Synthesis of Novel Thieno[2,3-*d*]pyrimidine, Thieno[2,3-*b*]pyridine and Thiazolo[3,2-*a*]thieno[2,3-*d*]pyrimidine Derivatives and Their Effect on the Production of Mycotoxins

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The thiophene derivative **1** reacts with the active methylene reagents **2a-c** to afford the thieno[2,3-*d*]pyrimidine derivatives **6a,b** and **8**, respectively. **1** reacts with phenacyl bromide **2d** to afford the *N*-alkylation product **9** and reacts with phenacyl thiocyanate **2e** to afford the *N*-(thiazol-2-yl) derivative **10**, which was further cyclized into thiazolo[3,2-*a*]thieno[2,3-*d*]pyrimidine derivative **12**. Compound **1** reacts also with the cinnamonitriles **3a,b** to afford the thieno[2,3-*b*]pyridines **15a,b**, respectively. **1** undergoes either acetylation or hydrolysis to afford the thieno[2,3-*b*]pyridine derivative **19** and the thiophene derivative **22**, respectively. Some of the new compounds show inhibitory effect to the production of mycotoxins and to fungal growth.

Synthese neuer Thieno[2,3-*d*]pyrimidine, Thieno[2,3-*b*]pyridine und Thiazolo[3,2-*a*]thieno[2,3-*d*]pyrimidine und ihr Einfluß auf die Mykotoxin-Bildung

Das Thiophenderivat **1** reagiert mit den aktiven Methylenverbindungen **2a-c** unter Bildung der Thieno[2,3-*d*]pyrimidine **6a,b** und **8**. **1** reagiert mit Phenacylbromid **2d** zum *N*-alkylierten Produkt **9** bzw. mit Phenacylthiocyanat **2e** zum *N*-Thiazolylderivat **10**. **10** wurde zum Thiazolo[3,2-*a*]thieno[2,3-*d*]pyrimidin **12** cyclisiert. - **1** reagiert auch mit den Zimtsäurenitrilen **3a,b** zu den Thieno[2,3-*b*]pyridinen **15a,b**. - **1** wird entweder acetyliert bzw. hydrolysiert zum Thieno[2,3-*b*]pyridin **19** bzw. zum Thiophenderivat **22**. Einige der neuen Verbindungen hemmen die Bildung von Mykotoxin und das Pilzwachstum.

Thiophenes and thieno fused heterocycles have received a considerable attention[1-3] due to their marked pharmaceutical activity[4]. In the last few years we have been involved in a programme aiming to synthesize potential biodegradable agrochemicals utilizing readily available starting materials[5-7]. In context with this work we report here the synthesis of the title compounds as well as their mycotoxigenic activity starting from 2-aminothiophene-3-carbonitrile derivative **1** (Scheme 1)[8,9].

1 reacts with malononitrile **2a** in refluxing DMF to give a product which was expected to be either **4** or **5** (Scheme 1)[1]. However, its IR spectrum did not reveal any cyano absorption bands, while a broad amino absorption band at $\nu = 3180$ cm^{-1} and a carbonyl absorption at $\nu = 1670$ cm^{-1} appeared. On this basis structures **4** and **5** were ruled out and structure **6a** was suggested for this product. The ^{1}H-NMR spectrum of this product confirmed it by a two methyl singlet (6H) at δ 2.4 ppm and a methylene singlet (2H) at δ 3.4 ppm besides an aromatic multiplet (10 H) at 7.4-8 ppm and a singlet (4H) at δ 8.1 ppm (NH_2 groups). Elemental analysis is in good agreement with a 2:1 adduct (Experimental Part).

Compound **6a** is assume to be formed by addition of the NH_2 groups of two molecules of **1** to the two CN groups of **2a** followed by addition of the newly formed NH_2 groups to the cyano functions of **1**.

In a similar way **1** reacts with ethyl cyanoacetate **2b** to afford the methylene bis-thieno[2,3-*d*]pyrimidine derivative **6b**.

Benzoylacetonitrile **2c** reacts with **1** to afford a 1:1 adduct. structures **7** and **8** were considered for this reaction product. On the basis of a methylene singlet at δ 3.4 ppm in the ^{1}H-NMR spectrum of this product, structure **8** was established. The other signals appeared as expected (cf. Experim. Part).

The reaction of **1** with phenacyl bromide **2d** led to *N*-alkylation, which was not further cyclized under our reaction conditions (cf. Experim. Part). Analytical and spectral data are in accordance with structure **9** for this product.

Phenacyl thiocyanate **2e** reacts with **1** in refluxing ethanol catalysed by HCl to afford a 1:1 adduct. The IR-spectrum revealed only one carbonyl absorption at ν 1665 cm^{-1} and one cyano absorption at ν 2190 cm^{-1}. The *N*-(thiazol-2-yl) derivative **10** or its tautomeric structure **11** were suggested for this product. The ^{1}H-NMR spectrum revealed a methyl singlet at δ 2.36 ppm, an aromatic multiplet (10 H) at δ 7.2-7.8 ppm and one NH singlet at δ 12.0 ppm. The thiazole 5-H appeared at δ 6.6 ppm. It seems that structure **10** predominates under acidic or neutral conditions, otherwise, if structure **11** were the predominant one, cyclization would have been expected to give **12**. As a corroboration to this, compound **10** was cyclized into **12** upon reflux in ethanol, catalysed by triethylamine. The cyclization was detected by the disappearance of the cyano absorption band in the IR spectrum of **12**.

A similar reaction has been reported[10]; however, the angular isomer is excluded in our case since **12** is obtained from the cyclization of **10** and not a direct product of the reaction.

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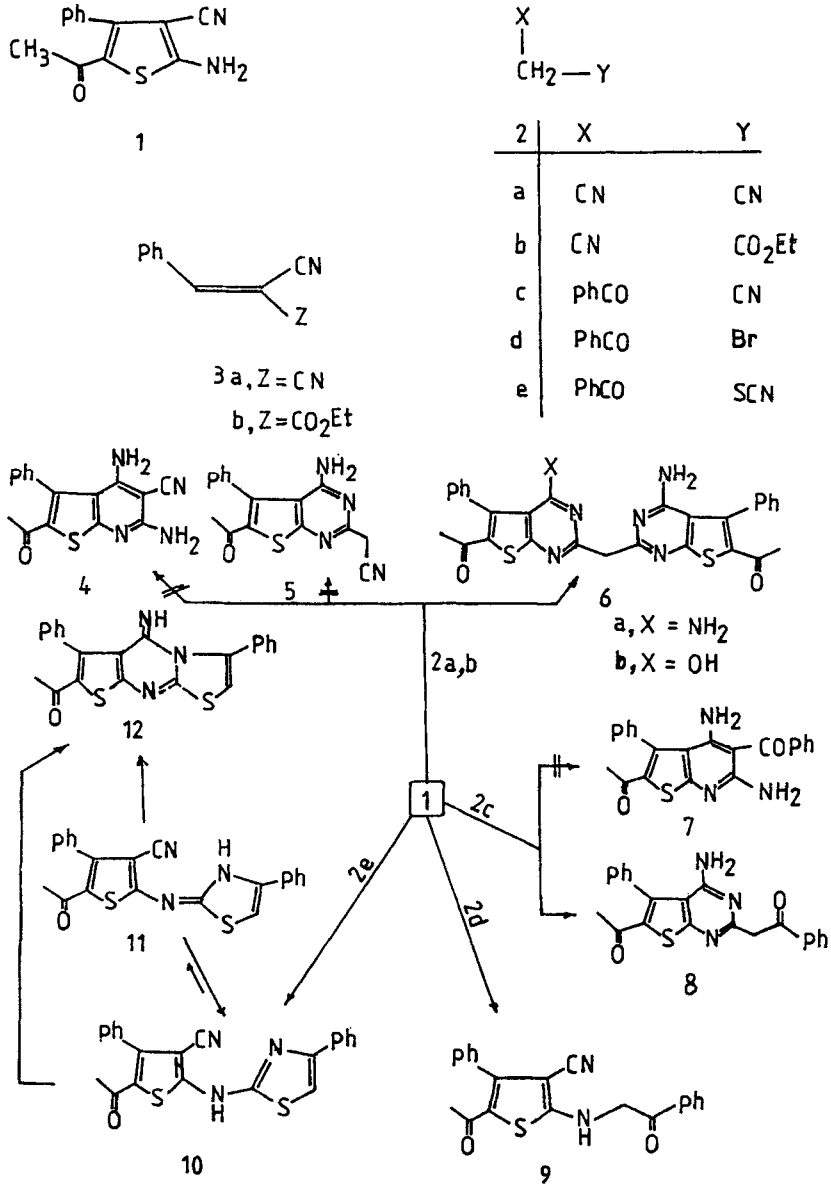


Scheme 1

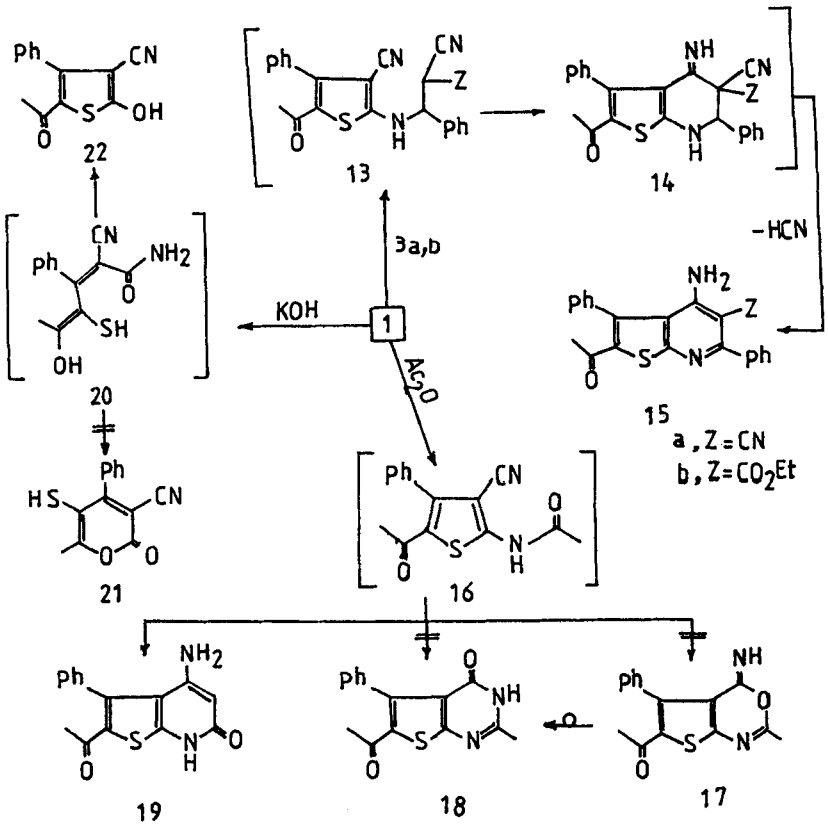
The reaction of **1** with the cinnamonitriles **3a,b** follows the same mechanistic pathway; addition of the NH_2 of **1** to the activated double bond of **3a,b** gives the acyclic intermediate **13** which cyclizes into **14**. This loses HCN to afford the final isolable products **15a,b**, respectively (Scheme 2).

Compound **1** undergoes *N*-acetylation on reflux in acetic anhydride, however the *N*-acetyl derivative **16** is apparently cyclized under the reaction conditions probably to the thienoxazine derivative **17** or its rearranged product: thienopyrimidine **18**. The ^{1}H-NMR spectrum of the product revealed, however, only one methyl singlet at δ 2.5 ppm. This means that the other CH_3 was involved in the cyclization step to afford the thienopyridine derivative **19** which was assigned to this reaction product.

Compound **1** undergoes hydrolysis and recyclization under the effect of KOH to afford a product, very similar to the starting compound **1** but having a different melting point.

Different structures can be postulated for this product which can arise from the hydrolysis intermediate **20** by elimination of H_2S, H_2O, or NH_3. Element test and elemental analysis revealed the presence of sulphur and suggest that ammonia is eliminated. Thus structures **21** and **22** were therefore only considered. The IR-spectrum revealed the carbonyl absorption at $\nu = 1655$ cm^{-1} which is more likely attributed to CH_3-CO-C=C rather than to a lactone C=O which is expected to appear at $\nu \approx 1750$ cm^{-1}. It showed also an absorption band at $\nu = 3451$ cm^{-1} which can be attributed to OH. Thus, structure **22** was preferred for this product. If the structure were **21**, an SH stretching band would have appeared near $\nu = 2550$ cm^{-1}.

The ^{1}H-NMR spectrum confirmed structure **22**. It revealed the OH singlet at δ 6.8 ppm. The SH signal in 21 should have appeared at much higher field (δ 2.8-3.6 ppm). Thus structure **22** was established for this rearrangement product. A similar result has been reported[11].



Scheme 1

Table 1: Effect of the prepared compounds on growth of *Aspergillus Parasiticus* NRRL 3145 and Aflatoxins (B_1, B_2, G_1, G_2) production

Compd. No.	Conc. mm/l	Final pH	Mycelial dry Wt(g/l)	Aflatoxins(mg/l) B_1	B_2	G_1	G_2	Total
Control	-	5.2	12	7.5	5.0	40.0	20.0	72.5
6a	0.1	5.0	10	3.9	2.2	26.0	9.0	41.1
	0.5	4.9	6.0	3.5	1.9	20.1	10.0	35.5
6b	0.1	5.1	10	6.5	3.8	36.0	18	64.3
	0.5	4.9	11	6.2	3.5	30.0	12	51.7
8	0.1	4.9	11.5	7.4	3.3	19.0	13	42.7
	0.5	4.8	12	6.3	3.2	15.0	10	34.5
9	0.1	4.9	11	7.5	4.5	19.5	25	56.5
	0.5	5.0	12	6.5	3.8	13.5	12	35.8
10	0.1	5.0	10.5	6.8	4.1	35.0	13	58.9
	0.5	4.9	9.0	6.3	3.9	30.0	11	51.2
12	0.1	5.0	10	5.4	2.7	24.0	10	42.1
	0.5	4.9	9.0	4.2	2.2	21.0	9.0	36.4
15a	0.1	4.8	7.5	7.0	4.1	30.0	14	55.1
	0.5	4.9	8.5	6.0	4.0	28.0	9.0	47.0
15b	0.1	4.9	9.5	6.9	4.0	33.0	19	62.9
	0.5	4.8	9.0	5.9	3.5	28.0	11	48.4
19	0.1	4.8	10	7.2	4.8	39.0	15	66.0
	0.5	4.9	9.5	6.3	4.1	30	11	51.4
22	0.1	5.0	11	7.2	3.9	33	15	59.1
	0.5	5.1	8.8	6.3	3.2	31	11	51.5

Table 2: Effect of the prepared compounds on growth of *Aspergillus Ochraceus* NRRL 3174 and ochratoxin A production

Compd. No.	Conc. mM/l	Final pH	Mycelial dry Wt(g/l)	Ochratoxin A (mg / l)
6a	0.1	4.7	7.5	1.8
	0.5	4.9	7.2	1.5
6b	0.1	4.9	9.6	2.0
	0.5	4.8	9.3	1.9
8	0.1	4.9	8.3	2.3
	0.5	4.5	8.5	2.4
9	0.1	4.9	7.1	1.8
	0.5	4.5	8.5	1.6
10	0.1	5.0	9.5	2.4
	0.5	5.0	9.2	2.0
12	0.1	4.9	9.5	1.9
	0.5	4.8	9.6	1.5
15a	0.1	4.7	6.0	1.9
	0.5	4.5	6.2	1.5
15b	0.1	5.1	9.2	2.3
	0.5	4.9	9.1	2.1
19	0.1	4.7	8.5	2.4
	0.5	4.9	8.3	2.4
22	0.1	4.7	8.9	2.6
	0.5	4.3	8.2	2.4

Mycotoxigenic Activity

The new compounds were tested for their effect on growth of *Aspergillus Parasiticus* NRRL 3145 and aflatoxins (B_1, B_2, G_1 and G_2) production, and also on growth of *Aspergillus Ochraceus* NRRL 3174 and ochratoxin A production at 0.1 and 0.5 mM/l medium.

Results are shown in tables 1 and 2. It is clear from table 1 that the compounds show a low inhibitory effect except compounds **6a**, **8**, and **12** which show significant inhibition to the first organism at both concentrations. Table 2 shows that compounds **6a**, **9**, **12**, and **15a** inhibit growth of *A. Ochraceus* at 0.5 mM/liter, while the other compounds did not show any effect at both concentrations. Thus, some of the new compounds seem to be promising as fungicides and antimycotoxigenic agents.

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Experimental Part

Melting points: uncorrected.- IR spectra: Pye-Unicam SP-1100; KBr pellets.- ^{1}H-NMR spectra: Varian EM-390 (90 MHz), DMSO-d_6, TMS as internal standard.- Microanalyses: Microanalytical Center, Cairo University.- Biological activity tests: Laboratory of pharmaceutical microbiology, National Center for Radiation Research and Technology, Cairo, Egypt.

Reaction of **1** *with active methylene reagents* **2a-c** *(general procedure)*

To a solution of **1** (2.42 g; 0.01 mole) in 20 ml of DMF were added 0.005 mole of either **2a** or **2b** or 0.01 mole of **2c** followed by 0.5 ml of piperidine. The reaction mixture was refluxed for 2 h then left to cool overnight. The precipitated solid products were recrystallized to give **6a**, **6b**, and **8**, respectively.

6a: *6-Acetyl-4-amino-2(6-acetyl-4-amino-5-phenylthieno[2,3-d]pyrimidin-2-yl)methyl-5-phenylthieno[2,3-d]pyrimidine.*

Mp. 345°C (DMF), 48%.- IR: 3180 (NH_2); 1670 (C=O).- ^{1}H-NMR: 2.4 (s, 6H, 2 CH_3); 3.4 (s, 2H, CH_2); 7.4-8.0 (m, 10 H, arom.); 8.1 (s, 4H, 2 NH_2).- $C_{29}H_{22}N_6O_2S_2$ (550.6) Calcd. C 63.3 H 4.0 N 15.3 Found C 63.0 H 3.9 N 14.8.

6b: *6-Acetyl-4-amino-2(6-acetyl-4-hydroxy-5-phenylthieno[2,3-d]pyrimidin-2-yl]methyl-5-phenylthieno[2,3-d]pyrimidine.*

Mp. 320°C (DMF), 43%.- IR: 3555 (OH); 3284 (br. NH and NH_2); 1665 (C=O).- ^{1}H-NMR: 2.36 (s, 6H, 2 CH_3); 3.35 (s, 2H, CH_2); 7.35-7.95 (m, 11 H, arom. + OH); 8.1 (s, 2H, NH_2).- $C_{29}H_{21}N_5O_3S_2$ (551.6) Calcd. C 63.1 H 3.8 N 12.7 Found C 62.8 H 4.2 N 13.1.

8: *6-Acetyl-4-amino-2-phenacyl-5-phenylthieno[2,3-d]pyrimidine.*

Mp. 320°C (DMF), 52%.- IR: 3282 (br. NH_2); 1690 and 1663 (two C=O).-^{1}H-NMR: 2.35 (s, 3H, CH_3); 3.4 (s, 2H, CH_2); 7.4-7.9 (m, 10 H, arom.); 8.15 (s, 2H, NH_2).- $C_{22}H_{17}N_3O_2S$ (387.5) Calcd. C 68.2 H 4.4 N 10.8 Found C 68.5 H 4.9 N 11.3.

5-Acetyl-2-(N-phenacyl)amino-4-phenylthiophene-3-carbonitrile (**9**)

To a solution of **1** (2.42 g; 0.01 mole) in 20 ml of ethanol was added **2d** (1.99 g; 0.01 mole) and K_2CO_3 (1.38 g; 0.01 mole dissolved in a minimum of water). The reaction mixture was refluxed for 2 h then left to cool over-

night. The solid product was recrystallized to afford **9**.- Mp. 290°C (AcOH), 58%.- IR: 3190 (br. NH); 2187 (CN); 1633 and 1695 (two C=O).- ^{1}H-NMR: 2.45 (s, 3H, CH_3); 3.4 (s, 2H, CH_2); 6.9 (br. s, 1H, NH); 7.3-7.9 (m, 10 H, arom.).- $C_{21}H_{16}N_2O_2S$ (360.4) Calcd. C 70.0 H 4.5 N 7.8 S 8.9 Found C 70.3 H 4.9 N 8.2 S 9.2.

5-Acetyl-2-(N-thiazol-2-yl)amino-4-phenylthiophene-3-carbonitrile (**10**)

A mixture of **1** (2.42 g; 0.01 mole) and **2e** (1.77 g; 0.01 mole) was dissolved in 20 ml of ethanol. To this solution was added 2 ml of conc. HCl. The reaction mixture was heated on a boiling water bath for 7 h, then left to cool overnight. The precipitated solid product was recrystallized to give **10**.- Mp. 185°C (EtOH), 62%.- IR: 3200 (br. NH); 2190 (CN); 1665 (C=O).- ^{1}H-NMR: 2.36 (s, 3H, CH_3); 6.6 (s, 1H, thiazole 5-H); 7.2-7.8 (m, 10 H, arom.); 12.0 (s, 1H, NH).- $C_{22}H_{15}N_3OS_2$ (401.5) Calcd. C 65.8 H 3.8 N 10.5 S 16.0 Found C 66.1 H 4.2 N 11.0 S 15.6.

6-Acetyl-4-imino-3,5-diphenylthiazolo[3,2-a]thieno[2,3-d]pyrimidine (**12**)

To a solution of **10** (2 g; 0.005 mole) in 20 ml of ethanol was added 0.5 ml of triethylamine as a catalyst. The mixture was refluxed for 2 h and then cooled to room temp. The precipitated solid was crystallized to afford **12**.- Mp. 330°C (DMF), 87%.- IR: 3220 (NH); 1663 (C=O).- $C_{22}H_{15}N_3OS_2$ (401.5) Calcd. C 65.8 H 3.8 N 10.5 Found C 65.7 H 4.0 N 10.2.

Thieno[2,3-b]pyridine derivatives **15a,b**

To a solution of **1** (2.42 g; 0.01 mole) and either **3a** or **3b** (0.01 mole) in ethanol/DMF mixture 1:1 (20 ml) were added 0.5 ml of triethylamine. The reaction mixture was refluxed for 3 h, left to cool overnight and the precipitated solids were recrystallized to afford **15a** and **15b**, respectively.

15a

Mp. 300°C (DMF), 55%.- IR: 3180 (NH_2); 2200 (CN); 1670 (C=O).- ^{1}H-NMR: 2.35 (s, 3H, CH_3); 7.3-7.95 (m, 10 H, arom.); 8.1 (s, 2H, NH_2).- $C_{22}H_{15}N_3OS$ (369.4) Calcd. C 71.5 H 4.1 N 11.4 Found C 71.3 H 4.5 N 11.7.

15b

Mp. 315°C (DMF), 54%.- IR: 3408 (br. NH_2); 1742 and 1659 (two C=O).- ^{1}H-NMR: 1.2 (t, 3H, CH_3); 2.18 (s, 3H, CH_3); 3.1 (q, 2H, CH_2); 7.3-7.9 (m, 10 H, arom.); 8.15 (s, 2H, NH_2).- $C_{24}H_{20}N_2O_3S$ (416.5) Calcd. C 69.2 H 4.8 Found C 69.7 H 4.5.

6-Acetyl-4-amino-5-phenyl-1,2-dihydrothieno[2,3-b]pyridin-2-one (**19**)

A solution of **1** (2.42 g; 0.01 mole) in 15 ml of acetic anhydride was refluxed for 3 h then left to cool overnight. The precipitated solid was recrystallized from DMF to give **19**.- Mp. 285°C (DMF), 72%.- IR: 3180 (br. NH_2 and NH); 1680 and 1650 (two C=O).- ^{1}H-NMR: 2.5 (s, 3H, CH_3); 5.8 (br. s, 2H, NH_2); 7.35-7.9 (m, 6H, arom. + CH); 8.05 (s, 1H, NH).- $C_{15}H_{12}N_2O_2S$ (284.3) Calcd. C 63.4 H 4.3 S 11.3 Found C 63.5 H 4.7 S 11.7.

5-Acetyl-2-hydroxy-4-phenylthiophene-3-carbonitrile (**22**)

In 15 ml of 15% KOH was suspended 2.42 g (0.01 mole) of **1**. The emulsion was refluxed for 1 h where it became clear and a precipitate reappeared during the reflux time. The reaction mixture was left to cool to room temp., the precipitate was recrystallized to give **22**.- Mp. 206°C (EtOH), 80%.- IR: 3451 (OH); 2170 (CN); 1655 (C=O).- ^{1}H-NMR: 2.3 (s, 3H, CH_3); 6.8 (s, 1H, OH); 7.1-7.9 (m, 5H, arom.).- $C_{13}H_9NO_2S$ (243.3) Calcd. C 64.2 H 3.7 S 13.2 Found C 64.5 H 4.1 S 13.5.

Mycotoxigenic Activity Test

1 ml portions spore suspension of *Aspergillus Parasiticus* NRRL 3145 or *Aspergillus Ochraceus* NRRL 3174, which were provided by the Northern Regional Research Center, Peoria, Illinois, U.S.A., were inoculated in 100 ml *Erlenmeyer* flasks. Each flask contained 20 ml of *Sabouraud's* broth fortified with 0.5% yeast extract, for the first organism, while yeast extract-succrose medium was used for the second organism. The media were supplemented with two conc. of the tested compounds (0.1 and 0.5 m mole/liter medium). Unsupplemented flasks served as control. The flasks were incubated for 7 days at 26 ± 1°C for the first organism and for 10 days at 28 ± 1°C for the second organism. After incubation, a known volume of the filtrate was extracted with chloroform according to *Hitokoto* et al.[12].

Detection and quantitative determinations of aflatoxins and ochratoxin A were performed according to AOAC[13]. Mycelial dry weight was estimated according to *Madhyastha* and *Bhadt*[14].

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