Synthesis and Cytostatic Activity of 4,7-Dihydroxythioaurone Derivatives. Effect of B Ring Substitution on the Activity

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Biological activity of thioaurones was not tested so far and the group constitute completely unexplored source of new molecules of pharmacological interest. We report synthesis and evaluation of cytotoxic activity of thioaurone derivatives bearing *p*-hydroquinone system in ring A. Their activity was found to depend strongly on substitution pattern, so eventually both the activity and pharmacokinetic parameters of the molecules could be tailored by further structural modifications.

Key words thioaurone; cytotoxicity; antitumor

Flavonoids have been known to exert the beneficial effects in various disease states, mainly cancer and cardiovascular disease. While their actual mechanism of action remains unknown, the flavonoids were claimed to positively influence intracellular redox processes, and recently several studies indicated the effect on cell signaling pathway.[1)] The affinity of flavonoids for a multitude of other cellular receptors has also been established.[2)] From the pharmacological point of view, such lack of selectivity is discouraging, as eventually, for any flavonoid medicine a number of side effects could be expected.

A possible approach to improve selectivity of flavonoids consist on significant modification of their core framework, and for this reason we have been interested in synthesis and potential antitumor activity of thioaurones. Their closest analogs, aurones, are known to posses significant activity.[3)] Biological effects of thioaurones, including those related to anticancer activity, were not studied yet,[4)] and to our knowledge, this is the first report describing their activity. We arbitrary decided to prepare derivatives **1** bearing *p*-hydroquinone system in the ring A (Fig. 1).

In this paper we report influence of the ring B substituents on *in vitro* cytotoxicity of compounds **1**.

Results

Chemistry Synthesis of 4-methoxy-7-piperidinocarbonyloxythioaurone derivatives **2** was described earlier,[5)] the obtained compounds are listed in Table 1.

Further modifications of the thioaurones **2** were performed by deprotection of the hydroxy substituents at positions 4 and/or 7 (Chart 1).

Selective deprotection of the hydroxy substituents at positions 4 and 7 of the thioaurones was relatively easy due to the differences between the protecting groups. Cleavage of the methoxy group at position 4 was done quantitatively using boron trifluoride/methyl sulfide.[6)] Typically, the 4-methoxy group was cleaved with excess of boron trifluoride/methyl sulfide complex in methylene chloride at 0 °C in 1—2 h. Overnight stirring at room temperature resulted in cleavage

Table 1. Structures and Cytotoxicity of 4-Methoxy-7-piperidinocarbonyloxythioaurones **2**

Compd. No.	Substituents in the ring B	IC_{50} value (μM)			
		HeLa	AZ-521	P815	WI-38
6	3′-OH	8.9	8.5	6.7	10.9
7	3′,4′-diOH	10.6	4.8	8.9	11.5
8	2′-Cl	12.0	17.0	23.8	>100
9	4′-Br	14.0	12.5	4.5	12.8
10	3′-Br; 4′,5′-diOCH$_3$	15.0	32.1	6.0	>50
11	4′-Cl	15.1	21.3	27.3	>100
12	2′,3′-diOCH$_3$	18.3	6.7	3.3	10.1
13	3′-Cl	20.3	30.4	>100	>100
14	4′-OH	31.2	31.2	77.7	57.2
15	—	44.3	77.4	>100	>100
16	Pyridinyl-4 ring	44.8	33.0	60.2	>100
17	4′-OCH$_3$	81.4	51.2	>100	99.5
18	3′,4′-diOCH$_3$	>100	>100	>100	>100
19	3′-OCH$_3$; 4′-OH	>100	44.4	>100	>100
20	3′-Br; 4′-OH; 5′-OCH$_3$	>100	12.6	14.7	18.2
21	4′-N(CH$_3$)$_2$	>100	36.3	>100	>100
22	4′-NO$_2$	>100	>100	>100	>100

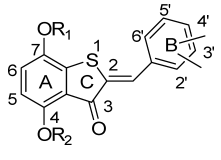

Fig. 1. General Formula of the Prepared Compounds (**1**)

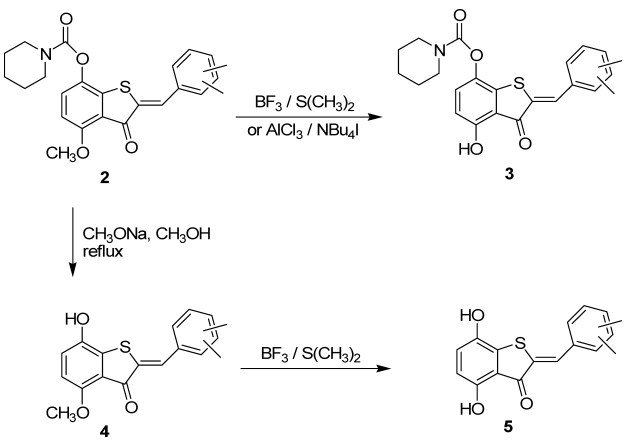

Chart 1. Deprotection of Thioaurones **2**

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Table 2. Structures and Cytotoxicity of 4-Hydroxy-7-piperidinocarbonyloxythioaurones **3**

Compd. No.	Substituents in the ring B	IC_{50} value (μM)			
		HeLa	AZ-521	P815	WI-38
23	3′-Br; 4′,5′-diOH	1.1	2.0	0.8	2.9
24	3′-Br; 4′,5′-diOCH$_3$	2.4	6.0	1.1	>50
25	3′,4′-diOH	2.7	3.9	2.2	7.8
26	3′,4′-diOCH$_3$	22.1	60.8	>89.7	>89.7

Table 3. Structures and Cytotoxicity of 7-Hydroxy-4-methoxythioaurones **4**

Compd. No.	Substituents in the ring B	IC_{50} value (μM)			
		HeLa	AZ-521	P815	WI-38
27	3′-Br; 4′,5′-diOCH$_3$	3.9	8.5	3.5	20.4
28	3′,4′-diOH	12.9	13.8	18.8	26.1
29	3′-Cl	13.6	28.4	31.7	>100
30	—	93.0	18.5	21.6	>100
31	3′,4′-diOCH$_3$	>100	30.9	>100	>100
32	4′-Cl	>100	>100	>100	>100
33	3′-Br; 4′-OH; 5′-OCH$_3$	>100	21.5	>100	29.6

of all methoxy substituents present in the molecule. Alternatively, metoxy substituents were cleaved with aluminum chloride–tetrabutylammonium iodide mixture, however the method was not as convenient as with BF_3–$S(CH_3)_2$, at least for the small scale reactions. The obtained compounds of general formula **3** are given in the Table 2.

The carbamate group at position 7 could be cleaved by aqueous sodium hydroxide but concerning purity of products better results were obtained using sodium methoxide in methanol. The reaction was quantitative and the yields depend essentially on the yield of isolation. The obtained compounds of general formula **4** are given in the Table 3.

The dihydroxy compounds **5** prepared by deprotection of both hydroxy groups are listed in Table 4.

Cytotoxic Activity The cytotoxic effects of synthesized compounds are presented in Tables 1—4, within each table the compounds are arranged according to their decreasing activity against the HeLa cell line. The presented data showed that thioaurones **1** are potential lead compounds for new anti-tumor agents, as significant number of the tested compounds demonstrated cytototoxic activity, and several compounds, *e.g.* **8**, **24**, **29** appeared to be cytotoxic to cancer cells but not to WI-38 cell line representing normal lung fibroblasts.

For compounds of general formula **2** (Table 1), a borderline between the active and inactive compounds was near the unsubstituted derivative **15**. Introduction of chlorine atom into any position of the B ring increased the activity. The two most active compounds **6** and **7** had free hydroxy substituents, however, methylation of the groups (compound **7** *versus* **18**, and **14** *versus* **17**) abolished completely the activity. Bromination of the inactive compound **18** lead again to an active molecule **10**, and mono-demethylation of **10** gave compound **20** of similar activity but with different selectivity against various cell lines.

The above structure–activity observations appeared invalid for dihydroxy compounds **5** (Table 4). In this case, the unsubstituted derivative **34** was the most active, the chlorinated compounds **35** and **36** were active, but hydroxylation of the ring B resulted in loss of the activity.

Table 4. Structures and Cytotoxicity of 4,7-Dihydroxythioaurones **5**

Compd. No.	Substituents in the ring B	IC_{50} value (μM)			
		HeLa	AZ-521	P815	WI-38
34	—	2.5	7.8	3.8	14.6
35	4′-Cl	2.8	12.4	4.4	7.2
36	3′-Cl	2.9	6.1	3.0	12.8
37	3′-Br; 4′,5′-diOCH$_3$	6.6	11.5	7.7	27.8
38	3′,4′-diOH	12.0	3.1	46.0	41.7
39	3′,4′-diOCH$_3$	>100	4.6	>100	>100

The mentioned above for compounds **2** influence of the methylation of the phenolic groups on the activity and cell specificity is of great interest, and is also evident for 4-hydroxy derivatives **3** (Table 2). The dihydroxy compound **23** exerted a strong cytotoxic effects against both cancer and normal cells (Table 2). Methylation of the hydroxyl functions (compound **24**) slightly diminished the cytotoxic effect against tumor cells, while the normal lung fibroblasts were not affected by **24**, at the tested range of concentrations. Debromination of the compound **23** (compound **25**) only slightly diminished the activity, while debromination of **24** (compound **26**) resulted in loss of both selectivity and activity, indicating the importance of bromine substitution. Interestingly, compounds selectively inactive against the normal cells WI-38 were usually substituted in the ring B with bromine or chlorine.

Conclusion

We report synthesis and evaluation of cytotoxic activity of thioaurone derivatives bearing *p*-hydroquinone system in ring A. Biological activity of thioaurones was not tested before, and the group constitutes completely unexplored source of new molecules of pharmacological interest. The present results demonstrated that thioaurones posses a promising cytotoxic activity. The activity was found to depend strongly on substitution pattern, so eventually both the activity and pharmacokinetic parameters of the molecules could be tailored by further structural modifications.

Experimental

Melting points were uncorrected. Infrared spectra were obtained from KBr pellets on Thermo Mattson Satellite instrument. The ^{1}H- and ^{13}C-NMR spectra were recorded on 200 MHz (Varian Gemini), 400 MHz (Varian Mercury) or 500 MHz (Varian Unity Plus) spectrometers. Elemental analyses were performed on Carlo-Erba 1108 instrument. Flash column chromatography was performed on silica gel 60 (Merck, less than 230 mesh). TLC was carried out on Merck 0.2 mm silica gel 60 F_{254} aluminum plates. Yields were not optimized. Compounds **6**—**22** and **28** were prepared as described previously.[5)]

Evaluation of Biological Activity Cell Lines and Culture Conditions: AZ-521, human gastric adenocarcinoma cell line was obtained from the Japanese Collection of Research Bioresources and cultured in EMEM cell culture medium supplemented with 10% fetal bovine serum (FBS). Hela, human uterocervical carcinoma, WI-38, human lung fibroblast, and P815 murine mastocytoma cell lines were purchased from the DaiNippon Pharm. Co., and were maintained in EMEM cell medium supplemented with 10% FBS, except P815 grown in the RPMI1640 medium plus 10% FBS.

Approximately 2×10^3 cells, suspended in a complete medium, were

plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 °C for 24 h. The test compounds at indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. After 72 h, the cell cultures were fixed with glutaraldehyde and then stained with crystal violet to determine the number of remaining cells. In the case of P815 cell line (growing as suspension), the cells were incubated for 1.5 h with WST-8 solution and the formed metabolic products were assayed at 450 nm by a microplate reader.

The cytotoxic effects were expressed as the IC_{50} values, the concentrations required to inhibit the growth of the cells by 50%.

General Procedure for Cleavage of 7-Piperidinocarbonyloxy Group by Sodium Methoxide A suitable 7-piperidinocarbonyloxy derivative (1 mmol) was dissolved in a solution of sodium methoxide (about 20 mmol) in methanol (10 ml) and the mixture was refluxed under argon, with stirring for 1—2 h. The obtained, dark mixture was cooled, diluted with water and acidified with hydrochloric acid. The formed precipitate was filtered, washed with water, dried, crystallized from 2-methoxyethanol, washed with methanol, and dried to give final products of general formula **4**.

2-(3′-Bromo-4′,5′-dimethoxybenzylidene)-7-hydroxy-4-methoxy-2,3-dihydrobenzo[*b*]thiophen-3-one (27) Prepared from **10**, yield 65%, brick-red solid, mp 240—242 °C dec. NMR (400 MHz, DMSO): δ 10.20 (br s, 1H, OH), 7.68 (s, 1H, H-a), 7.58 (d, 1H, J=2.0 Hz, H-2′), 7.46 (d, 1H, J=2.0 Hz, H-6′), 7.11 (d, 1H, J=8.8 Hz, H-6), 6.83 (d, 1H, J=8.8 Hz, H-5), 3.92 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3). IR (KBr) cm^{-1}: 3180, 1649, 1570, 1492, 1281, 1238, 1047. *Anal.* Calcd for $C_{18}H_{15}O_5SBr$: C, 51.08; H, 3.57; S, 7.58. Found: C, 51.20; H, 3.46; S, 7.68.

2-(3′-Chlorobenzylidene)-7-hydroxy-4-methoxy-2,3-dihydrobenzo-[*b*]thiophen-3-one (29) Prepared from **13**, yield 58%, brick-red solid, mp >240 °C dec. NMR (500 MHz, DMSO): δ 10.21 (s, 1H, OH), 7.81 (s, 1H, H-2′), 7.74 (d, 1H, J=7.3 Hz, H-6′), 7.72 (s, 1H, H-a), 7.58 (t, 1H, J=7.8 Hz, H-5′), 7.53 (d, 1H, J=7.8 Hz, H-4′), 7.10 (d, 1H, J=8.8 Hz, H-6), 6.83 (d, 1H, J=8.8 Hz, H-5), 3.83 (s, 3H, OCH_3). IR (KBr) cm^{-1}: 3361, 1666, 1579, 1495, 1272, 1237, 1038. *Anal.* Calcd for $C_{16}H_{11}O_3SCl$: C, 60.28; H, 3.48; S, 10.06. Found: C, 60.17; H, 3.47; S, 10.22.

2-Benzylidene-7-hydroxy-4-methoxy-2,3-dihydrobenzo[*b*]thiophen-3-one (30) Prepared from **15**, dark cherry solid, yield 41%, mp 270—280 °C dec. NMR (200 MHz, DMSO): δ 10.20 (s, 1H, OH), 7.74 (s, 1H, H-a), 7.78 (d, 2H, J=6.9 Hz, H-2′, H-6′), 7.4—7.6 (m, 3H, H-3′, H-4′, H-5′), 7.10 (d, 1H, J=8.8 Hz, H-6), 6.83 (d, 1H, J=8.8 Hz, H-5), 3.83 (s, 3H, OCH_3). IR (KBr) cm^{-1}: 3278, 1663, 1579, 1501, 1289, 1270, 1242, 1049. *Anal.* Calcd for $C_{16}H_{12}O_3S$: C, 67.59; H, 4.26; S, 11.26. Found: C, 67.42; H, 4.23; S, 11.35.

2-(3′,4′-Dimethoxybenzylidene)-7-hydroxy-4-methoxy-2,3-dihydrobenzo[*b*]thiophen-3-one (31) Prepared from **18**, yield 77%, brick-red solid, mp 277—282 °C. NMR (200 MHz, acetone): δ 9.05 (br s, 1H, OH), 7.73 (s, 1H, H-a), 7.34—7.46 (m, 2H, H-2′, H-6′), 7.16 (d, 1H, J=8.3 Hz, H-5′), 7.13 (d, 1H, J=8.8 Hz, H-6), 6.80 (d, 1H, J=8.8, H-5), 3.94 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3). IR (KBr) cm^{-1}: 3249, 1642, 1583, 1559, 1512, 1266, 1239, 1039. *Anal.* Calcd for $C_{18}H_{16}O_5S$: C, 62.78; H, 4.68; S, 9.31. Found: C, 62.58; H, 4.67; S, 9.24.

2-(4′-Chlorobenzylidene)-7-hydroxy-4-methoxy-2,3-dihydrobenzo-[*b*]thiophen-3-one (32) Prepared from **11**, yield 65%, dark brown solid, mp 258—261 °C. NMR (400 MHz, DMSO): δ 7.79 (d, 2H, J=8.6 Hz, H-2′, H-6′), 7.70 (s, 1H, H-α), 7.60 (d, 2H, J=8.6 Hz, H-3′, H-5′), 7.03 (d, 1H, J=8.7 Hz, H-6), 6.78 (d, 1H, J=8.7 Hz, H-5), 3.81 (s, 3H, OCH_3), 3.5 (br s, OH). IR (KBr) cm^{-1}: 3466, 1667, 1581, 1491, 1282, 1240, 1042. *Anal.* Calcd for $C_{16}H_{11}O_3SCl\times0.5H_2O$: C, 58.63; H, 3.69; S, 9.78. Found: C, 58.45; H, 3.39; S, 9.97.

2-(3′-Bromo-4′-hydroxy-5′-methoxybenzylidene)-7-hydroxy-4-methoxy-2,3-dihydrobenzo[*b*]thiophen-3-one (33) Prepared from **20**, brick-red solid, yield 65%, mp 255—260 °C, dec. NMR (200 MHz, DMSO): δ 10.35 (br s, 1H, OH), 10.17 (s, 1H, OH), 7.66 (s, 1H, H-α), 7.55 (d, 1H, J=1.9 Hz, H-2′), 7.39 (d, 1H, J=1.9 Hz, H-6′), 7.09 (d, 1H, J=8.8 Hz, H-6), 6.81 (d, 1H, J=8.8 Hz, H-5), 3.91 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3). IR (KBr) cm^{-1}: 3464, 3179, 1645, 1577, 1500, 1282, 1046. *Anal.* Calcd for $C_{17}H_{13}O_5SBr$: C, 49.89; H, 3.20; S, 7.83. Found: C, 49.67; H, 3.30; S, 7.73.

General Procedure for Selective Cleavage of 4-Methoxy Substituent by BF_3/Me_2S Complex A suitable 4-methoxy derivative (1 mmol) was suspended in dry methylene chloride (6 ml), cooled to 0 °C (ice-water bath) and deoxygenated with argon. Next, boron trifluoride–methyl sulfide complex (Aldrich) (1.4 ml, 13 mmol) was added and the mixture was stirred in the cooling bath, under argon for 1—2 h. The reaction mixture was quenched with water and hydrochloric acid, usually with small addition of methanol to facilitate solidification of the product. Methylene chloride was evaporated under vacuum and the mixture was filtered. The obtained solid was washed with water, dried, crystallized from 2-methoxyethanol, washed with methanol and dried to give products of general formula **3** or **5**.

2-(3′-Bromo-4′,5′-dimethoxybenzylidene)-4,7-dihydroxy-2,3-dihydrobenzo[*b*]thiophen-3-one (37) Prepared from **27**, purple solid, yield 49%, mp 253—254 °C dec. NMR (400 MHz, DMSO): δ 9.9 (br s, 2H, OH), 7.75 (s, 1H, H-α), 7.60 (d, 1H, J=1.8 Hz, H-2′), 7.48 (d, 1H, J=1.8 Hz, H-6′), 7.04 (d, 1H, J=8.8 Hz, H-6), 6.65 (d, 1H, J=8.8 Hz, H-5). IR (KBr) cm^{-1}: 3250, 1567, 1491, 1459, 1297, 1209. *Anal.* Calcd for $C_{17}H_{13}O_5SBr$: C, 49.89; H, 3.20; S, 7.83. Found: C, 50.05; H, 3.09; S, 8.01.

2-(3′,4′-Dimethoxybenzylidene)-4,7-dihydroxy-2,3-dihydrobenzo-[*b*]thiophen-3-one (39) Prepared from **31**, claret solid, yield 75%, mp 245 °C. NMR (500 MHz, DMSO): δ 10.00 (s, 1H, OH), 9.80 (s, 1H, OH), 7.80 (s, 1H, H-α), 7.41 (dd, 1H, J_1=8.3 Hz, J_2=1.9 Hz, H-6′), 7.37 (d, 1H, J=1.9 Hz, H-2′), 7.16 (d, 1H, J=8.3 Hz, H-5′), 7.02 (d, 1H, J=8.3 Hz, H-6), 6.62 (d, 1H, J=8.3 Hz, H-5), 3.85 (s, 6H, 2×OCH_3). IR (KBr) cm^{-1}: 3250, 1589, 1559, 1510, 1462, 1284, 1215, 1145. *Anal.* Calcd for $C_{17}H_{14}O_5S$: C, 61.81; H, 4.27; S, 9.69. Found: C, 62.00; H, 4.16; S, 9.48.

General Procedure for Cleavage of All Methoxy Substituents by BF_3/Me_2S Complex A suitable methoxy derivative (1 mmol) was suspended in dry methylene chloride (6 ml), cooled to 0 °C (ice-water bath) and deoxygenated with argon. Next, boron trifluoride–methyl sulfide complex (Aldrich) (13 mmol per one methoxy substituent) was added and the mixture was stirred at room temperature, under argon for 15 h. The reaction mixture was quenched with water and hydrochloric acid, usually with small addition of methanol to facilitate solidification of the product. Methylene chloride was evaporated under vacuum and the mixture was filtered. The solid on filter was washed with water, dried, crystallized from 2-methoxyethanol, washed with methanol and dried to give products of general formula **3** or **5**.

2-(3′,4′-Dihydroxybenzylidene)-4-hydroxy-7-piperidinocarbonyloxy-2,3-dihydrobenzo[*b*]thiophen-3-one (25) Prepared from **18**, yellow solid, yield 40%, mp 243—247 °C. NMR (500 MHz, DMSO): δ 10.4 (br s, 1H, OH), 9.8 (br s, 2H, 2×OH), 7.71 (s, 1H, H-α), 7.34 (d, 1H, J=8.8 Hz, H-6), 7.20 (d, 1H, J=1.9 Hz, H-2′), 7.13 (dd, 1H, J_1=8.3 Hz, J_2=1.9 Hz, H-6′), 6.90 (d, 1H, J=8.3 Hz, H-5′), 6.76 (d, 1H, J=8.8 Hz, H-5), 3.61 (br s, 2H, piperidine), 3.43 (br s, 2H, piperidine), 1.63 (br s, 4H, piperidine), 1.56 (br s, 2H, piperidine). IR (KBr) cm^{-1}: 3374, 1681, 1650, 1570, 1508, 1433, 1236. *Anal.* Calcd for $C_{21}H_{19}NO_6S$: C, 61.00; H, 4.64; N, 3.39; S, 7.74. Found: C, 60.85; H, 4.53; N, 3.31; S, 7.58.

2-Benzylidene-4,7-dihydroxy-2,3-dihydrobenzo[*b*]thiophen-3-one (34) Prepared from **30**, brown solid, yield 52%, mp above 250 °C dec. NMR (400 MHz, DMSO): δ 9.8 (br s, 2H, 2×OH), 7.80 (s, 1H, H-α), 7.77 (d, 2H, J=7.7 Hz, H-2′, H-6′), 7.55 (t, 2H, J=6.9 Hz, H-3′, H-5′), 7.47 (t, 1H, J=7.0 Hz, H-4′), 7.02 (d, 1H, J=8.6 Hz, H-6), 6.63 (d, 1H, J=8.6 Hz, H-5). IR (KBr) cm^{-1}: 3363, 1649, 1582, 1566, 1461, 1298, 1198. *Anal.* Calcd for $C_{15}H_{10}O_3S$: C, 66.65; H, 3.73; S, 11.86. Found: C, 66.44; H, 3.60; S, 12.04.

2-(4′-Chlorobenzylidene)-4,7-dihydroxy-2,3-dihydrobenzo[*b*]thiophen-3-one (35) Prepared from **32**, brown-orange solid, yield 59%, mp above 270 °C dec. NMR (400 MHz, DMSO): δ 9.9 (br s, 2H, 2×OH), 7.81 (d, 2H, J=8.4 Hz, H-2′, H-6′), 7.79 (s, 1H, H-α), 7.63 (d, 2H, J=8.4 Hz, H-3′, H-5′), 7.04 (d, 1H, J=8.8 Hz, H-6), 6.65 (d, 1H, J=8.8 Hz, H-5). IR (KBr) cm^{-1}: 3385, 1644, 1578, 1486, 1383, 1345, 1228. *Anal.* Calcd for $C_{15}H_9O_3SCl$: C, 59.21; H, 2.98; S, 10.52. Found: C, 59.35; H, 2.83; S, 10.44.

2-(3′-Chlorobenzylidene)-4,7-dihydroxy-2,3-dihydrobenzo[*b*]thiophen-3-one (36) Prepared from **29**, yield 65%, cherry solid, mp above 230 °C dec. NMR (500 MHz, DMSO): δ 10.01 (br s, 1H, OH), 9.91 (br s, 1H, OH), 7.82 (s, 1H, H-2′), 7.77 (s, 1H, H-α), 7.74 (d, 1H, J=7.8 Hz, H-6′), 7.58 (t, 1H, J=7.8 Hz, H-5′), 7.53 (d, 1H, J=8.3 Hz, H-4′), 7.03 (d, 1 H, J=8.8 Hz, H-6), 6.65 (d, 1H, J=8.8 Hz, H-5). IR (KBr) cm^{-1}: 3254, 1638, 1572, 1457, 1284, 1195. *Anal.* Calcd for $C_{15}H_9O_3SCl$: C, 59.21; H, 2.98; S, 10.52. Found: C, 59.07; H, 2.80; S, 10.78.

2-(3′,4′-Dihydroxybenzylidene)-4,7-dihydroxy-2,3-dihydrobenzo-[*b*]thiophen-3-one (38) Prepared from **31**, yield 93%, claret solid, mp 253—254 °C dec. NMR (500 MHz, DMSO): δ 9.8 (br s, 4H, 4×OH), 7.68 (s, 1H, H-α), 7.28 (d, 1H, J=1.9 Hz, H-2′), 7.15 (dd, 1H, J_1=8.3 Hz, J_2=1.9 Hz, H-6′), 7.00 (d, 1H, J=8.3 Hz, H-6), 6.89 (d, 1H, J=8.3 Hz, H-5′), 6.61 (d, 1H, J=8.3 Hz, H-5). IR (KBr) cm^{-1}: 3383, 1589, 1549, 1517, 14447, 1292, 1192. *Anal.* Calcd for $C_{15}H_{10}O_5S$: C, 59.59; H, 3.33; S, 10.61. Found: C, 59.56; H, 3.56; S, 10.20.

Cleavage of Methoxy Substituents by Aluminum Chloride/Tetrabutylammonium Iodide. 2-(3′,4′-Dimethoxybenzylidene)-4-hydroxy-7-piperidinocarbonyloxy-2,3-dihydrobenzo[*b*]thiophen-3-one (26) 2-(3′,4′-Dimethoxybenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydrobenzo[*b*]thiophen-3-one (**18**) (110 mg, 0.24 mmol), aluminum chloride

(65 mg, 0.48 mmol), tetrabutylammonium iodide (260 mg, 0.72 mmol) in dry benzene (2 ml) under argon were stirred at 50 °C for 2 h. The reaction mixture was cooled in ice and decomposed by addition of water and hydrochloric acid. The formed suspension was extracted with ethyl acetate (80 ml), the organic layer was washed with water and brine, dried (Na_2SO_4) and evaporated to dryness. The residue was purified on silica gel column in chloroform, the main fraction was evaporated, and the residue was washed with methanol to give (44 mg, 41%) of 2-(3′,4′-dimethoxybenzylidene)-4-hydroxy-7-piperidinocarbonyloxy-2,3-dihydrobenzo[*b*]thiophen-3-one (**26**) as a yellow solid, mp 204—206 °C. NMR (200 MHz, DMSO): δ 10.6 (br s, 1H, OH), 7.82 (s, 1H, H-α), 7.36 (br s, 3H, H-6, H-2′, H-6′), 7.16 (d, 1H, J=8.0 Hz, H-5′), 6.76 (d, 1H, J=8.8, H-5), 3.84 (s, 6H, 2×OCH_3), 3.60 (br s, 2H, piperidine), 3.42 (br s, 2H, piperidine), 1.63 (br s, 6H, piperidine). IR (KBr) cm^{-1}: 3431, 1722, 1567, 1509, 1421, 1273, 1219, 1145. *Anal.* Calcd for $C_{23}H_{23}NO_6S$: C, 62.57; H, 5.25; N, 3.17; S, 7.25. Found: C, 62.29; H, 5.17; N, 3.04; S, 7.41.

2-(3′-Bromo-4′,5′-dihydroxybenzylidene)-4-hydroxy-7-piperidinocarbonyloxy-2,3-dihydrobenzo[*b*]thiophen-3-one (23) and 2-(3′-Bromo-4′,5′-dimethoxybenzylidene)-4-hydroxy-7-piperidinocarbonyloxy-2,3-dihydrobenzo[*b*]thiophen-3-one (24) 2-(3′-Bromo-4′,5′-dimethoxybenzylidene)-4-methoxy-7-piperidinocarbonyloxy-2,3-dihydrobenzo[*b*]thiophen-3-one (**10**), (400 mg, 0.75 mmol), aluminum chloride (450 mg, 3.38 mmol), tetrabutylammonium iodide (554 mg, 1.5 mmol) in dry benzene (10 ml) under argon were refluxed for 2.5 h. The reaction mixture was cooled in ice and decomposed by addition of water with hydrochloric acid and methanol. Next, the organic solvents were evaporated under vacuum and the formed suspension was filtered to give black solid (480 mg).

The solid was suspended in mixture of chloroform (5 ml) and ethyl acetate (0.5 ml), and filtered. The solid was washed on filter with chloroform, and crystallized from 2-methoxyethanol–chloroform mixture to give crude 2-(3′-bromo-4′,5′-dihydroxybenzylidene)-4-hydroxy-7-piperidinocarbonyloxy-2,3-dihydrobenzo[*b*]thiophen-3-one (190 mg, 50%). Re-crystallization from 2-methoxyethanol–methanol gave pure 2-(3′-bromo-4′,5′-dihydroxybenzylidene)-4-hydroxy-7-piperidinocarbonyloxy-2,3-dihydrobenzo[*b*]thiophen-3-one×0.5H_2O (**23**) as an orange solid (140 mg, 37%), mp 250—252 °C dec. NMR (400 MHz, DMSO): δ 10.4 (br s, 3H, 3×OH), 7.69 (s, 1H, H-α), 7.44 (d, 1H, J=2.0 Hz, H-2′), 7.36 (d, 1H, J=8.8 Hz, H-6), 7.22 (d, 1H, J=2.0 Hz, H-6′), 6.78 (d, 1H, J=8.8 Hz, H-5), 3.60 (br s, 2H, piperidine), 3.44 (br s, 2H, piperidine), 1.65 (br s, 4H, piperidine), 1.58 (br s, 2H, piperidine). IR (KBr) cm^{-1}: 3330, 1691, 1566, 1427, 1237, 1187. *Anal.* Calcd for $C_{21}H_{18}NO_6SBr\times0.5H_2O$: C, 50.30; H, 3.82; N, 2.80; S, 6.38. Found: C, 50.08; H, 3.96; N, 2.59; S, 6.10.

The first filtrate [mixture of chloroform (5 ml) and ethyl acetate (0.5 ml)] was evaporated and the residue was separated on silica gel column in chloroform–ethyl acetate 100 : 1 mixture. The first fraction (60 mg) was separated again on silica gel column in toluene–chloroform 3 : 1 mixture to give 2-(3′-bromo-4′,5′-dimethoxybenzylidene)-4-hydroxy-7-piperidinocarbonyloxy-2,3-dihydro-benzo[*b*]thiophen-3-one (**24**) as an orange solid (20 mg, 5%), mp190—192 °C. NMR (400 MHz, $CDCl_3$): δ 9.80 (s, 1H, OH), 7.78 (s, 1H, H-α), 7.52 (d, 1H, J=2.2 Hz, H-2′), 7.30 (d, 1H, J=9.0 Hz, H-6), 7.15 (d, 1H, J=2.2 Hz, H-6′), 6.75 (d, 1H, J=9.0 Hz, H-5), 3.95 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.67 (br s, 2H, piperidine), 3.54 (br s, 2H, piperidine), 1.65 (br s, 6H, piperidine). IR (KBr) cm^{-1}: 3425, 1720, 1575, 1489, 1427, 1223, 1146. *Anal.* Calcd for $C_{23}H_{22}NO_6SBr$: C, 53.07; H, 4.26; N, 2.69; S, 6.15. Found: C, 53.12; H, 4.36; N, 2.40; S, 6.09.

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