

3-(Substituted Methyl)-2-phenyl-4*H*-1-benzothiopyran-4-ones

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Received April 3, 1985

A series of 3-(substituted methyl)-2-phenyl-4*H*-1-benzothiopyran-4-ones (thioflavones) and thioflavone 1,1-dioxides was prepared to test for antimicrobial activity and for antitumor activity. It was shown that an introduction of a substituted methyl group in the 3-position of thioflavone resulted in significant antimicrobial activity against *Trichophyton*s. 3-(Acetoxymethyl)thioflavone shows the most antimicrobial potency *in vitro* against *Trichophyton mentagrophytes*. Most of the thioflavone 1,1-dioxides showed antimicrobial activity against fungi. Five of the 40 related compounds demonstrated weak antitumor activity against P-388 lymphocytic leukemia.

J. Heterocyclic Chem., **22**, 1593 (1985).

It is well known that flavonoids are an important group of naturally occurring bioactive compounds [2], but few 2-phenyl-4*H*-1-benzothiopyran-4-ones (thioflavones) have so far been tested for any antimicrobial activity. Some thioflavones and their guanyldiazide derivatives exhibit bronchodilator, schistosomicide, anti-malarial, anti-fungal, and anti-viral activities, and are useful as agricultural bactericides as described in the literature [3-9], however, systematic studies of the structure-antimicrobial activity relationships of thioflavones have not been reported as yet.

We have attempted to prepare novel thioflavones and related compounds as potential antimicrobially active compounds [10,11]. We found that in the screening test for antimicrobial activity of thioflavones and related compounds the sulfone derivative **12a** or sulfoxide derivative of thioflavone show effective antimicrobial activity against fungi and molds, and 3-(hydroxymethyl)thioflavone showed significant activities against two kinds of *Trichophyton*s *in vitro*.

Scheme I

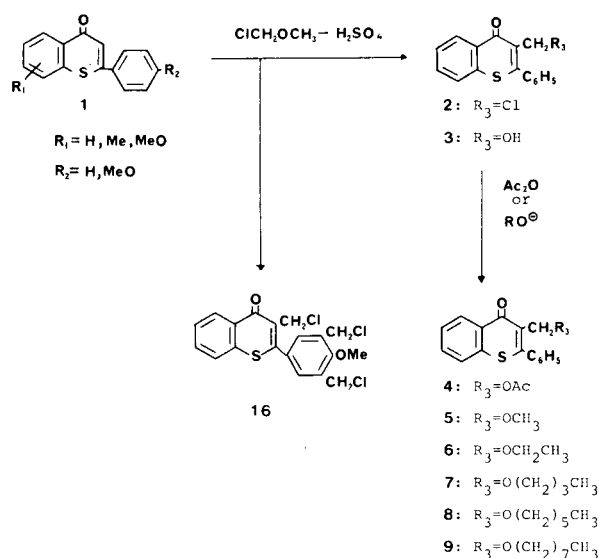


Table I

Properties of 3-(Alkoxyethyl)thioflavones

Compound	Yield %	Formula	Elemental Analysis (%)		¹ H-NMR (deuteriochloroform) (δ, ppm)
			Calcd./Found	C	
5 [a]	65	C ₁₇ H ₁₄ O ₂ S	72.31	5.00	3.38 (s, 3H), 4.32 (s, 2H), 7.34-7.70 (m, 8H), 8.63 (m, 1H)
			72.15	4.66	
6 [b]	64	C ₁₈ H ₁₆ O ₂ S	72.94	5.44	1.15 (t, 3H, J = 7.5 Hz), 3.48 (q, 2H, J = 7.5 Hz), 4.30 (s, 2H), 7.36-7.64 (m, 8H), 8.56 (m, 1H)
			72.86	5.41	
7	66	C ₂₀ H ₂₀ O ₂ S	74.04	6.21	0.88 (t, 3H, J = 5 Hz), 1.20-1.70 (m, 4H), 3.47 (t, 2H, J = 5 Hz), 4.36 (s, 2H), 7.48-7.68 (m, 8H), 8.65 (m, 1H)
			73.90	6.45	
8	89	C ₂₂ H ₂₄ O ₂ S	74.96	6.86	0.80-1.70 (m, 11H), 3.45 (t, 2H, J = 6 Hz), 4.32 (s, 2H), 7.35-7.72 (m, 8H), 8.56 (m, 1H)
			74.64	7.03	
9	51	C ₂₄ H ₂₈ O ₂ S	75.75	7.42	0.89 (m, 3H), 1.14-1.67 (m, 12H), 3.48 (t, 2H, J = 7 Hz), 4.38 (s, 2H), 7.52-7.76 (m, 8H), 8.68 (m, 1H)
			75.63	7.60	

[a] Mp 90-92°. [b] Mp 75-77°.

In this study, we made novel alkoxymethyl and acetoxymethyl thioflavones at the 3-position and tested them for antimicrobial activity. Sulfone derivatives of these compounds and related compounds then were prepared and tested for antimicrobial activity and antitumor activity.

The chemistry of the thioflavone system and its oxides is well known [12-19]. Thioflavones **1a-1g** were prepared by condensation of the appropriate benzenethiol and ethyl benzoylacetate in polyphosphoric acid [15]. Hydroxythioflavones **1h-1k** were prepared by the previously reported method [11]. The key intermediates **2** and **3** were prepared by chloromethylation of **1a** [20]. Compounds **5-9** were easily obtained by treatment of **2** with the corresponding alcohol and sodium (Table I). Thioflavone 1,1-dioxides **10-13** were prepared in 60-80% yields by oxidation of the corresponding thioflavone derivatives with hydrogen peroxide in glacial acetic acid. The yields and mp's of these compounds are summarized in Table II. The structure of thioflavone 1,1-dioxides was confirmed on the basis of mass spectra characterized by abundant molecular ion, as shown in Table II. Compounds **14** and **15** were prepared by known methods [9,21].

Antimicrobial analysis of the 3-(substituted methyl)thioflavone with an agar or broth dilution assay is compared in Table III. All compounds tested herein were inactive against Gram-negative bacteria (*Escherichia coli* IFO 3545 and *Pseudomonas aeruginosa* IAM 1007).

Thioflavone **1a** was inactive against all of microorganisms tested *in vitro*, however, an introduction of a substi-

tuted methyl group in the 3-position of thioflavone resulted in significant activities against two kinds of *Trichophyton* as is evident from the table. Unfortunately, none of the 3-(substituted methyl)thioflavones exhibited superior activity over the reference compound, tolinaftate. The most active thioflavones in this series are the hydroxymethyl substituted compound **3** and the acetoxymethyl substituted compound **4** against *Trichophyton rubrum* and *Trichophyton mentagrophytes*, respectively. Comparison of the activities of compounds **6-9** shows that the longer alkoxymethyl substitution in the 3-position resulted in decrease in activity against *Trichophyton*s. 3-(Substituted

Scheme II

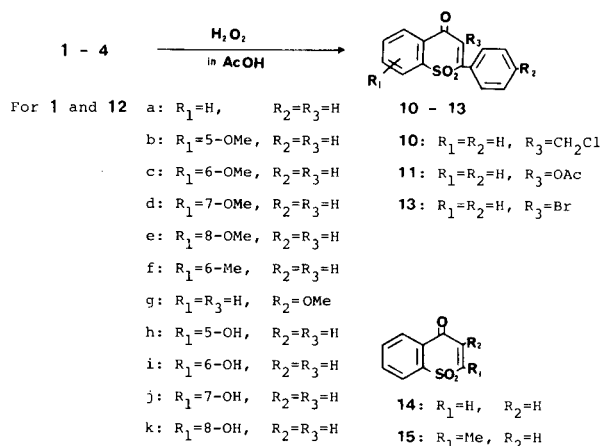


Table II

Properties of Thioflavone 1,1-Dioxide Derivatives

No.	Mp °C	Recrystallization Solvent	Yield %	Formula	Elemental Analysis (%)		Mass m/z (relative intensities)
					Calcd./Found	C H	
10	178-180	C ₆ H ₆ -Hexane	44	C ₁₆ H ₁₁ O ₃ SCl	60.29 60.36	3.48 3.39	320 (M ⁺ + 2, 5), 318 (M ⁺ , 14), 284 (50), 282 (100)
11	113-116	C ₆ H ₆	51	C ₁₈ H ₁₄ O ₃ S	63.15 62.91	4.12 4.03	300 (M ⁺ - 42, 18), 282 (20), 236 (76), 208 (100)
12b	149-150	EtOH	63	C ₁₆ H ₁₂ O ₄ S	63.99 64.13	4.03 4.03	300 (M ⁺ , 100), 236 (15)
12c	173-175	EtOH	80	C ₁₆ H ₁₂ O ₄ S	63.99 63.89	4.03 3.87	300 (M ⁺ , 100), 252 (25), 236 (14)
12d	144-146	EtOH	76	C ₁₆ H ₁₂ O ₄ S	63.99 63.76	4.03 3.89	300 (M ⁺ , 100), 193 (14)
12e	155-157	EtOH	74	C ₁₆ H ₁₂ O ₄ S	63.99 63.80	4.03 3.97	300 (M ⁺ , 100), 179 (19)
12g	159-160	EtOH	65	C ₁₆ H ₁₂ O ₄ S	63.99 63.61	4.03 3.75	300 (M ⁺ , 56), 236 (8), 234 (22), 165 (100)
12h	185-187	EtOH-H ₂ O	75	C ₁₅ H ₁₀ O ₄ S	62.93 62.90	3.52 3.34	286 (M ⁺ , 100), 221 (10), 194 (12)
12i	212-213	EtOH-H ₂ O	63	C ₁₅ H ₁₀ O ₄ S	62.93 62.65	3.52 3.27	286 (M ⁺ , 100), 238 (40), 194 (25))
12j	217-219	EtOH-H ₂ O	76	C ₁₅ H ₁₀ O ₄ S	62.93 62.78	3.52 3.21	286 (M ⁺ , 100), 194 (21)
12k	185-187	EtOH-H ₂ O	59	C ₁₅ H ₁₀ O ₄ S	62.93 63.14	3.52 3.20	286 (M ⁺ , 100), 165 (59)

methyl) substitution also improved the activity against *P. crustosum*.

In an attempt to relate antimicrobial and antitumor activity to the sulfone substitution, various sulfone derivatives were made. A comparison of the antimicrobial properties of sulfone analogs is shown in Table IV.

The replacement of the sulfur atom of the substituted thioflavones **1b-1k**, as well as thioflavone **1a**, with the sulfone functional group leads to a significant improvement of the antimicrobial activity, in particular against fungi and molds. Comparison of the activities of compounds **12a**, **14**, and **15** against *S. cerevisiae* and *C. utilis* shows that replacing the hydrogen atom in **14** by the methyl group in **15** resulted in an approximately 30-fold increase in activity and that replacing the methyl group in **15** by the phenyl group in **12a** resulted in an 8-fold increase in activity. It is conceivable that the lipophilic affinity of the

phenyl group enhanced the activity of **12a**. A comparison between the activities of the pairs of compounds **2**, **10** and **4**, **11** shows that replacement of the sulfur atom of 3-(substituted methyl) thioflavones by the sulfone group results in decrease in activity against *Trichophyton* (500-fold or 60-fold).

The antitumor activities of selected thioflavones are summarized in Table V. Most of the other thioflavones prepared herein did not exhibit antitumor activity against P-388 lymphocytic leukemia. Contrary to what could be expected on the basis of previous results with thiochromone 1,1-dioxides [12] the 3-(substituted methyl)thioflavones **10** and **11** exhibited no activity. tris(Chloromethyl)thioflavone **16** showed the highest T/C. Some thioflavone 1,1-dioxides caused only very modest increase in survival time (T/C = ~ 120%).

The methoxy derivatives of thioflavone 1,1-dioxides (LD₁₀₀: 250-2000 mg/kg, LD₀: 125-1000 mg/kg) were gene-

Table III
Antimicrobial Activity of 3-(Substituted methyl)thioflavones

No.	R ₃	(S.c.)	(C.u.)	MIC, µg/ml (organism) [a]					(T.m.)	(C.a.)
				(P.c.)	(R.c.)	(T.r.)				
2	Cl	[b]	[b]	12.5	[b]	0.195		0.195		[b]
3	OH	[b]	[b]	6.25	[b]	0.0488		0.195		[b]
4	OCOCH ₃	[b]	[b]	25.0	[b]	0.195		0.0488		[b]
5	OCH ₃	[b]	[b]	25.0	[b]	0.781		0.781		[b]
6	OCH ₂ CH ₃	[b]	[b]	12.5	[b]	0.195		0.0977		[b]
7	O(CH ₂) ₃ CH ₃	[b]	[b]	[b]	[b]	0.195		0.195		[b]
8	O(CH ₂) ₅ CH ₃	[b]	[b]	[b]	[b]	6.25		1.56		[b]
9	O(CH ₂) ₇ CH ₃	[b]	[b]	[b]	[b]	50.0		12.5		[b]
	Tolnaftate	[c]	[c]	[c]	[c]	0.00305		0.0244		> 100

[a] S.c., *Saccharomyces cerevisiae* IFO 0202; C.u., *Candida utilis* OUT 6020; P.c., *Penicillium crustosum* Thom; R.c., *Rhizopus chinensis* IFO 4745; T.r., *Trichophyton rubrum* IFO 5467; T.m., *Trichophyton mentagrophytes* IFO 6202; C.a., *Candida albicans*. [b] > 800 µg/ml. [c] Not determined.

Table IV
Antimicrobial Activity of Thioflavone 1,1-Dioxides

No.	MIC, µg/ml (organism) [a]								
	(B.s.)	(S.a.)	(S.c.)	(C.u.)	(P.c.)	(R.c.)	(T.r.)	(T.m.)	(C.a.)
10	[b]	800	25.0	25.0	25.0	200	50.0	100	800
11	400	400	6.25	6.25	25.0	50.0	12.5	25.0	200
12a	[b]	800	3.13	1.56	12.5	[b]	6.25	6.25	25.0
12b	800	50.0	3.13	3.13	12.5	[b]	6.25	12.5	50.0
12c	[b]	[b]	[b]	[b]	[b]	[b]	6.25	12.5	200
12d	[b]	200	[b]	1.56	[b]	[b]	1.56	3.13	[b]
12e	200	200	25.0	50.0	[b]	[b]	6.25	6.25	[b]
12f	[b]	[b]	[b]	6.25	[b]	[b]	3.13	12.5	100
12g	[b]	[b]	3.13	1.56	[b]	[b]	3.13	6.25	[b]
12h	[b]	[b]	3.13	3.13	[b]	[b]	50.0	50.0	[b]
12i	200	200	12.5	12.5	50.0	[b]	25.0	12.5	100
12j	100	50.0	25.0	25.0	[b]	[b]	3.13	12.5	100
12k	400	200	25.0	12.5	50.0	[b]	1.56	6.25	100
14	[b]	[b]	800	800	800	800	200	400	[b]
15	[b]	[b]	25.0	25.0	50.0	400	200	200	800

[a] B.s., *Bacillus subtilis* K 49; S.a., *Staphylococcus aureus* NCTC 8530. Other organisms were explained as footnote [a] in Table III. [b] > 800 µg/ml.

Table V

Antitumor Activity of Thioflavones in Mice Bearing
Lymphocytic Leukemia P-388 [a]

Compound	Dose mg/kg per day	Median Survival time days Control	Treated mice	T/C %
16	100	10.7	13.8	128
12d	200	9.5	11.7	123
12e	240	10.2	12.3	120
12i	50	10.7	13.0	121
12k	50	10.7	13.0	121

[a] Tests were done on 3PS31 by the NCI according to the protocol described in Instruction 14, Screening Data Summary Interpretation and Outline of Current Screen, Drug Evaluation Branch, Drug Research and Development Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Tumor Inoculum: CD₂F(CDF₁) mice were injected ip with 10⁶ ascites cells. Evaluation: MST = median survival time in days. %T/C = MST treated/MST control × 100. %T/C 125% is considered significant antitumor effect. %T/C = 120 is minimal activity.

rally less toxic than the corresponding hydroxy derivatives (LD₁₀₀: 200-500 mg/kg, LD₀: 125-250 mg/kg), except for the 5-substituted derivative (LD₀: >4000 mg/kg). The smaller toxicity of 12h might be due to an intramolecular hydrogen bond between the hydrogen atom of the hydroxy group and the carbonyl oxygen.

EXPERIMENTAL

All melting points are uncorrected. Proton nmr spectra were taken on a JEOL JNM-MH-100 spectrometer with tetramethylsilane as an internal standard. Infrared spectra were recorded on a Shimadzu IR-420 spectrometer using potassium bromide pellets. Elemental analyses were recorded on a Yanaco CHN recorder MT-2. Mass spectra were recorded on a Hitachi RMU-6E mass spectrometer operating at 80 eV. In the acute toxicity test, the lethal dose (LD₁₀₀) and the highest nontoxic dose (LD₀) were determined after a single intraperitoneal injection to ICR mice (6 weeks old).

3-Acetoxyethyl-2-phenyl-4H-1-benzothiopyran-4-one (4).

3-(Hydroxymethyl)thioflavone **3** (3.5 g, 0.013 mole) was heated at reflux for 2 hours in 15 ml of acetic anhydride. The solution was poured into ice-water, and the resulting solid was filtered and recrystallized from ligroin (bp 75-120°) to give compound **4** (66%), mp 115-117°; ir: 1730, 1610 cm⁻¹ (C=O); ¹H-nmr (deuteriochloroform): 1.90 (s, 3H), 4.90 (s, 2H), 7.27-7.47 (m, 8H), 8.50 (m, 1H).

Anal. Calcd. for C₁₈H₁₄O₃S: C, 69.66; H, 4.55; Found: C, 69.34; H, 4.39.

3-Alkoxyethyl-2-phenyl-4H-1-benzothiopyran-4-ones (5-9).

These compounds were formed by heating 3-(chloromethyl)thioflavone **2** (1.5 g, 0.0052 mole) with the corresponding alcohol (40 ml) and sodium for 3-17 hours at reflux. The remaining alcohol was evaporated *in vacuo*, and the resulting solid was recrystallized from methanol-water or ligroin (bp 75-120°). Compounds **7-9** were distilled by Kugelrohr and identified by means of ¹H-nmr spectra. The physical properties of compounds **5-9** are summarized in Table I.

General Procedure for the Preparation of 2-Phenyl-4H-1-benzothiopyran-4-one 1,1-Dioxides.

Hydrogen peroxide (30%, 5.4 g, 0.048 mole) was added dropwise to a solution of thioflavone (**1-4**, 0.009 mole) in 80 ml of glacial acetic acid. The resulting solution was stirred for 1 hour at 100°. After cooling, the reaction mixture was diluted with ice-water and the resulting solid was filtered and washed with water. Recrystallization from solvent listed in Table II gave thioflavone 1,1-dioxides. Compound **11** was prepared from **3** or **4** in 39% or 51% yield. The mp's of the known compounds were as follows: **12a**, mp 136-137° (lit [14] 136°); **12f**, mp 161-163° (lit [14] 156-157°).

Antimicrobial Activity.

Minimum inhibitory concentration (MIC) for various microorganisms was determined by a microplate broth dilution technique (bacteria, fungi and molds) and by microplate agar dilution technique (*Trichophyton* and *Candida albicans*). Serial two-fold dilutions of the test substance were prepared in 50 µl of a standard broth medium in the wells of microplate. Each well was then inoculated with 50 µl of standardized cell suspension to yield a final microbial concentration of ~10⁵ cell/ml. The microplates were incubated at 37° for 24 hours for bacteria, 25° for 24 hours or 48 hours for fungi and molds, 25° for 96 hours or 168 hours for *Trichophyton*, and 25° for 24 hours for *Candida albicans*. The lowest concentration of test substance that inhibited visible growth was considered to be the MIC.

Tests against P-388 Leukemia.

Tests vs. P-388 leukemia were performed at the National Cancer Institute, Silver Springs, MD, as explained in footnote [a] of Table V. The day of death of each of the treated and untreated mice was determined by daily observations. The ratio T/C% reflects the life span of a group of 6 treated test mice as compared to a group of 30 inoculated untreated control mice. 40 Thioflavone derivatives have been tested, and 5 compounds which showed an increase of life span over 20% (T/C% = 120) are listed in Table V.

Acknowledgment.

We are grateful to Dr. S. Taniguchi and his staff of Shinnippon Pharmaceutical Co. Ltd. for the *in vitro* testing data and the acute toxicity data. We wish to thank Dr. I. Szele for helpful preparation of this manuscript.

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