# Synthesis and Antibacterial Activity of 2-Phenyl-4*H*-benzo[*b*]thiopyran-4-ones (Thioflavones) and Related Compounds

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A number of 2-phenyl-4*H*-benzo[*b*]thiopyran-4-ones (thioflavones) and related compounds have been prepared to test their antibacterial activity. The flavone derivatives were also prepared to compare with their antibacterial activity. It was found that hydroxythioflavones were easily prepared by demethylation of methoxythioflavones with aluminium chloride. In the test of antimicrobial activity, methoxy- or hydroxythioflavones were found to be inactive. It is suggested that the sulfone or sulfoxide of thioflavone is required for antimicrobial activities against yeast funguses and molds. These thioflavone derivatives exhibit low acute toxicity.

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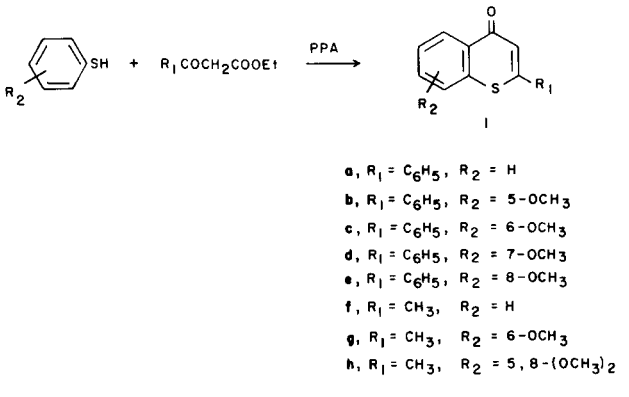
2-Phenyl-4*H*-benzo[*b*]thiopyran-4-one (thioflavone) derivatives are the thio analogs of flavonoid derivatives which are an important group of naturally occurring pharmacologically effective compounds. Few connections between chemical structure of thioflavones and pharmacological effects have so far studied systematically.

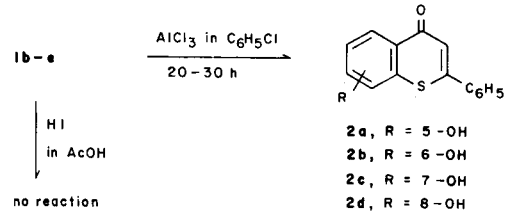
Most of flavonoid compounds which are biologically and pharmacologically active substances have hydroxyl groups in the flavonoid skeleton [1]. Few reports are available on the preparation of hydroxyl derivatives of thioflavonoid compounds, probably because of difficulty of preparation. We now found the convenient method to prepare hydroxythioflavones including demethylation of methoxythioflavones with aluminium chloride. We prepared various thioflavones, 2-methyl-4*H*-benzo[*b*]thiopyran-4-ones (2-methylthiochromones) and related compounds to test antibacterial activity.

The present paper describes the convenient method to prepare new hydroxythioflavones, benzo[*b*]thiopyrylium salts and thioflavone *S*-oxides, and their antibacterial activities and $LD_{50}$. These are also compared with other known flavone derivatives. Methoxyl substituted thioflavones and 2-methylthiochromones were obtained by a method similar to the preparation of thiochromone and thioflavone reported by Bossert (Scheme I) [2].

Scheme I




Scheme II




8-Hydroxythioflavone (**2d**) has been prepared by demethylation of **1e** with hydrogen iodide [3]. However, demethylation of methoxythioflavones under the conditions similar to that of flavonoid compounds with hydrogen iodide in acetic acid [4] failed to give a product. We now found that hydroxythioflavones were easily prepared in good yields by treatment of methoxythioflavones with aluminium chloride using chlorobenzene as a solvent (Table I).

Scheme III

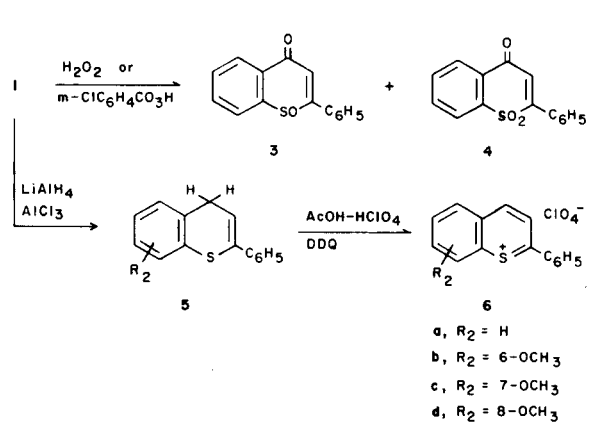


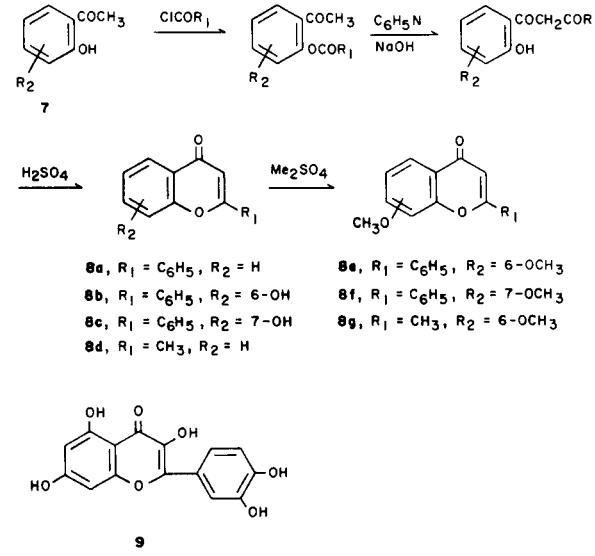
Table I

| Compound | Mp (°C) | Yield (%) | Analysis [a] (%) Found C | H |
|---|---|---|---|---|
| **2a** | 156-158 | 82 | 70.72 | 3.41 |
| **2b** | 288-289 | 72 | 71.22 | 3.49 |
| **2c** | 270-270.5 | 75 | 70.61 | 3.45 |
| **2d** | 288-290 (lit [3] 292) | 74 | 70.58 | 3.43 |

[a] *Anal.* Calcd. for $C_{15}H_{10}O_2S$: C, 70.85; H, 3.96.

Thioflavone 1-oxide (**3**) has been prepared by several steps from thiochromanone derivatives [5]. Prior use of *m*-chloroperbenzoic acid or hydrogen peroxide selectively afforded only thioflavone 1,1-dioxide (**4**) [6]. We found that if an equimolar amount of hydrogen peroxide or *m*-chloroperbenzoic acid was used, sulfur atom of **1a** was oxidized to give **3** and **4** as scheme III. These oxidized products could be easily separated by chromatography on silica gel. This method may be a convenient method to prepare the thioflavone 1-oxide. 2-Phenylbenzo[*b*]thiopyrylium perchlorates were prepared by the previously reported method [7] using reduction of thioflavone with aluminium hydride and subsequent hydride abstraction from 2-phenyl-4*H*-1-thiochromene (**5**) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).

Scheme IV





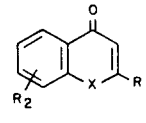


Flavone and 2-methylchromone derivatives **8** were generally obtained from *o*-hydroxyacetophenone derivatives **7** by a method similar to the preparation of the flavone reported by Wheeler [9] (Scheme IV).

Most of compounds reported herein were screened *via in vitro* antimicrobial activity against Gram-positive and Gram-negative bacteria, yeast funguses, and molds. The

Table II

MIC (μg/ml) of Thioflavones and Related Compounds

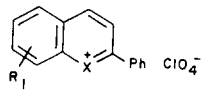



| No. | X | $R_1$ | $R_2$ | *B. subtilis* K 49 | *S. aureus* NCTC 8530 | *S. cerevisiae* IFO 0203 | *C. utillis* OUT 6020 | *P. crustosum* Thom | *R. chinensis* IFO 4745 |
|---|---|---|---|---|---|---|---|---|---|
| (**1a-1e**, **2a-2d**) | S | Ph | H, $OCH_3$, OH | [a] | [a] | [a] | [a] | [a] | [a] |
| **1f** | S | $CH_3$ | H | 400 | [a] | 800 | 800 | 400 | 400 |
| **1g** | S | $CH_3$ | 6-$OCH_3$ | 200 | [a] | 400 | 400 | 100 | 200 |
| **1h** | S | $CH_3$ | 5,8-$(OCH_3)_2$ | 800 | [a] | [a] | [a] | [a] | 800 |
| **3** | SO | Ph | H | 100 | 400 | 12.5 | 25 | 100 | 100 |
| **4** | $SO_2$ | Ph | H | 800 | 800 | 3.13 | 1.56 | 12.5 | [a] |
| **8a** | O | Ph | H | 50 | 800 | 100 | [a] | 50 | 50 |
| **8b** | O | Ph | 6-OH | [a] | [a] | 200 | 800 | [a] | [a] |
| (**8c**, **8e**, **8d**) | O | Ph | 7-OH, 6-$OCH_3$, H | [a] | [a] | [a] | [a] | [a] | [a] |
| **8f** | O | Ph | 7-$OCH_3$ | 25 | [a] | [a] | [a] | [a] | [a] |
| **8g** | O | $CH_3$ | 6-$OCH_3$ | 400 | [a] | 800 | [a] | 400 | 800 |
| **9** | | | Quercetine | 400 | 800 | [a] | [a] | [a] | [a] |

[a] >800 μg/ml. MIC of all compounds against Gram-negative bacteria of *E. coli* (IFO 3545) and *P. aeruginosa* (IAM 1007) was >800μg/ml.

Table III

MIC (μg/ml) of Benzo[*b*]thiopyrylium Perchlorates and Flavinium Perchlorate




| No. | X | $R_1$ | *B. subtilis* K 49 | *S. aureus* NCTC 8530 | *S. cerevisiae* IFO 0203 | *C. utillis* OUT 6020 | *P. crustosum* Thom | *R. chinensis* IFO 4745 |
|---|---|---|---|---|---|---|---|---|
| **6a** | S | H | 100 | [a] | 800 | [a] | [a] | 800 |
| **6b** | S | 6-$OCH_3$ | [a] | [a] | [a] | [a] | [a] | 800 |
| **6c** | S | 7-$OCH_3$ | [a] | [a] | 400 | [a] | [a] | 50 |
| **6d** | S | 8-$OCH_3$ | [a] | [a] | [a] | [a] | [a] | [a] |
| **10** | O | H | [a] | [a] | 400 | [a] | 800 | 800 |
| Streptomycine | | | 50 | 100 | — | — | — | — |
| Cycloheximide | | | — | — | 1.56 | 3.13 | 100 | 50 |

[a] >800 μg/ml. MIC of all compounds against Gram-negative bacteria of *E. coli* (IFO 3545) and *P. aeruginosa* (IAM 1007) was >800 μg/ml.

results are summarized in Tables II and III. The results of the known flavone derivatives are compared. Generally, thioflavone and hydroxythioflavone derivatives exhibit no activity and 2-methylthiochromones **1f** and **1g** exhibit only slight activity. It was found that replacement of sulfur atom of thioflavone into sulfoxide or sulfone group increased significantly the antimicrobial activities against yeast funguses and molds.

On the other hand, flavone **8a** exhibits a stronger antibacterial activity against some kinds of microorganisms than thioflavone. Substitution of methyl group in 2-position of flavone reduced activity as observed in Table II. In the benzo[*b*]thiopyrylium salts, only 7-methoxy derivative **6c** exhibits somewhat antimicrobial activity against *R. Chinenis*, but other compounds are inactive. From these results, it is apparent that a sulfone or sulfoxide of thioflavone is required for antimicrobial activity as well as for antitumor activity [6].

Table IV

Acute Toxicity of Thioflavones and Benzo[*b*]thiopyrylium Salts

| Compound | $LD_{50}$ [a] (g/Kg) [b] | [c] |
|---|---|---|
| **1a** | >4.0 | 1.7 |
| **1c** | >4.0 | >4.0 |
| **1d** | >4.0 | >4.0 |
| **8a** | 2.5 | 0.61 |
| **8e** | >4.0 | >4.0 |
| **8f** | >4.0 | 1.2 |
| **9** | >4.0 | >4.0 |
| **6a** | 0.87 | 0.22 |
| **10** | 3.2 | 0.47 |

[a] 95% Confidence limits. [b] Oral administration. [c] Intraperitoneal injection.

The results of acute toxicity of thioflavone, flavone and their pyrylium salts are summarized in Table IV.

Generally, flavone and thioflavone derivatives exhibit no acute toxicity. It was observed that benzo[*b*]thiopyrylium salt had a slighter toxicity than the corresponding flavinium salt. From results of acute toxicity and antibacterial test, it may be expected that thiopyran derivatives as thioflavone and thiochromone derivatives may be a new group of pharmacologically active compounds.

## EXPERIMENTAL

All the melting points are uncorrected. Proton nmr spectra were taken on a JEOL JNM-MH-100 spectrometer with tetramethylsilane as an internal standard. 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. Infrared spectra were recorded on a Shimazu IR-420 spectrometer, and uv spectra were recorded on a Shimazu UV-240 spectrometer.

General Procedure for Preparation of 2-Phenyl-4*H*-benzo[*b*]thiopyran-4-ones (Thioflavones) and 2-Methyl-4*H*-benzo[*b*]thiopyran-4-ones (Thiochromones).

These compounds were generally prepared by Bossert's method [2]. To a warm polyphosphoric acid (50 g) was added a mixture of appropriate benzenethiol (0.027 mole) and ethyl benzoylacetate (or ethyl acetoacetate) (0.034 mole). The mixture was then stirred and heated to 100° for 1 hour. After cooling, the mixture was poured into an ice-water solution. The resulting solid was filtered and recrystallized from ethanol.

Compounds **1b** and **1d** were prepared as follows. The reaction mixture which was prepared from *m*-methoxybenzenethiol and ethyl benzoylacetate was chromatographed on silica gel using benzene/acetone as an eluent to give **1b** (18%) and **1d** (33%).

These melting points are as follows and the agreement between calculated and found values of elemental analyses for these compounds was within ± 0.3%.

Thioflavones were **1a**, mp 125-127° (lit [2] 124-126°), **1b**, mp 204-205°, **1c**, mp 155-157° (lit [2] 157°), **1d**, mp 137-139° (lit [2] 150°), and **1e**, mp 128-129° (lit [3] 129-130°).

2-Methylthiochromones were **1f**, mp 103-104° (lit [2] 105°), **1g** mp

101-102° (lit [8] 102-103°), and **1h**, mp 145-147° (lit [8] 146-148°).

General Procedure for Demethylation of Methoxythioflavones to Give Hydroxythioflavones.

The mixture of appropriate methoxythioflavone (3.1 g, 0.012 mole) and aluminium chloride (5.6 g, 0.042 mole) in chlorobenzene (100 ml) was refluxed for 20 hours. After cooling, the reaction mixture was poured into dilute hydrochloric acid and then chlorobenzene was removed by steam distillation. The residue was filtered and washed with water and recrystallized from ethanol to give hydroxythioflavone derivatives (Table I). These compounds **2a-2d** had typical spectral data (ms: m/e 254 ($M^+$, 100); ir (potassium bromide): 3360-3400 $cm^{-1}$ (OH)).

Thioflavone 1-Oxide **3** and 1,1-Dioxide **4**.

Method A.

To a solution of thioflavone (1.19 g, 5 mmoles) in acetic acid (10 ml) was added 0.57 g of 30% aqueous hydrogen peroxide solution. The mixture was heated for 6 hours at 70°. After cooling, the reaction mixture was poured into an ice-water solution and then resulting solid was filtered and washed with water. The crude products were dissolved in benzene and then chromatographed on silica gel using benzene/acetone (20/1) as an eluent to give 0.13 g of **3** (10%), mp 133-135° (lit [5] 134°); ir (potassium bromide): 1640 (CO), 1060 and 1035 $cm^{-1}$ (SO); and 0.43 g of **4** (32%), mp 136.5-137° (lit [5] 136°); ir (potassium bromide): 1645 (CO), 1280 and 1145 $cm^{-1}$ ($SO_2$). Compound **1a** was recovered (47%).

Method B.

A solution of *m*-chloroperbenzoic acid (1.72 g, 1 mmole) in chloromethane (20 ml) was added dropwise to a solution of thioflavone (2.38 g, 1 mmole) in chloroform at 0°. The reaction mixture was stirred for 3 hours at room temperature (~15°) and allowed to stand overnight and then washed well with 30 ml of 5% aqueous sodium hydrogen carbonate and with water. The chloromethane solution was dried over magnesium sulfate and removed *in vacuo*. The resulting solid was dissolved in benzene and then chromatographed on silica gel using benzene/acetone (20/1) to give **3** (12%) and **4** (30%). Compound **1a** was recovered (50%).

8-Methoxy-2-phenylbenzo[*b*]thiopyrylium Perchlorate **6d**.

To a stirred suspension at 25° of aluminium lithium hydride (0.19 g, 5 mmoles) and aluminium chloride (0.66 g, 5 mmoles) in THF (30 ml) was added dropwise 8-methoxythioflavone (**1e**) (1.34 g, 5 mmoles) in THF (15 ml). The reaction mixture was stirred for 100 minutes at 25°, and then water (1.0 ml) and concentrated sulfuric acid (2.5 ml) was added. After filtration, the filtrate was extracted with ether to give 8-methoxy-2-phenyl-4*H*-1-thiochromene **5d** (66%), mp 87-89°; nmr (deuteriochloroform): $\delta$ 4.01 (s, 3H, $OCH_3$), 3.65 (d, 2H, $J_{3,4}$ = 5 Hz), 6.35 (t, 1H, J = 5 Hz, H-3), 6.86-7.31 (m, 3H, Ar-H), 7.43-7.54 (m, 3H, Ar-H), and 7.72-7.90 (m, 2H, Ar-H); ms: m/e 254 ($M^+$, 100), 253 (66), 239 (18), 221 (11), 210 (17) and 177 (47).

*Anal.* Calcd. for $C_{16}H_{14}OS$: C, 75.56; H, 5.55. Found: C, 75.96; H, 5.47.

A solution of DDQ (0.68 g) in glacial acetic acid (3 ml) was added to a solution of **5d** (0.6 g) in glacial acetic acid (3 ml) under the stirring. After 30 minutes 60% perchloric acid (3.3 g) was added and the mixture was stirred for 30 minutes. The resulting solid was again separated. The collected solid was recrystallized from glacial acetic acid to give **6d** (62%), mp 181-183°; nmr (trifluoroacetic acid): $\delta$ 4.26 (s, 3H, $OCH_3$), 7.53-7.80 (m, 4H, Ar-H), 7.92-8.16 (m, 4H, Ar-H), 8.73 (d, 1H, J = 10 Hz) and 9.24 (d, 1H, J = 10 Hz); uv (sulfuric acid): $\lambda$ 284, 321 and 406 nm.

*Anal.* Calcd. for $C_{16}H_{13}ClO_5S$: C, 54.47; H, 3.71. Found: C, 54.21; H, 3.63.

Compounds **6a-6c** were used which we previously prepared [7].

Flavone and 2-Methylchromone Derivatives **8**.

Hydroxyflavone and 2-methylchromone derivatives were prepared by the Wheeler's method. Methoxyl substituted flavone and 2-methylchromone derivatives were ordinarily obtained by treatment of the corresponding hydroxyl derivatives with dimethyl sulfate. These melting points are as follows. Flavones were **8a**, mp 95-97° (lit [9] 95-97°), **8b**, mp 234-235° (lit [10] 231-232°), **8c**, mp 231-233° (lit [11] 240°), **8e**, mp 164-165° (lit [12] 154°), and **8f**, mp 103-105° (lit [10] 110°). 2-Methylchromones were **8d**, mp 70-71° (lit [13] 70-71°) and **8g**, mp 107-108° (lit [14] 102-105°).

Other Materials.

Quercetine **9** was the special grade of commercial origin. Flavinium salt **10**, mp 178-180° (lit [15] 174°) was prepared by the Wizinger and Tobel's method.

Antimicrobial Activity Test and Acute Toxicity Test.

The MIC was determined by the dilution method of broths using streptomycine and cycloheximide as the references. $LD_{50}$'s were determined by means of the standard oral administration or intraperitoneal injection using mice.

Acknowledgement.

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## REFERENCES AND NOTES

[1] M. Gobor, "Flavonoids and Bioflavonoids, 1981", L. Frarkas, F. Kallay and H. Wagner, Elsevier Scientific Publishing Co. Amsterdam, 1982, p 363.

[2] F. Bossert, *Ann. Chem.*, **680**, 40 (1964).

[3] S. Ruhemann, *Ber.*, **46**, 3392 (1913).

[4] M. Nakayama, T. Horie, M. Makino, S, Hayashi and S. Ganno, *Nippon Kagaku Kaishi*, 1390 (1978).

[5] R. Bognar, J. Balint and M. Rakosi, *Ann. Chem.*, 1529 (1977).

[6] M. H. Holshouser, L. J. Loeffler and I. H. Hall, *J. Med. Chem.*, **24**, 853 (1981).

[7] H. Nakazumi, T. Ueyama, T. Endo and T. Kitao, *Bull. Chem. Soc. Japan*, **56**, 1251 (1983).

[8] H. Nakazumi and T. Kitao, *ibid.*, **50**, 939 (1977).

[9] T. S. Wheeler, "Organic Synthesis", Vol 32, John Wiley and Sons, Inc. New York, 1952, p 72.

[10] S. Hattori, *Acta. Phytochim.*, **4**, 45 (1928).

[11] J. Allan and R. Robinson, *J. Chem. Soc.*, **125**, 2193 (1924).

[12] H. Simonis and S. Danischewski, *Ber.*, **59**, 2916 (1926).

[13] S. Binecki and E. Kesler, *Acta. Pol. Pharm.*, **13**, 503 (1956).

[14] P. F. Wiley, *J. Am. Chem. Soc.*, **73**, 4205 (1951).

[15] R. Wizinger and H.v. Tobel, *Helv. Chim. Acta*, **40**, 1305 (1957).