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SYNTHESIS, ANTIMICROBIAL AND ANTIAFLATOXIGENIC ACTIVITIES OF SOME BENZOFURAN CONTAINING 1,2,4-TRIAZOLE, 1,3,4-THIADIAZOLE AND OXADIAZOLE DERIVATIVES

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Alkaline hydrolysis of 9-methoxypsoralene-4-sulfonamide derivatives (Ia-d) using potassium hydroxide and dimethyl sulfate, afforded 3-propenoic acid derivative (IIa-d) which in turn were converted to 3-propenoic acid chloride (IIIa-d) by the action of thionyl chloride in boiling toluene. Condensation of the latter compounds with thiosemicarbazide gave the corresponding carbonyl thiosemicarbazide derivatives (IVa-d). Cyclization of the latter compounds using boiling 5% sodium hydroxide solution gave 5-mercapto-1,2,4-triazolyl-3 derivatives (Va-d), using O-phosphoric acid gave 5-amino-1,3, 4-thiadiazolyl-3-derivatives (VIa-d) and using DCC gave 5-amino-1, 3, 4-oxadiazolyl-2-derivatives (VIIa-d), respectively. Also, the antimicrobial and antiaflatoxigenic activities of compounds (Va-d), (VIa-d) and (VIIa-d) were investigated.

Key words: Xanthotoxin-4-sulfonamides, Benzofuran derivatives, 1,2,4-Triazole; 1,3,4-Thiadiazole, Oxadiazole derivatives.

Introduction

It has been reported that benzofuran derivatives possess bacteriostatic, bactericidal, fungistatic and fungicidal activity [1-9]. On the other hand, the sulfonamide derivatives exhibit remarkable bacteriostatic action [10,11]. Moreover, substituted triazole, oxadiazole and thiadiazole are known to possess bacteriostatic and bactericidal activity [12-18] and are used as antifungal agents [19-21]. Also, oxadiazole derivatives and related compounds are used as potential hypoglycemic agents [22]. Therefore, it becomes of interest to prepare some new benzofuran derivatives containing sulfonamide and heterocyclic moieties which can be prepared from the naturally occurring substance xanthotoxin (9-methoxypsoralene) and test their antimicrobial as well as antiaflatoxigenic activities.

Experimental

Preparation of 3-[5-(6,7-dimethoxy benzofuryl-4-sulfonamido)]propenoic acid (IIa-d). One gram of 9-methoxypsoralene-4-sulfonamides (Ia-d) was dissolved in 50 ml of acetone and dimethyl sulfate 10 ml was added, followed by 50 ml 20% potassium hydroxide. After refluxing for 15 min another 10 ml of dimethyl sulfate was added followed by 25 ml of 20% potassium hydroxide. Reflux was continued for 2 hr. The solution was then cooled and acidified with dilute hydrochloric acid. The acetone was removed in vacuo and the product was collected, dried and then crystallized from methanol. The physical and analytical data are illustrated in Table 1.

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Preparation of 3-[5-(6,7-dimethoxy benzofuryl-4-sulfonamido)] propenoic acid chloride (IIIa-d). One gram of propenoic acid (IIa-d) was suspended in 20 ml dry toluene and 5 ml thionyl chloride was added. After refluxing for 3 hr, toluene and excess thionyl chloride were removed under vacuo and the obtained product was crystallized from chloroform. The physical and analytical data are illustrated in Table 1.

Preparation of 3-[5-(6,7-dimethoxy benzofuryl-4-sulfonamido)]propenoyl thiosemicarbazides (IVa-d). Equimolecular ratio (0.001 mol) of the acid chloride (IIIa-d) and thiosemicarbazide in 20 ml dioxane and triethylamine were refluxed for 2 hr. After cooling, water was added and the formed product was filtered off, washed with water, dried and then crystallized from methanol. The physical and analytical data of compounds (IVa-d) are illustrated in Table 1.

1-[5-(6,7-Dimethoxy benzofuryl-4-sulfonamido)]-2-(5-mercapto-1,2,4-triazol-3-yl) ethene (Va-d). A mixture of (0.001 mol) carbonyl thiosemicarbazido derivatives (IVa-d) and 5% sodium hydroxide (4 ml) was refluxed for 2 hr. After cooling, the reaction mixture was neutrallized with dilute hydrochloric acid and the formed product was collected and crystallized from ethanol. The physical and analytical data are illustrated in Table 1.

1-[5-(6,7-Dimethoxy benzofuryl-4-sulfonamido)]-2-(5-amino-1,3,4-thiadiazol-2-yl) ethene (VIa-d). To 0.001 mol of carbonyl thiosemicarbazido derivatives (IVa-d), was added gradually with stirring orthophosphoric acid (3 ml) and the reaction mixture was then heated at 120°C for 6 hr. The reaction mixture was cooled and neutralized with ammonia solution. The formed product was collected, dried and then crystallized from ethanol. The physical and analytical data of

the obtained products are illustrated in Table 1.

1-[5-(6,7-Dimethoxy benzofuryl-4-sulfonamido)]-2-(5-amino -1,3,4-oxadiazol-2-yl) ethene (VIIa-d). A mixture of carbonyl thiosemicarbazido derivatives (IVa-d) (0.001 mol) in dry ethanol (10 ml) and DCC (0.28 g, 0.0015 mol) was refluxed for 7 hr. After cooling, the separated product was washed with aqueous ethanol and recrystallized from ethanol. The physical and analytical data of the obtained products are illustrated in Table 1.

TABLE 1. THE PHYSICAL AND ANALYTICAL DATA OF THE PREPARED COMPOUNDS.

Compd. No.	Molecular formula Molecular weight	M.P. (°C)	Yield (%)	Analysis Calcd. / Found C%	H%	N%
IIa	$C_{15}H_{17}NO_7S$ 355	150-2	55	50.70	4.78	3.94
				50.45	4.90	4.10
b	$C_{17}H_{21}NO_7S$ 383	180-2	60	53.61	5.84	3.65
				53.50	5.30	3.20
c	$C_{18}H_{21}NO_7S$ 395	147-9	55	54.68	5.31	3.54
				54.30	5.10	4.00
d	$C_{17}H_{19}NO_8S$ 397	143-5	55	51.38	4.78	3.52
				51.55	4.45	3.85
IIIa	$C_{15}H_{16}ClNO_6S$ 373.5	175-7	40	48.19	4.28	3.75
				47.90	3.90	3.60
b	$C_{17}H_{20}ClNO_6S$ 401.5	195-7	45	50.81	4.98	3.48
				50.25	4.60	3.50
c	$C_{18}H_{20}ClNO_7S$ 413.5	180-2	50	52.24	4.84	3.38
				52.60	4.50	3.50
d	$C_{17}H_{18}ClNO_7S$ 415.5	183-5	40	49.09	4.33	3.36
				49.35	4.20	3.55
IVa	$C_{16}H_{20}N_4O_6S_2$ 428	220-2	65	44.86	4.67	13.08
				44.55	4.45	13.50
b	$C_{18}H_{24}N_4O_6S_2$ 456	255-7	60	47.37	5.26	12.28
				47.55	5.55	12.25
c	$C_{19}H_{24}N_4O_6S_2$ 468	213-5	55	48.71	5.13	11.96
				48.55	5.45	11.65
d	$C_{18}H_{22}N_4O_7S_2$ 470	219-21	45	55.96	4.68	11.91
				55.65	4.75	11.65
Va	$C_{16}H_{18}N_4O_5S_2$ 410	260-2	35	46.83	4.39	13.66
				46.55	4.65	13.30
b	$C_{18}H_{22}N_4O_5S_2$ 438	295-7	40	49.31	5.02	12.78
				49.65	4.90	12.55
c	$C_{19}H_{22}N_4O_5S_2$ 450	255-7	40	50.67	4.89	12.44
				50.55	4.55	12.50
d	$C_{18}H_{20}N_4O_6S_2$ 452	264-6	45	47.79	4.42	12.39
				47.45	4.65	12.89
VIa	$C_{16}H_{18}N_4O_5S_2$ 410	265-6	55	47.83	4.39	13.66
				46.55	4.45	13.34
b	$C_{18}H_{22}N_4O_5S_2$ 438	302-4	50	49.31	5.02	12.78
				49.35	5.45	12.65
c	$C_{19}H_{20}N_4O_5S_2$ 450	261-3	40	50.67	4.89	12.44
				50.55	4.48	12.76
d	$C_{18}H_{20}N_4O_6S_2$ 452	286-7	50	47.79	4.42	12.39
				47.64	4.68	12.78
VIIa	$C_{16}H_{18}N_4O_6S$ 394	255-7	45	48.73	4.56	14.21
				48.54	4.76	14.44
b	$C_{18}H_{22}N_4O_6S$ 422	269-71	40	51.18	5.21	13.27
				51.43	5.45	13.53
c	$C_{19}H_{22}N_4O_6S$ 434	305-7	45	52.53	5.07	12.90
				52.66	4.97	12.75
d	$C_{18}H_{20}N_4O_7S$ 436	310-12	40	49.54	4.58	12.84
				49.66	4.33	12.65

Preliminary antimicrobial activity screening. The antimicrobial activity test was performed according to the cup plate method adopted with some modification [28]. The compounds (Va-d), (VIa-d) and (VIIa-d) were tested against two strains of gram-positive and two strains of gram-negative bacteria, one strain of yeast and one strain of fungi.

From the data obtained (Table 2), it is clear that compounds (Va,b), (VIa,b) and (VIIa,b) possess high activity towards gram-positive bacteria (*B. subtilis*) and also against yeast (*C. albicans*). On the other hand, all the triazole, thiadiazole and oxadiazole derivatives possess moderate activity towards yeast, fungi and gram-positive bacteria (*S. aureus*). Also, all compounds possess slight activity towards gram-negative bacteria (*E. coli* and *P. aeruginosa*).

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.%% yeast). Extraction of aflatoxins was performed according to Hitokoto *et al.* [29]. Estimation of aflatoxins was performed using TLC plates according to AOAC method [30]. The mycelial dry weight was estimated according to Madhyastha and Bhat [31].

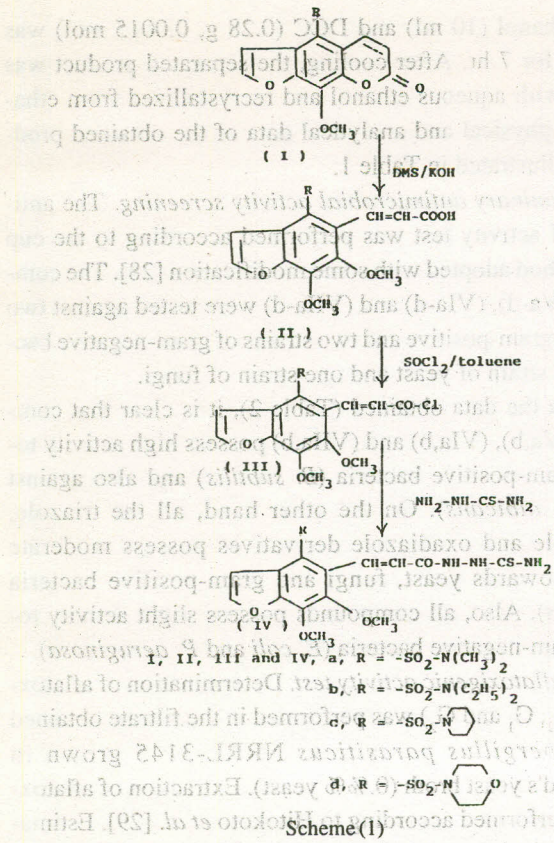
In the determination of aflatoxins (B_1, B_2, G_1 and G_2), two concentrations of the tested compounds were used (0.1 and 0.5 mmol/l medium). From the data obtained (Table 3), it is clear that, at a concentration of 0.1 mmol/l of compounds (Va-d, VIa-d, and VIIa-d), the reduction %age of total aflatoxins (B_1, B_2, G_1 and G_2) ranged between 80.53-73.15, 76.51-73.15 and 80.53-68.45, respectively. On the other hand, at a concentration of 0.5 mmol of the same compounds, the reduction %age ranged between 79.19-34.89, 75.83-45.63 and 69.12-45.63, respectively.

In general, the triazole, thiadiazole and oxadiazole compounds (Va-d, VIa-d and VIIa-d) may have potential use as bacteriostatic, fungistatic or fungitoxicant agents.

Results and Discussion

The naturally occurring xanthotoxin (9-methoxypsoralene) was chlorosulfonated to yield 9-metho-xypsoralen-4-sulfonyl chloride [23] which in turn was reacted with secondry amines namely, dimethyl, diethylamine, piperidine and/or morpholine to give the intermediates 9-methoxypsoralen-4-sulfonamide derivatives (Ia-d) (Scheme 1) [24].

It was previously reported that the lactone ring of xanthotoxin is opened upon the action of potassium



Scheme (1)

hydroxide and dimethyl sulfate to give the corresponding 3-[5-(6, 7-dimethoxy benzofuryl)] propenoic acid [25-26].

In the present work, xanthotoxin-4-sulfonamide derivatives (Ia-d) were subjected to the above mentioned conditions to give the corresponding 3-[5-(6, 7-dimethoxy benzofuryl-4-sulfonamide)] propenoic acid (IIa-d), respectively. The structure of the obtained compounds were confirmed by their correct elemental analyses (Table 1), IR and ^{1}H-NMR spectra.

The IR spectra of compounds IIa-d showed absorption bands at cm^{-1}, at 3350-3500 (OH, group), at 2890-3000 (CH, str.), at 1680-1700 (C=O, str.), at 1140-1150 and 1370-1385 (SO_2-N) and at 1050-1080 (C-O, furan).

The ^{1}H-NMR spectrum of compound IIa (DMSO-d_6) revealed signals (δ ppm) at 2.8 (6H, s, $N(CH_3)_2$), at 4.1 and 4.3 (6H, s,2-OCH_3), at 6.3 and 6.5 (2H, dd, CH=CH), at 6.8 (1H, d, H-3 furan) and at 7.2 (1H, d, H-2 furan).

Compounds IIa-d were refluxed in dry toluene and excess of thionyl chloride to give the corresponding 3-propenoic acid chloride derivatives IIIa-d, respectively. The structures of the obtained compounds were confirmed by their correct elemental analyses and IR spectra. Also, all compounds IIIa-d gave positive halogen test.

The IR spectra of compounds IIIa-d showed no absorption bands for hydroxyl groups and showed bands at 1720-1750 cm^{-1} (C=O, str.), besides the other characteristic bands for the rest of the molecules.

The condensation reaction of the acid chloride derivatives IIIa-d with thiosemicarbazide in refluxing dioxane gave the corresponding carbonyl thiosemicarbazide derivatives IVa-d, respectively (Scheme 1). The structures of the prepared compounds were confirmed by their correct elemental analyses, IR spectra. The IR spectra of compounds IVa-d showed absorption bands (cm^{-1}), at 3200-3300 (NH, str.), at 3300-3320 and 3400-3420 (two bands for NH_2), at 1250-1260 (C=S, str.), and at 1135-1160 and 1360-1385 (SO_2-N).

It was previously reported that, cyclization of carbonyl thiosemicarbazide derivatives using 5% sodium hydroxide gives 1,2,4-triazole-5-mercapto-3-derivatives, cyclodehydration of carbonyl thiosemicarbazide using orthophosphoric acid yields 5-amino-1, 3, 4-thiazole-3-derivatives and cyclodehydration of carbonyl thiosemicarbazide derivatives using dicyclohexylcarbodiimide (DCC) gives 5-amino-1, 3, 4-oxadiazole derivatives [27].

Cyclization of the carbonyl thiosemicarbazide derivatives IVa-d in refluxing sodium hydroxide solution (5%) led to the formation of 5-(5-mercapto-1,2,4-triazol-3-ethenyl)-6, 7-dimethoxy benzofuran-4-sulfonamides (Va-d), respectively (Scheme 2). The structures of the obtained compounds were confirmed by their correct elemental analyses (Table 1), IR and ^{1}H-NMR spectra.

The IR spectra of compounds Va-d showed absorption bands (cm^{-1}), at 3310-3340 (NH, str.), at 2580-2600 (SH, str.), at 1140-1150 and 1370-1385 (SO_2-N) and at 1050-1090 (C-O, furan).

The ^{1}H-NMR spectrum of compound Vb (DMSO-d_6) revealed signals (δ ppm) at 1.7 (6H, t, $2CH_3$), at 2.7 (4H, q, $2CH_2$), at 4.8 and 4.2 (6H, s, $2OCH_3$), at 6.3 and 6.5 (2H, dd, CH=CH), at 6.7 (1H, d, H-3 furan) and at 7.2 (1H, d, H-2 furan).

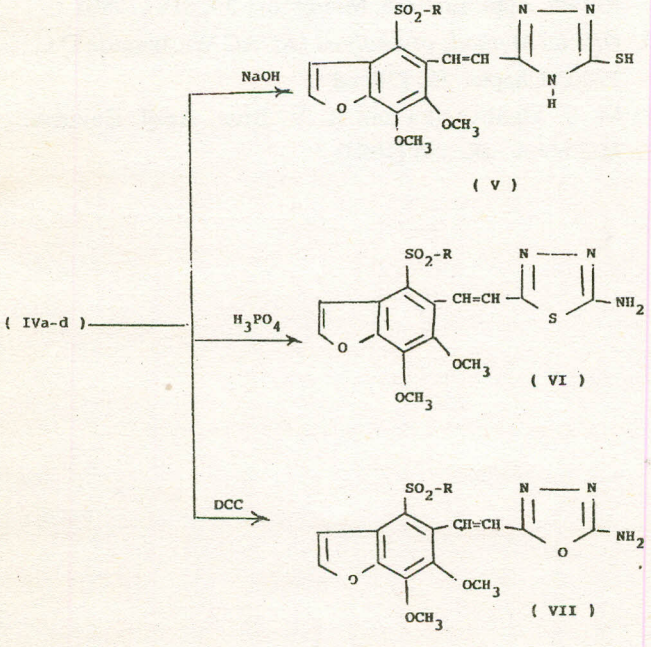
Cyclodehydration of carbonyl thiosemicarbazide derivatives IVa-d using orthophosphoric acid gave 5-(5-amino- 1, 3, 4-thiadiazol-2-ethenyl)-6,7-dimethoxy benzofuran-4-sulfonamide derivatives (VIa-d), respectively (Scheme 2). On the other hand, cyclodehydration of compounds IVa-d using dicyclohexylcarbodiimide (DCC) led to the formation of 5-(5-amino-1,3,4-oxadiazole-2-ethenyl)-6, 7-dimethoxy benzofuran-4-sulfonamide derivatives (VIIa-d), respectively (Scheme 2). The structure of the obtained compounds were confirmed beside their correct elemental analyses (Table 1), by IR and ^{1}H-NMR.

The IR spectra of compounds VIa-d and VIIa-d showed no absorption bands for carbonyl and thiocarbonyl groups, and showed absorption bands (cm^{-1}); at 3340-3360, 3360-3380 (two bands NH_2, str.), at 1590-1630 (C=N, str.), at 1580-

TABLE 2. PRELIMINARY SCREENING OF ANTIMICROBIAL ACTIVITY OF THE PREPARED COMPOUNDS.

Compd. No.	Micro-organism/Inhibition zone					
	1	2	3	4	5	6
a	+++	+++	+++	+++	+++	++
b	-	-	-	+	-	+
Va	+++	++	+	+	+++	++
b	+++	++	+	+	+++	++
c	+	+	+	+	++	++
d	+	+	+	+	++	++
VIa	+++	++	+	+	++	++
b	+++	++	+	+	++	++
c	++	++	+	+	++	++
d	++	++	+	+	++	++
VIIa	+++	++	+	+	+++	++
b	+++	++	+	+	+++	++
c	++	++	+	+	++	++
d	++	++	+	+	++	++

Microorganisms: 1-*Bacillus subtilis*. 2-*Staphylococcus aureus*. 3-*Escherichia coli*. 4-*Pseudomonas aeruginosa*. 5-*Candida albicans*. 6-*Aspergillus flavus*. The antimicrobial activity was measured in the following manner: +++ = Highly active (inhibition zone> 12 mm); ++ = Moderately active (inhibition zone 9-12 mm); + = Slightly active (inhibition zone 6-9 mm); a = -9-Methoxypsoralene (xanthotoxin); b = -3-[5-(6,7-dimethoxy benzofuryl)] propenoic acid. Compounds a and b are the original compounds.



V, VI and VII, a, R = $-N(CH_3)_2$

b, R = $-N(C_2H_5)_2$

c, R = $-N$

d, R = $-N$ O

Scheme (2)

TABLE 3. ANTIAFLATOXIGENIC ACTIVITY OF COMPOUNDS (Va-d), (VIa-d) and (VIIa-d).

Compd No.	Conc. mM/L	Final pH	Mycelial dry weight (g/L)	Aflatoxin B	µg/L G	Toxin total
Control	-	5.0	10	4400	10500	14900
Va	0.1	4.9	8	1000	3000	4000
	0.5	4.9	8	2500	5400	9700
b	0.1	4.9	9	1100	2800	3900
	0.5	4.8	9	2350	5500	7850
c	0.1	5.0	8	800	2100	2900
	0.5	4.9	8	1200	1900	3100
d	0.1	4.9	9	900	2800	3700
	0.5	4.8	8	1300	2200	3500
VIa	0.1	4.9	9	1100	2900	4000
	0.5	4.8	10	2600	5500	8100
b	0.1	4.9	10	1300	2200	3500
	0.5	4.7	9	2500	5600	8100
c	0.1	4.9	10	1100	3100	4200
	0.5	4.7	9	1300	2300	3600
d	0.1	5.0	8	1200	2400	3600
	0.5	5.0	10	1700	2200	3900
VIIa	0.1	5.0	10	1320	2230	3550
	0.5	5.0	9	1400	3200	4600
b	0.1	4.9	9	1400	2700	4700
	0.5	5.0	10	2400	5700	8100
c	0.1	4.8	8	900	2100	3000
	0.5	5.0	9	2300	3400	5700
d	0.1	4.9	10	900	2000	2900
	0.5	4.9	9	1900	3900	5800

Aflatoxin B represents the total toxin B_1 and B_2;.Aflatoxin G represents the total toxin G_1 and G_2.

1620 (C=C, str.), at 1140-1150 and 1360-1385 (SO_2-N) and at 1010-1030 (C-O furan).

The ^{1}H-NMR spectrum of compound VIc (DMSO-d_6) revealed signals (δ ppm) at 1.3 (2H, m, CH_2), at 1.65 (4H,m,2CH_2), at 2.9 (4H,t, 2CH_2) at 4.1 and 4.3 (6H, s, 2OCH_3), at 6.4 and 6.6 (2H, dd, CH=CH), at 6.8 (1H, d, H-3 furan) and at 7.4 (1H, d, H-2 furan).

The ^{1}H-NMR spectrum of compound VIId (DMSO-d_6) revealed signals (δ ppm) at 2.7 (4H, t, CH_2), at 3.6 (4H, t, 2CH_2), at 3.9 and 4.1 (6H, s, 2OCH_3), at 6.3 and 6.6 (2H, dd, CH=CH), at 6.9 (1H, d, H-3 furan) and at 7.1 (1H, d, H-2 furan).

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