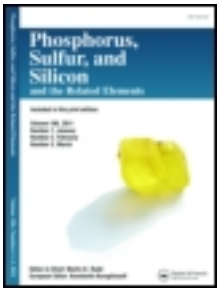

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SYNTHESIS AND BIOLOGICAL EVALUATION OF 1,2,3-THIA AND SELENADIAZOLES-4-DERIVATIVES

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SYNTHESIS AND BIOLOGICAL EVALUATION OF 1,2,3-THIA AND SELENADIAZOLES-4-DERIVATIVES

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Visnagin-9-sulfonyl chloride (**Ic**) reacted with *p*-aminoacetophenone to give visnagin-9-N-(*p*-acetophenyl) sulfonamide (**II**), which in turn was reacted with semicarbazide hydrochloride in the presence of sodium acetate to give the corresponding visnagin-9-N-(*p*-acetophenyl semicarbazone) sulfonamide (**IV**). The latter compound was cyclized through oxidation with thionyl chloride to yield 1,2,3-thiadiazol-4-derivative (**V**), while the oxidative cyclization with selenium dioxide led to the formation of 1,2,3-selenadiazol-4-derivative (**VI**). Visnaginone (**VIIa**) or khellinone (**VIIb**) was reacted with *p*-aminoacetophenone in absolute ethanol to give the Schiff's bases (**VIIIa,b**), respectively. The latter compounds were condensed with semicarbazide hydrochloride to yield the corresponding semicarbazone derivatives (**IXa,b**), respectively. The oxidative cyclization of **IXa** or **IXb** using thionyl chloride gave 1,2,3-thiadiazol-4-derivatives (**Xa**) and (**Xc**), while the oxidative cyclization of **IXa** or **IXb** using selenium dioxide gave 1,2,3-selenadiazol-4-derivatives (**Xb**) and (**Xd**), respectively. The antimicrobial and antiaflatoxigenic activities of thia and selenadiazoles were also investigated.

Key words: Thiadiazoles, selenadiazoles, antimicrobial activity.

INTRODUCTION

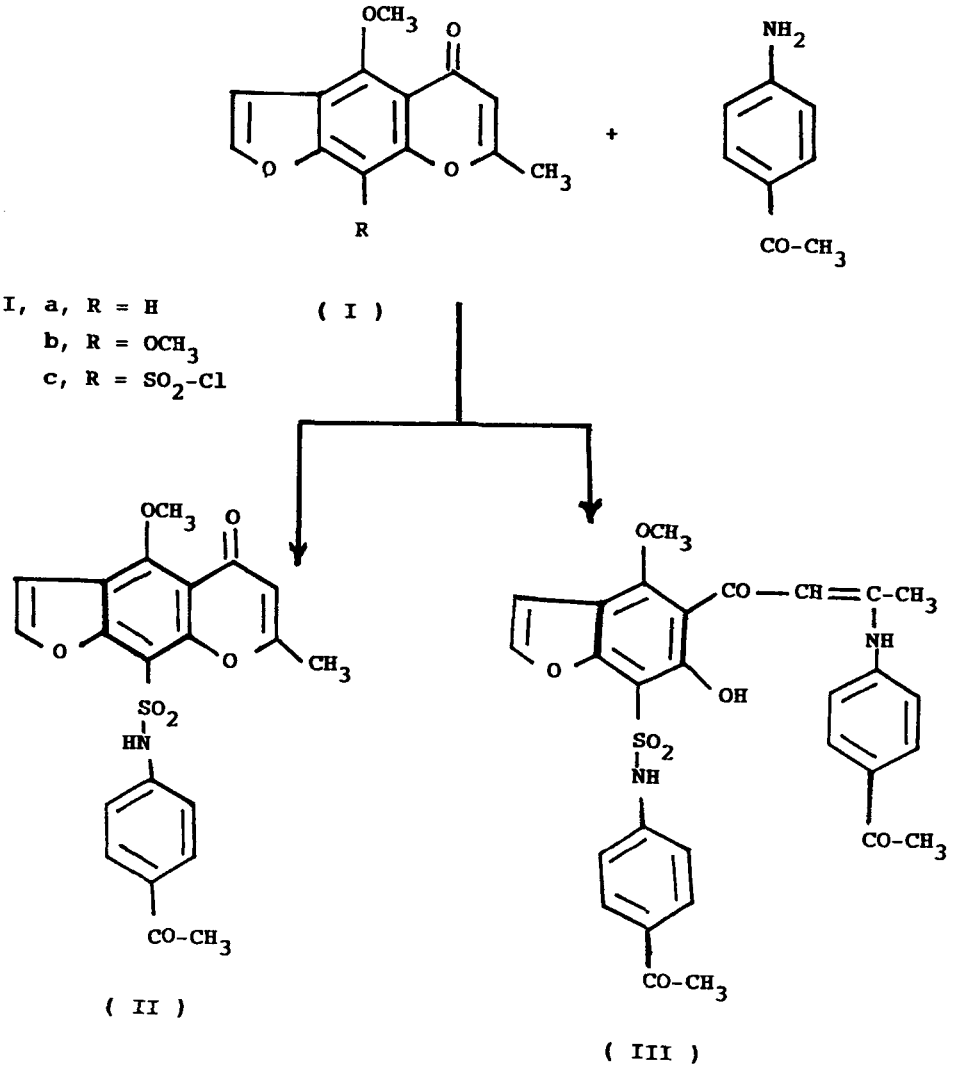
Visnagin (**Ia**) isolated from *Ammi visnaga*, has been known to exhibit diverse biological and physiological activities.[1–6] Also, visnagin-9-derivatives possess antimicrobial and antiaflatoxigenic activities.[7–9] Benzofuran derivatives possess antimicrobial and antiaflatoxigenic activities.[10,11] The 1,2,3-thia and selenadiazol-4-derivatives were found to possess antibacterial and antifungal activities.[12–17] It is of interest to synthesize visnagin and benzofuran containing 1,2,3-thia and selenadiazoles to evaluate their antimicrobial and antiaflatoxigenic activities in a trial to obtain new derivatives with high biological activity.

DISCUSSION

Previous studies reported that, visnagin (**Ia**) reacts with primary aliphatic or aromatic amines and not with secondary amines, the reaction involves the opening of the γ-pryone ring and the formation of the corresponding 6-hydroxy-4-methoxy-5-(1-oxo-3-alkyl or aryl imino butyryl) benzofuran.[18] Also, the reaction of visnagin-9-sulfonyl chloride reacts with *p*-toluidine to give visnagin-9-N-(*p*-tolyl) sulfonamide as a major product together with 6-hydroxy-4-methoxy-5-[3-(*p*-tolyl) amino-1-oxo-2-butenyl]-7-(*p*-tolyl) benzo furansulfonamide as a minor product.[19]

In the present work, visnagin-9-sulfonyl chloride[19,20] (**Ic**) reacted with 2 moles of *p*-aminoacetophenone in boiling dioxane for 3 hours to yield visnagin-9-N-(*p*-acetophenyl) sulfonamide (**II**) in a yield about 65% as a major product and a minor product about 20% yield identified as 6-hydroxy-4-methoxy-5-[3-(*p*-N-acetonphenyl amino)-1-oxo-2-butenyl]-7-N-(*p*-acetophenyl) benzofuransulfonamide (**III**) (Scheme 1). Compound **III** gave positive ferric chloride test.

The structures of compounds **II** and **III** were confirmed by their correct elemental analyses (Table I), IR and ^{1}HNMR spectra. The IR spectrum of **II** showed bands at cm^{-1}, at 3300 (NH, str.), at 2900 (CH, str.), at 1675 (C=O, acetyl), at 1620 (C=O, pyrone), at 1590 (C=C, str.), at 1120 and 1080 (C—O, pyrone and furan) and two bands at 1150 and 1385 (SO_2—N). The IR of **III** showed absorption bands at cm^{-1}, at 3450 (OH, str.), at 3200, 3100 (NH, str.), at 2850 (CH, str.), at 1670, 1680



Scheme 1

TABLE I

The physical and analytical data of the prepared compounds

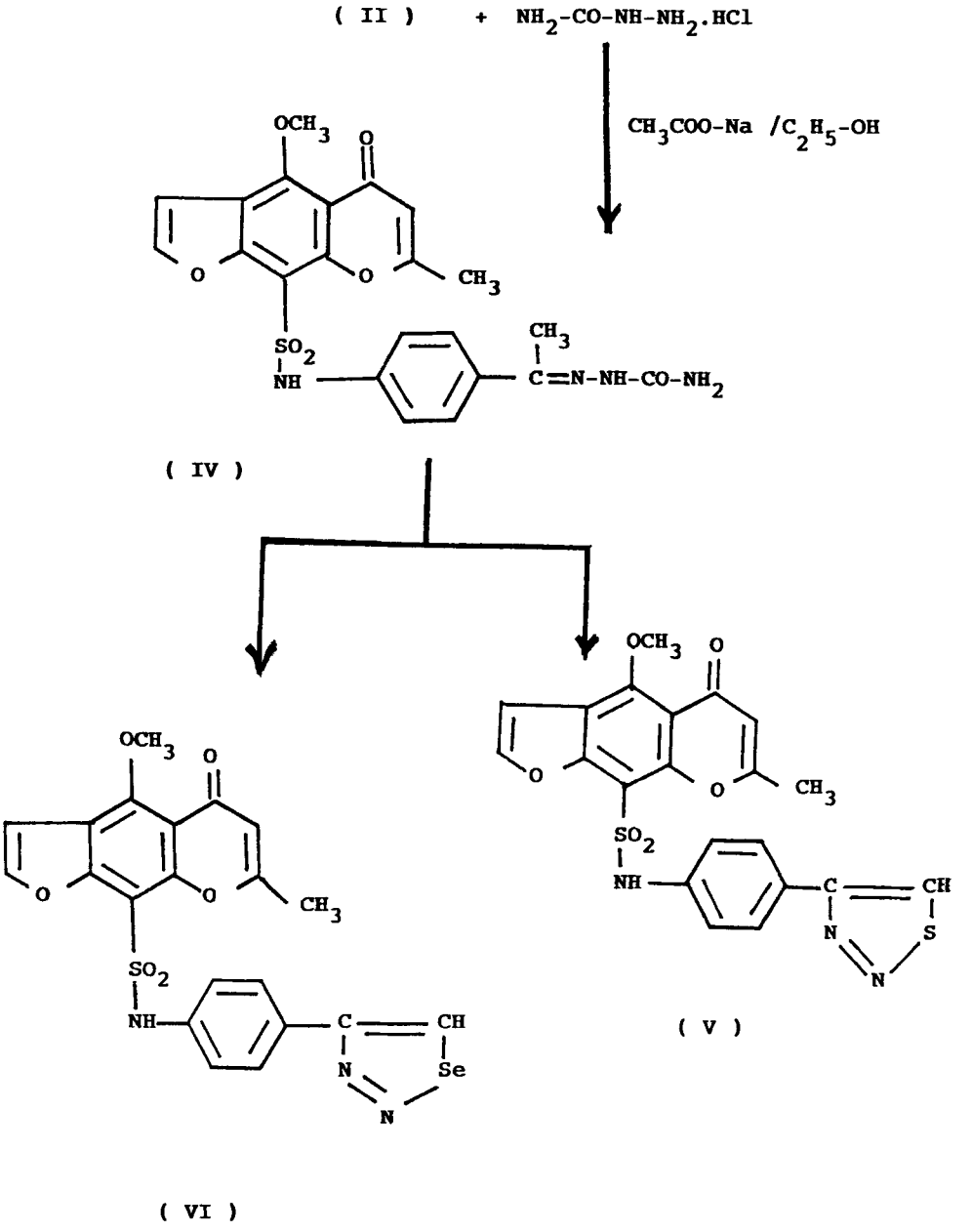
Compound No.	Molecular formula Molecular weight	yield %	M.P. °C	Analysis Calcd./Found C%	H%	N%
II	$C_{21}H_{17}NO_7S$ (427)	65	215-17	59.01	3.98	3.27
				58.75	4.10	2.90
III	$C_{29}H_{27}N_2O_8S$ (563)	20	165-7	61.81	4.79	4.97
				61.55	5.00	5.08
IV	$C_{22}H_{20}N_4O_7S$ (484)	50	265-7	54.54	4.13	11.57
				54.45	4.50	11.25
V	$C_{21}H_{15}N_3O_6S_2$(469)	30	301-3	53.73	3.19	8.95
				53.45	3.44	8.55
VI	$C_{21}H_{15}N_3O_6SSe$(516)	40	310-12	48.83	2.90	8.13
				48.55	2.55	8.55
VIIIa	$C_{19}H_{17}NO_4$ (323)	40	145-7	70.58	5.26	4.33
				70.44	5.55	4.65
b	$C_{20}H_{19}NO_5$ (353)	40	140-2	67.98	5.38	3.96
				68.09	5.44	4.09
IXa	$C_{20}H_{20}N_4O_4$(380)	45	190-2	63.15	5.26	14.73
				62.99	5.44	14.65
b	$C_{21}H_{22}N_4O_5$(410)	40	180-2	61.46	5.36	13.65
				61.77	5.44	13.32
Xa	$C_{19}H_{15}N_3O_3S$(365)	30	297-9	62.46	4.10	11.50
				62.33	3.90	11.32
b	$C_{20}H_{17}N_3O_4S$(395)	25	285-7	60.75	4.30	10.63
				60.88	4.22	10.77
c	$C_{19}H_{15}N_3O_3Se$(412)	25	315-7	55.33	3.64	10.19
				55.65	3.87	10.43
d	$C_{20}H_{17}N_3O_4Se$(442)	25	310-12	54.29	3.84	9.50
				54.61	3.99	9.21

(C═O, acetyl), at 1600 (C═C, str.), at 1090 (C—O, furan), and two bands at 1145 and 1375 (SO_2—N).

The ^{1}HNMR spectrum of **II** (DMSO-d_6) revealed signals (δ = ppm) at 8.2 (1H, d, H-2), five aromatic protons multiplet centered at 7.5, (4H, phenyl and H-3), at 6.2 (1H, s, H-6), at 4.2 (3H, s, OCH_3), at 2.5 (3H, s, CH_3) and at 2.35 (3H, s, CH_3). The ^{1}HNMR spectrum of **III** DMSO-d_6) revealed signals (δ ppm) at 8.1 (1H, d, H-2), at 7.45 (1H, d, H-3), at 6.3 and 6.1 (2H, dd, CH═CH), at 7.5–7.7, multiplet (8H, aromatic), at 4.1 (3H, s, OCH_3), at 2.9 (3H, s, CH_3) and at 2.3 (6H, s, $2CH_3$).

The condensation of compound **II** with semicarbazide hydrochloride in the pres-

ence of sodium acetate (Angla's method)[21] gave the corresponding semicarbazone derivative (**IV**) (Scheme 2). The IR spectrum of **IV** showed absorption bands at cm^{-1}, at 3605, 3477 and 3135 (NH and NH_2, str.), at 2976 (CH, str.), at 1762 (C=O, str. amide), at 1635 (C=O, pyrone), at 1599 (C=N, str.), at 1585 (C=C, str.), at 1080 (and 1024 (C—O, pyrone and furan), and two bands at 1148 and 1352 (SO_2—N).



Scheme 2

Oxidative cyclization of **IV** with thionyl chloride[22] led to the formation of visnagin-9-N-[4-(1′,2′,3′-thiadiazol-4′-yl) phenyl] sulfonamide (**V**). The oxidative cyclization of **IV** with selenium dioxide in glacial acetic acid[23] gave visnagin-9-N-[4-(1′,2′,3′-selenadiazol-4′-yl) phenyl] sulfonamide (**VI**) (Scheme 2). The physical and analytical data of compounds **V** and **VI** are illustrated in Table I.

The IR spectra of compounds **V** and **VI** showed absorption bands at cm^{-1}, at 3385 and 3340 (NH, str.), at 1661 and 1665 (C=O, pyrone), at 1607 and 1620 (C=N, str.), at 1570 and 1580 (C=C, str.), at 1100 and 1120 (C—O, pyrone), at 1040 and 1080 (C—O, furan), and two bands for each at 1159, 1166 and 1375 and 1385 (SO_2—N).

The ^{1}HNMR spectrum of compound **V** (DMSO-d_6) revealed signals (δ ppm) at 11.3 (1H, s, NH), at 8.2 (1H, d, H-2 furan), at 8.0 (1H, s, H-5′ thiadiazole), at 7.7 (2H, d, H-2, 6 phenyl), at 7.45 (1H, s, H-6 pyrone), at 7.2 (2H, d, H-3, 5 phenyl), at 6.15 (1H, d, H-3 furan), at 4.0 (3H, s, OCH_3) and at 2.45 (3H, s, CH_3).

Alkaline hydrolysis of visnagin (**Ia**) gave visnaginone (**VIIa**)[24] and of khellin (**Ib**) gave khellinone (**VIIb**).[25] The condensation of compound **VIIa** or **VIIb** with *p*-aminoacetophenone in absolute ethanol and in the presence of few drops of glacial acetic acid gave the corresponding Schiff's bases (**VIIIa, b**), respectively (Scheme 3). Condensation of the latter compounds with semicarbazide hydrochloride in the presence of sodium acetate led to the formation of the corresponding semicarbazone derivatives (**IXa** and **IXb**), respectively (Scheme 3). The structures of the obtained compounds were confirmed by their correct elemental analyses (Table I), IR and ^{1}HNMR spectra.

The IR spectrum of compound **VIIIa** showed absorption bands at cm^{-1}, 3540 (OH, str.), at 1680 (C=O, str.), at 1620 (C=C, str.), at 1600 (C=N, str.), at 1080 (C—O, furan). The IR spectrum of **IXb** showed absorption bands at cm^{-1}, at 3600 (OH, str.), at 3450, 3320 and 3100 (NH, NH_2, str.), at 1675 (C=O, amide, str.), at 1630 (C=C, str.), at 1595 and 1600 (C=N, str.), at 1070 (C—O, furan).

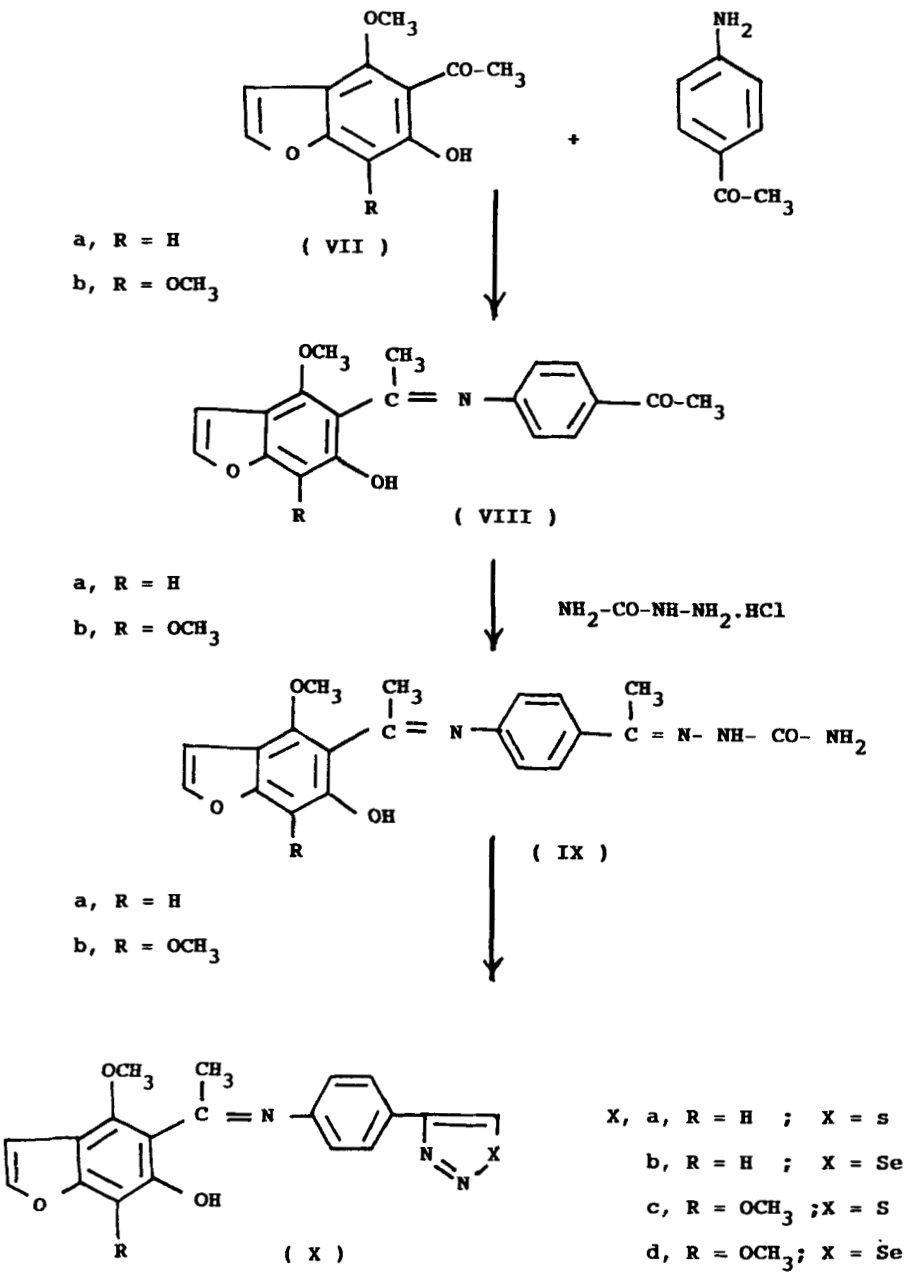
The ^{1}HNMR spectrum of **VIIIb** (DMSO-d_6) revealed signals (δ ppm) at 8.0 (1H, d, H-2), at 7.7 (1H, d, H-3), at 7.3–7.5 (4H, m, phenyl), at 4.0 and 4.1 (6H, ss, $2OCH_3$), at 2.9 (3H, s, CH_3) and at 2.2 (3H, s, CH_3).

The oxidative cyclization of compounds **IXa,b** using thionyl chloride led to the formation of the corresponding 1,2,3-thiadiazol-4-derivatives (**Xa,c**), respectively. The oxidative cyclization of compounds **IXa,b** using selenium dioxide in glacial acetic acid gave the corresponding 1,2,3-selenadiazol-4-derivatives (**Xb,d**) respectively (Scheme 3). The structures of the obtained compounds were confirmed by elemental analyses (Table I), IR and ^{1}HNMR spectra.

The IR spectra of compounds **Xa** and **Xd** showed absorption bands at cm^{-1}, at 3550 and 3520 (OH, str.), at 1630 and 1650 (C=N, str.), at 1610 and 1595 (N=N, str.), at 1600 and 1610 (C=C, str.) at 1080 and 1090 (C—O, furan).

The ^{1}HNMR spectrum of compound **Xa** (DMSO-d_6) revealed signals (δ ppm) at 8.0 (1H, d, H-2), at 7.8 (1H, s, H-5′-thiadiazole), at 7.6 (1H, d, H-3), at 7.1 (1H, s, H-7), at 7.4–7.5 (4H, m, phenyl) at 4.1 (3H, s, OCH_3) and at 2.4 (3H, s, CH_3).

The ^{1}HNMR spectrum of compound **Xc** (DMSO-d_6) revealed signals (δ ppm) at 7.9 (1H, d, H-2), at 7.8 (1H, s, H-5′ thiadiazole), at 7.55 (1H, d, H-3), at 7.3–7.45 (4H, m, phenyl), at 4.0 and 4.1 (6H, ss, $2OCH_3$) and at 2.35 (3H, s, CH_3).



Scheme 3

EXPERIMENTAL

Melting points are uncorrected and were taken on Stuart Scientific MP, SMP1 (UK). Infrared spectra were recorded on an IMR 16 infrared spectrophotometer Karl Zeies and Mattson 1000, FTIR spectrophotometer. The ^{1}HNMR spectra were determined on Jeol EX-270 MHz spectrometer.

Preparation of Compounds II *and* III

Visnagin-9-sulfonyl chloride (Ic) (0.01 mol) and *p*-aminoacetophenone (0.02 moles) in 20 ml dioxane were refluxed for three hours in the presence of few drops of triethylamine. After cooling, the product

was filtered off, washed with water, dried and crystallized from ethanol/water to give compound **II**. The filtrate was concentrated to 1/3 of its volume and water was added and the formed product was filtered off, washed with water and crystallized from methanol/water to give compound **III**.

Preparation of the Schiff's Bases **VIIIa,b**

Visnaginone (**VIIa**) or khellinone (**VIIb**) (0.01 mol) and *p*-amino acetophenone (0.01 mol) in 50 ml absolute ethanol and 1 ml of glacial acetic acid, were refluxed for 7 hours on a water bath. After cooling and adding water, the product was filtered off, air dried and crystallized from ethanol-water. The physical and analytical data are shown in Table I.

Preparation of Semicarbazone Derivatives **IV**, **IXa** *and* **IXb**

A mixture of 0.11 g (0.001 mol) semicarbazide hydrochloride and 0.082 g (0.001 mole) crystalline sodium acetate in 10 ml water, was added while stirring to a solution of (0.001 mol) of **II**, **VIIIa** or **VIIIb** in 50 ml (95%) ethanol. Stirring was continued for 15 min and the mixture was cooled in

TABLE II

The antimicrobial activity screening of 1,2,3-thia- and selenadiazol-4-derivatives

Compound No.	Micro-organism/inhibition zone 1	2	3	4	5	6
Reference	+++	+++	+++	+++	+++	+++
V	+++	+++	++	++	+++	+++
VI	+++	+++	++	++	++	+++
Xa	++	++	-	-	+++	+++
b	++	++	-	-	++	++
c	++	++	-	-	++	+++
d	++	++	-	-	++	++

All the compounds including the reference (chloroamphenicol) were tested in a concentration of 500 ug/disk (0.5 cm, diameter).

+++ = Highly active (inhibition zone > 12 mm).

++ = Moderately active (inhibition zone 9-12 mm).

+ = Slightly active (inhibition zone 6-9 mm).

- = Not sensitive.

Micro-organisms: 1-Bacillus subtilis 2-Staphylococcus aureus
3-Escherichia coli 4-Pesudomonas aeruginosa
5-Candida albicans 6-Aspergillus flavus

TABLE III

Antiaflatoxigenic activity of 1,2,3-thia and selenadiazol-4-derivatives using two concentrations 0.1 and 0.5 mM/L medium of *Aspergillus parasiticus* NRRL-3145

Compound No.	Final pH	Mycelial dry weight g/L	Total aflatoxins (B+G) µg/L	% Reduction of total toxin
Control	5.0	10	15500	100.00
V	5.1	9	3700	76.12
	4.9	10	7600	50.96
VI	5.0	10	3800	75.48
	5.1	9	6900	55.48
Xa	4.9	11	4000	74.19
	5.1	10	8300	46.45
b	4.8	10	2400	84.51
	5.0	11	5800	62.58
c	4.9	9	2600	83.22
	5.1	10	8600	44.51
d	4.9	11	2700	82.58
	5.1	9	5100	67.09

Total aflatoxins represents the toxins ($B_1 + B_2 + G_1 + G_2$). Assuming that the percentage of the toxin in control is 100, the percentage of reduction in toxin content under the action of the test compound is calculated as follows:

$$\% \text{ Reduction of the total toxin} = 100 - \left[\frac{\text{Total toxin}}{\text{Control}} \times 100 \right]$$

refrigerator (5°C). The precipitated product was filtered off, washed with cold water, dried and crystallized from ethanol to give the title compounds. The physical and analytical data are shown in Table I.

Preparation of 1,2,3-thiadiazol-4-derivatives **V**, **Xa** *and* **Xc**

To (0.001 mol) of compound **IV**, **IXa** or **IXb**, was added gradually (0.027 mol) (2 ml) of thionyl chloride at ice bath temperature and then allowed to stand for 30 min. Chloroform was added (30 ml) and the reaction mixture was decomposed with saturated solution of sodium carbonate. The organic layer was separated and dried over anhydrous sodium sulfate. After evaporation of the solvent, the obtained product was crystallized from dimethylformamide to give the title compounds. The physical and analytical data of the obtained compounds are shown in Table I.

Preparation of 1,2,3-selenadiazol-4-derivatives **VI**, **Xb** *and* **Xd**

Compound **IV**, **IXa** or **IXb** (0.00 mol) was dissolved in 10 ml boiling glacial acetic acid. To the boiling solution, 0.14 g (0.007 mol) of powdered selenium dioxide was added portionwise while stirring. After complete addition, boiling and stirring were continued for one hour. The reaction mixture was then filtered while hot, and the filtrate was cooled and then poured onto ice-water. The formed product was

filtered off, washed with water, dried and crystallized from ethanol-water to give the title compounds. The physical and analytical data are shown in Table I.

Biological Activity Test

1. Antimicrobial Activity Test: The antimicrobial activity test was performed using the disk diffusion method.[26] From the data obtained (Table II), it is clear that compounds **V** and **VI** possess high activity as reference towards Gram-positive bacteria and fungi. Compounds **Xa–d** possess moderate activity towards Gram-positive bacteria and fungi. Compounds **V** and **VI** possess moderate activity towards Gram-negative bacteria, while compounds **Xa–d** were inactive towards Gram-negative bacteria. Compounds **V** and **Xa** possess high activity towards yeast, while the others were found to possess moderate activity towards yeast.

2. Antiaflatoxigenic Activity Test: Determination of aflatoxins (B_1, B_2, G_1 and G_2) was performed in the filtrate obtained from *Aspergillus parasiticus* NRRL-3145 grown in Sabouraud's yeast broth (0.5% yeast). Extraction of aflatoxins was performed according to Hitokoto *et al.* (1980).[27] Estimation of aflatoxins was performed using TLC plates according to AOAC method.[28] The mycelial dry weight was estimated according to Madhystha and Bhat (1984).[29]

For the determination of aflatoxins production by the fungus *Aspergillus parasiticus*, two concentrations of the tested compounds were used (0.1 and 0.5 mM/L medium). From the data obtained in Table III, it is clear that, compounds **Xa** and **Xc** at a concentration of 0.5 mM/l medium were to be the most active towards fungus, since the percentage reductions were found to be 46.45 and 44.51, respectively. On the other hand, all the compounds at a concentration of 0.1 mM/L were found to be slightly active towards the fungus since, the percentage reduction were more than 75%. In general, all the compounds under test were found to be slightly active towards the fungus and have not potential use as antifungitoxicant agent but have moderate activity towards the fungi and may have potential use as fungistatic agents.

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