s, 1H, $-SO_2HN$), 6.75 (s, 1H side-chain methine=CH), 10.60 (s, 1H, -NH), 8.25—6.85 (m, the rest of aromatic protons) (Table 3) (Scheme 2).

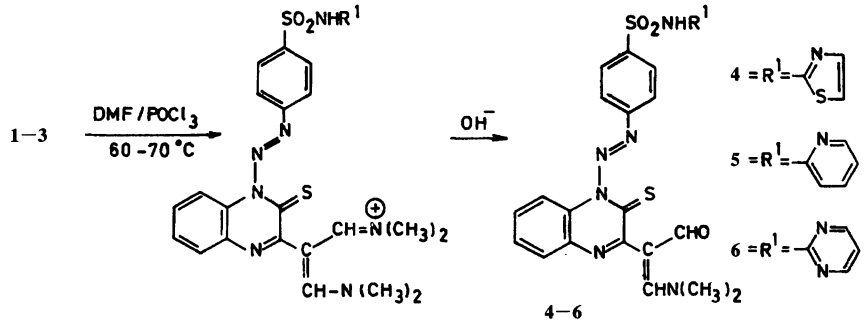
Also, the structures of compounds **4**—**6** were confirmed by their conversion with hot diluted acid to afford the corresponding malondialdehydes **7**—**9**, which gave a pale yellowish brown coloration with ferric chloride (Scheme 3).

The malonaldehydes **7**—**9** and/or 3-aminoacrylaldehydes **4**—**6** on treatment with hydroxylamine, hydrazine hydrate, and phenylhydrazine in boiling ethanol gave the corresponding new azo dyes **10**—**18** containing heterocyclic substitutents at 3 position of the 2(1*H*)-quinoxalinethione moiety. The structures of compounds **10**—**18** were confirmed by their correct elemental analyses data (Table 1) and IR spectra which showed the disappearance of the carbonyl bands related to ν-CHO group. The ^{1}H NMR spectra in $CDCl_3$ showed the absence of signals for $-N(CH_3)_2$ group and the presence of signals related to the rest of all protons (Table 3) (Scheme 4).

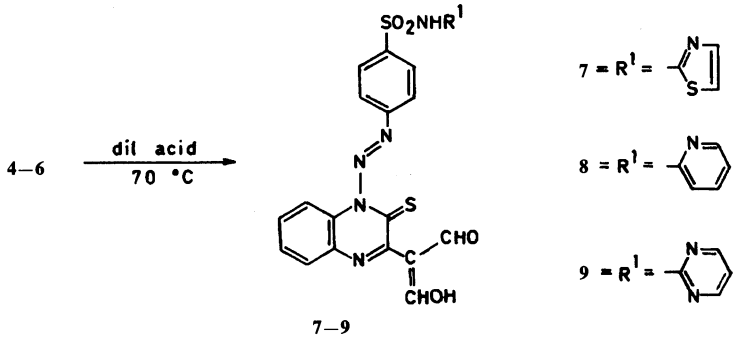
Furthermore, condensation of **4**—**6** or **7**—**9** with some heterocyclic secondary amines, morpholine, piperidine, piperazine, proceeded easily in warm ethanol giving the expected aminomethylene compounds **19**—**27**. The structures of these compounds were established by their elemental analyses data (Table 1). The IR spectra of these compounds were also in agreement with their structures, showing the bands at 1730—1710 cm^{-1} (CHO), at 1555, 1260 cm^{-1} C=S at 1375 cm^{-1} ($-SO_2NH-$), (Table 2).

The ^{1}H NMR spectrum of compounds **20** in $CDCl_3$ showed signals at δ=3.56 and 3.47 due to the morpholine ring $-N-CH_2-$ besides the signals due to other protons and at δ=8.50 (s, 1H, $-SO_2-NH-$) (Table 3) (Scheme 5).

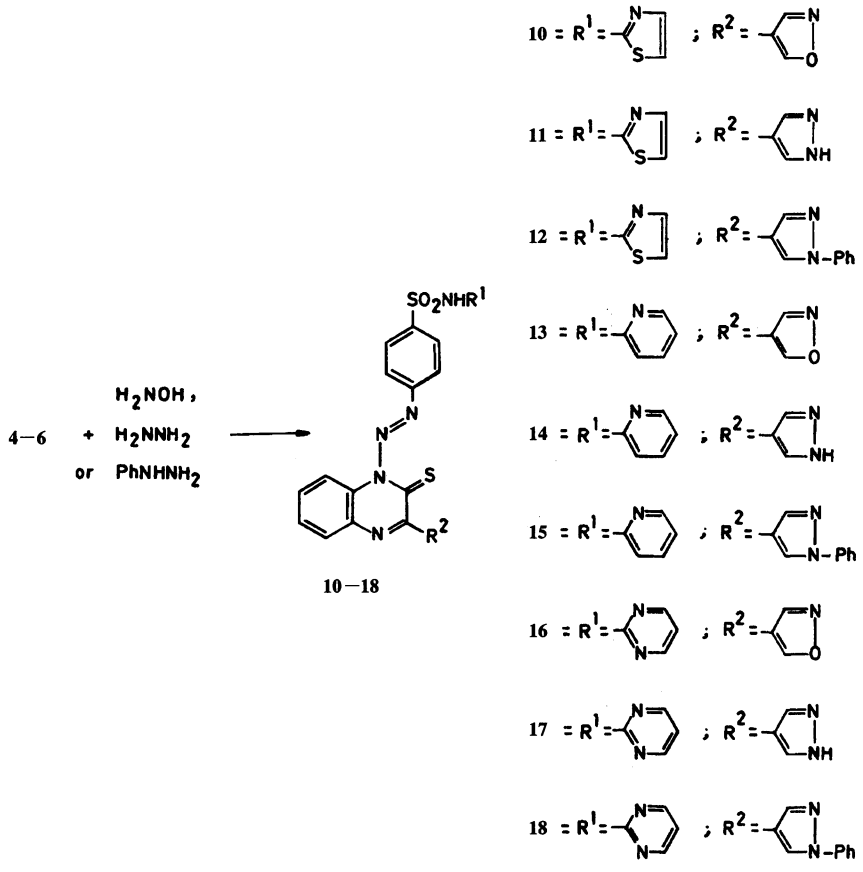
On treatment of isoxazole compounds **10**, **13**, and **16** with sodium hydroxide gave 3-(1-cyano-2-oxoethyl)-substituted 2(1*H*)-quinoxalinethione dyes (**28**—**30**) as shown the characteristic strong absorption band at 2228 cm^{-1} ν-C≡N and at 1695 cm^{-1} ν-CHO in their IR



Scheme 2.



Scheme 3.

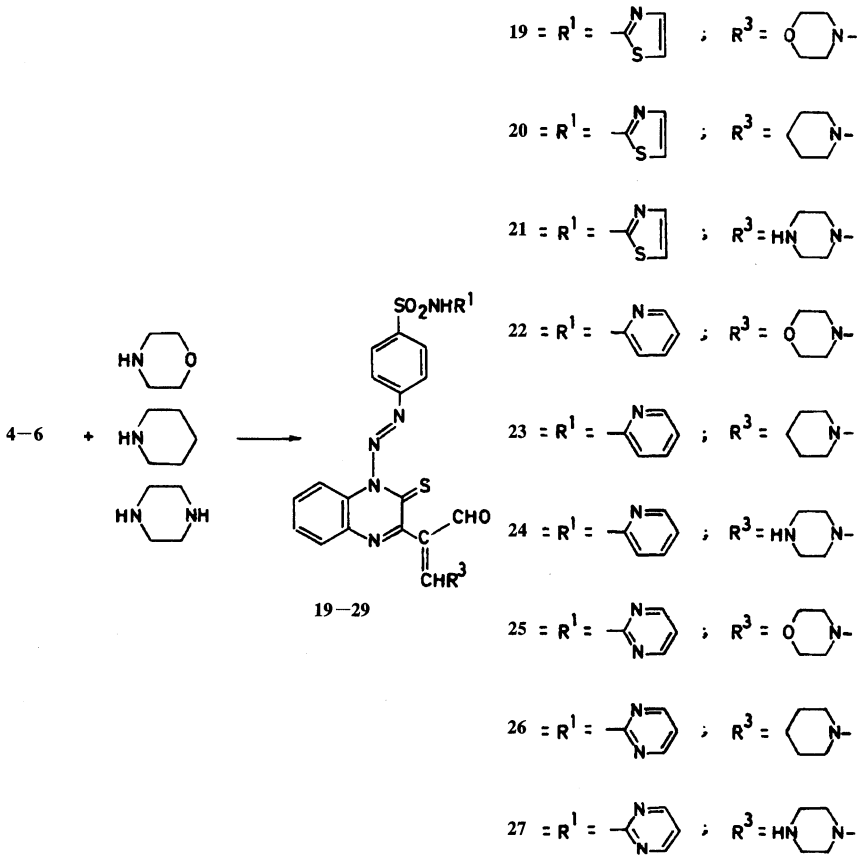


Scheme 4.

Table 3. ^{1}H NMR Spectra of Some Novel Synthesized Compounds (Chemical Shifts in δ/ppm)[a)]

Compd No.	Aromatic protons	$-N<^{CH_3}_{CH_3}$	$-CH_3$	α,β-Unsaturated $-CHO$	$>NH$	Acrolein-methine $=CHN<$	Enolic OH malonaldehyde	$-N<^{CH_2-}_{CH_2-}$	$-SO_2NH-$
	(m)	(s)	(s)	(s)	(s)	(s)	(s)	(t)	(s)
2	8.15—7.00(12H)	—	2.18(3H)	—	—	—	—	—	8.35(1H)
5	8.20—7.10(12H)	3.35(6H)	—	9.15(1H)	—	6.75(1H)	—	—	8.40(1H)
7	8.18—7.10(10H)	—	—	9.25(1H)	—	6.80(1H)	4.90(1H)	—	8.35(1H)
13	8.15—7.00(14H)	—	—	—	—	—	—	—	8.25(1H)
14	8.20—7.00(14H)	—	—	—	9.80(1H)	—	—	—	8.35(1H)
17	8.18—6.95(13H)	—	—	—	9.80(1H)	—	—	—	8.30(1H)
23	8.18—6.95(12H)	—	—	9.20(1H)	—	6.82(1H)	—	2.52(2t,4H, 2αCH$_2$), 3.33(2t,4H,2βCH$_2$), 3.38(t,2H,γCH$_2$).	8.30(1H)
24	8.20—7.00(12H)	—	—	9.25(1H)	9.85(1H)	6.85(1H)	—	2.55(2t,4H, 2αCH$_2$), 3.35(2t,4H,2βCH$_2$).	8.35(1H)
27	8.15—6.95(11H)	—	—	9.25(1H)	9.89(1H)	6.80(1H)	—	2.50(2t,4H,2αCH$_2$) 3.32(2t,4H,2βCH$_2$).	8.30(1H)

a) Deutrated solvent ($CDCl_3$).

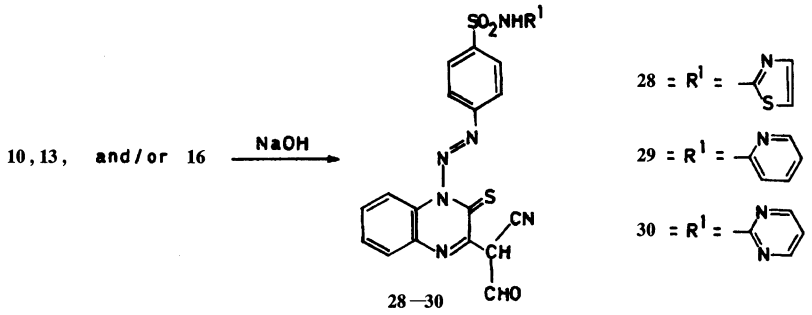


Scheme 5.

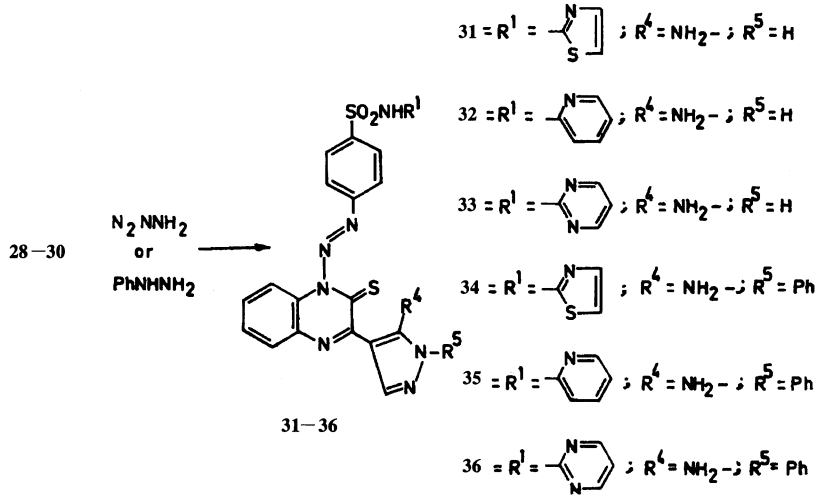
spectra (Scheme 6).

Reaction of cyano aldehyde compounds **28**—**30** with hydrazine hydrate and phenylhydrazine in acetic acid gave the corresponding 5-amino-1*H*-pyrazol-4-yl-derivatives (**31**—**33** and **34**—**36**). The IR spectra of these compounds were also in agreement with the structures indicating the presence of a sharp $-NH_2$ band at 3455 cm^{-1} in addition to the absorption band for C=S group of 2(1*H*)-quinoxalinethione moiety (Scheme 7).

Antimicrobial Activity. The antibacterial results revealed that the synthesized compounds exhibited variable and pronounced activities (inhibition zones ranged from 70—150 mm) against bacteria used. It seems of interest to note that these compounds are highly ac-



Scheme 6.



Scheme 7.

tive against at least four bacteria used namely: *Bacillus anthracis*, *Bacillus cereus*, *Kelbsiella pneumoniae*, and *Serratia rhodnii*. All synthesized compounds showed strong activities (inhibition zones ranged from 70—130 mm) against *Bacillus anthracis* except compounds **24**, **25**, and **27**.

Furthermore, 2(1*H*)-quinoxalinethione sulfa drugs and their heterocyclo-substituted derivatives are comparatively more active than the free *p*-aminobenzenesulfonamide derivatives (inhibition zones ranged from 20—80 mm) against all bacteria under investigation (Table 4).

Antifungal Activity. From the antifungal results, it was found that all synthesized compounds showed variable effects (inhibition zones ranged from 10—170 mm) except compounds **4**, **5**, **7**, **11**, **12**, **19**, **21**, **25**, and **26** are quite potent against all fungi under investigation. Compounds **1**, **9**, **22**, and **23** are highly active (inhibition zones ranged from 75—270 mm) against three fungi namely: *Aspergillus flavus*, *Aspergillus ochraceus*, and *Penicillium chrysogenum*. Interestingly, all the synthesized compounds are bactericidal more than fungicidal effects (Table 4).

Experimental

Melting points were determined on koflor melting point apparatus and are uncorrected. Elemental analyses were performed on a Perkin–Elmer 240 E Microanalyzer. IR spectra were recorded on a Pye-Unicam SP-200G infrared spectrophotometer (KBr Pellets technique). ^{1}H NMR spectra were recorded on a Varian EM-390 MHz instrument in the suitable deuterated solvent ($CDCl_3$), using TMS as an internal reference.

4-(Substituted-sulfamoyl)benzenediazonium acetates were prepared by diazotization of 4-(2-thiazolylsulfamolyl)aniline, 4-(2-pyridylsulfamoyl)aniline, and/or 4-(2-pyrimidinylsulfamoyl)aniline (2.45 g, 0.01 mol), dissolved in acetone and 40 ml of 50% acetic acid, with sodium nitrite (0.7 g, 0.01 mol) at 0—5°C.

Synthesis of 3-Methyl-1-[4-(substitutedsulfamoyl)-phenylazo]-2(1*H*)-quinoxalinethione Dyes (1—3). To an ice cold solution of 3-methyl-2(1*H*)-quinoxalinethione (1.80 g, 0.01 mol) in 25 ml of 10 % sodium hydroxide solution, a cold acetic acid solution of the diazonium salt was added dropwise with stirring, the reaction mixture was further stirred for 2 h at 5—10°C. When a yellowish brown precipitate separated in acidic medium, an excess of cold water was added. The product was collected, washed well with water, and crystallized from methanol. The physical and elemental analysis data are given in Table 1.

3- (2- Dimethylamino- 1- formylvinyl)- 1- (4- sulfamoylphenylazo)-2(1*H*)-quinoxalinethione Dyes (4—6). To *N*,*N*-dimethylformamide (10 ml) cooled to −5°C, $POCl_3$ (0.085 mol) was added dropwise and the solution mixture was left to stand for 15 min till the solution became reddish yellow color. To this, the quinoxalinethione

Table 4. Antimicrobial Screening of the Synthesized Compounds **1**—**36** (Inhibition Zones/mm)[a)]

Compd No.	Antibacterial Activity						Antifungal Activity				
	Bacillus anthracis, DSM 344	*Bacillus cereus*, DSM 345	*Staphylo-coccurs aureus*, DSM 346	*Kelbsiella Pneumoniae*, DSM 681	*Serratia rhadnii*, DSM 1608	*Micro-coccus luteus*, DSM 348	*Aspergillus ochraceus*, Wilhelm AUCC-230	*Aspergillus flavus*, Link AUCC-164	*Penicillium chrysogenum*, Thom AUCC-530	*Rhodotorula rubra*, Demme & Lodder AUCC-5730	*Candida albicans*, Robin Berkhout AUCC-1720
1	60(-ve)	70(50)	-ve(40)	-ve(-ve)	-ve(50)	30(80)	60(-ve)	270(-ve)	200(-ve)	-ve(-ve)	-ve(-ve)
2	100(20)	105(-ve)	95(10)	100(-ve)	60(40)	-ve(-ve)	-ve(-ve)	20(-ve)	20(-ve)	90(-ve)	-ve(-ve)
3	120(-ve)	100(-ve)	100(-ve)	-ve(-ve)	-ve(60)	-ve(-ve)	-ve(-ve)	-ve(-ve)	-ve(-ve)	85(-ve)	-ve(-ve)
4	140	130	20	150	80	85	85	10	90	110	90
5	85	90	-ve	105	50	-ve	70	-ve	100	100	40
6	130	95	-ve	110	80	70	-ve	-ve	20	80	-ve
7	80	120	-ve	130	90	80	90	100	89	85	-ve
8	100	95	40	105	105	-ve	70	65	-ve	40	-ve
9	90	110	-ve	120	80	-ve	80	90	95	-ve	-ve
10	85	90	-ve	105	85	-ve	20	-ve	-ve	-ve	-ve
11	70	80	40	40	-ve	80	70	50	80	95	-ve
12	90	110	-ve	85	95	70	85	75	95	120	-ve
13	105	80	-ve	-ve	75	-ve	-ve	80	-ve	-ve	70
14	90	70	85	-ve	60	70	75	60	-ve	75	60
15	50	60	70	85	-ve	50	-ve	80	60	-ve	80
16	90	100	-ve	120	85	105	-ve	-ve	30	100	-ve
17	85	90	-ve	130	140	70	-ve	85	95	-ve	-ve
18	120	140	80	100	80	70	-ve	90	110	-ve	-ve
19	95	100	85	130	90	60	90	-ve	85	90	70
20	80	130	-ve	100	-ve	85	-ve	-ve	70	80	50
21	90	105	-ve	110	-ve	90	-ve	95	85	95	75
22	105	120	70	120	-ve	105	80	85	75	-ve	-ve
23	110	130	80	140	90	110	170	160	170	-ve	-ve
24	-ve	110	20	-ve	40	100	-ve	60	-ve	-ve	60
25	-ve	75	50	-ve	-ve	95	70	120	110	130	-ve
26	95	70	60	80	70	80	120	90	85	100	95
27	-ve	90	-ve	-ve	85	-ve	-ve	85	-ve	-ve	80
28	130	150	45	30	40	70	105	290	220	-ve	-ve
29	150	160	105	75	95	65	95	40	30	105	40
30	140	180	170	110	120	105	85	-ve	-ve	90	-ve
31	95	100	-ve	90	-ve	-ve	70	-ve	-ve	105	20
32	85	95	-ve	85	90	65	-ve	-ve	-ve	60	-ve
33	105	110	65	105	85	-ve	-ve	-ve	65	-ve	-ve
34	65	85	-ve	-ve	30	-ve	-ve	90	95	60	-ve
35	70	95	80	-ve	-ve	60	85	50	105	-ve	-ve
36	85	105	70	95	65	-ve	70	105	85	-ve	-ve

a) (Values)=Inhibition zones of the free *p*-aminobenzenesulfonamide derivatives. (-ve)=Compounds not active biologically. (DSM)=Deutsche Sammlung von Microorgamifmen (German Collection of Microorganisms). (AUCC)=Assiut University Culture Collection.

azo dye (**1**—**3**) (0.04 mol) dissolved in *N*,*N*-dimethylformamide (15 ml), was added dropwise with stirring. The reaction mixture was left to stand for 20 min with stirring, then heated to 70°C for 6—7 h. The cooled reaction mixture was poured into ice-cold water and treated with 5% sodium hydrogencarbonate solution (100 ml). The reddish yellow solid that separated was filtered, washed thoroughly with cold water, and crystallized from aqueous ethanol. The mp and yields are described in Table 1.

3-(1-Formyl-2-hydroxyvinyl)-1-(4-sulfamoylphenylazo)-2(1*H*)-quinoxalinethione (7—9). The 3-aminoacrylaldehyde derivatives **4**—**6** (1 g) taken in dilute HCl (20 ml) were heated to 60°C for 25 min. It was then filtered off, cooled and basified with 2% sodium hydrogencarbonate solution. The brownish yellow solid that separated was filtered, washed well with cold water, and crystallized from aqueous ethanol. Yields and melting points of the products are listed in Table 1.

3-[4-Isoxazolyl, 4-(1*H*-pyrazol-4-yl), or 4-(1-phenyl-1*H*-pyrazol-4-yl]-1-(4-sulfamoylphenylazo)-2(1*H*)-quinoxalinethione Dyes (10—18). To a solution of aminoacryldehyde derivatives (**4**—**6**) in ethanol (25 ml) was added equimolar quantity (0.01 mol) of hydroxylamine hydrochloride, hydrazine hydrate, and/or phenylhydrazine, respectively. The reaction mixture was refluxed for 1 h, cooled, concentrated and poured onto crushed ice. The precipitate solid was filtered, washed with water several times, and crystallized from methanol. Yields and melting points are given in Table 1.

3-[1-Formyl-2-(morpholinyl, piperidinyl, and/or 1-

piperazinyl)vinyl]-1-(4-sulfamoylphenylazo)-2-(1*H*)-quinoxalinethione Dyes (19—27). To the aminoacryldehyde derivatives (**4**—**6**) (0.01 mol) taken in ethanol (35 ml) was added on (0.01 mol) quantity of some secondary amines (morpholine, piperidine and piperazine). The mixture was gently heated on a water bath for 40 min, the solid that separated after concentration was filtered, washed with cold ethanol, dry diethyl ether and crystallized from methanol. Yields and melting points of the products are presented in Table 1.

3-(1-Cyano-2-oxoethyl)-1-(4-sulfamoylphenylazo)-2(1*H*)-quinoxalinethione Dyes (28—30). The isoxazole compounds **10**, **13**, and/or **16** (1 g, 0.04 mol) taken in 5% aqueous sodium hydroxide 20 ml was heated till a clear solution was obtained (30 min). It was then cooled and acidified with hydrochloric acid. A reddish yellow solid separated out was filtered, washed thoroughly with water, and crystallized from aqueous ethanol. Yields and melting points of the products are listed in Table 1.

3-(5-Amino-1*H*-pyrazol-4-yl and/or 1-phenyl-5-amino-1*H*-pyrazol-4-yl)-1-(4-sulfamoylphenylazo)-2-(1*H*)-quinoxalinethione Dyes (31—36). A mixture of compounds **28**, **29**, and/or **30** (1 g, 0.04 mol) and hydrazine hydrate (80%, 0.4 ml) or phenylhydrazine (0.3 ml) dissolved in acetic acid (20 ml) was heated under reflux for 2 h. The reaction mixture was concentrated, cooled, and poured onto crushed ice. The aminopyrazolyl (**31**—**33**) and aminophenylpyrazolyl-substituted compounds (**34**—**36**) obtained as deep yellow solid were filtered, washed with water, and crystallized from methanol. Yields and melting points of the products are presented in Table 1.

Biological Screening. The disc-diffusion method was used to measure the antimicrobial activity (Sleigh & Timbury, 1981[22—24]). The tested compounds were dissolved in pure *N,N*-dimethylformamide and added at a concentration of 0.5 mg/disk (Whatman No. 3 filter paper, 0.5 cm diameter). The antibacterial spectrum of the different compounds was tested with six straions of bacteria: *Bacillus anthracis* DMS 344, *Bacillus cereus*, DSM 345, *Staphylococcus aureus* DSM 346, *Klebsiella pneumoniae* DSM 681, *Serratia rhadnii* DSM 1608, and *Micrococcus lutues* DSM 348. The antifungal effect of the same compounds was tested with five species of fungi namely: *Aspergillus ochraceus* Wilhelm AUCC-230, *Aspergillus flavus*, Link AUCC-134, *Penicillium chrysogenum* Thom AUCC-230, *Aspergillus flavus*, Link AUCC-164, *Penicillium chrysogenum* Thom AUCC-530, *Rhodotorula rubra* Demme & Lodder AUCC-5730 and *Candida albicans* Robin Cerkhout AUCC-1720. The culture medium for bacteria was nutrient agar (NA) (composed of beef extract 3 g, peptone 5 g, agar 15 g/L and adjusted to ph 7 before sterilization at 121°C for 20 min). Glucose–Czapek's agar medium ($NaNO_3$, 2 g; KH_2PO_4, 1 g; $MgSO_4$, 0.5 g; KCl, 0.5 g; glucose, 10 g; agar, 15 g/L of distilled water) was used for fungi. The inoculated plates were incubated at 37±1°C for 24—48 h in case of bacteria and at 28°C for 7—8 d in case of fungi. The inhibition zones of microbial growth produced by different compounds were measured.[22—24]

References

1) S. Pati, "The Chemistry of Hydrazo, Azo, and Azoxy Groupsi," Part 1, Wiley, New York (1975).
2) S. Moeschlin and U. Schnider, *New Eng. J. Med.*, **1963**, 269.
3) I. M. A. Awad, A. A. M. Aly, A. M. Abdel-Alim, R. A. Abdel-Aal, and S. M. Ahmed, *J. Inorg, Biochem.*, **33**, 77 (1988).
4) I. M. A. Awad, A. A. M. Aly, A. A. Abdel Hafes, and Kh. M. Hassan, *J. Chin. Chem. Soc. (Taipei)*, **36**, 107 (1989).
5) I. M. A. Awad and Kh. M. Hassan, *Phosphorus Sulfur Silicon*, **44**, 135 (1989).
6) I. M. A. Awad and A. A. M. Aly, *Synth. React. Inorg. Met. Org. Chem.*, **21**, 275 (1991).
7) I. M. A. Awad, *Dyes Pigments*, **17**, 123 (1991).
8) I. M. A. Awad, "33rd IUPAC Congress," Budapest, 17—22 August 1991, Abstr., No. 4048, 152.
9) I. M. A. Awad, "33rd IUPAC Congress," Budapest, 17—22 August 1991, Abstr., No. 4230, 174.
10) M. Sirki, Djordjevice, *Anal. Chem.*, **43**, 1375 (1971).
11) S. Shibata, K. Gota, and E. Kamata, *Anal. Chim. Acta*, **55**, 234 (1971).
12) A. A. M. Aly and I. M. A. Awad, *Phosphorus Sulfur Silicon*, **50**, 135 (1991).
13) S. K. Mohanty, R. Sridhar, S. Y. Padmanavan, R. Sounder, and A. S. Mittra, *Indian J. Chem.*, Sect. B, **15**, 1146 (1977).
14) I. M. A. Awad, *J. Chem. Technol. Biotechnol.*, **53**, 227 (1992).
15) I. M. A. Awad and Kh. M. Hassan, *Phosphorus Sulfur Silicon*, **45**, 155 (1989).
16) I. M. A. Awad and Kh. M. Hassan, *Phosphorus Sulfur Silicon*, **47**, 311 (1990).
17) I. M. A. Awad and Kh. M. Hassan, *Collect. Czech. Chem. Commun.*, **54**, 706 (1990).
18) I. M. A. Awad and Kh. M. Hassan, *J. Chim. Chem. (Taipei)*, **37**, 599 (1990).
19) I. M. A. Awad, *Monatsh. Chem.*, **121**, 1023 (1990).
20) I. M. A. Awad, *Bull. Chem. Soc. Jpn.*, **65**, 1652 (1992).
21) I. M. A. Awad, *Phosphorus Sulfur Silicon*, proof, in-print (1992).
22) J. D. Sleight and M. C. Timbury "Notes on Medical-Bacteriology," Churchill, Livingstone, U.S.A. (1981), p. 43.
23) E. S. Moss and A. L. Mcquon "Atlas of Medical Myeology," 3rd ed, the Willams and Wilkinc Co., Baltimore, U.S.A. (1969), p. 366.
24) K. K. Chaturvedi, N. K. Jain, P. Jain, and R. Kaushal, *Indian Drugs*, **15**, 57 (1978).