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Synthesis and Anti-inflammatory Activity of 7-Methyl-4-hydroxybenzothiphene-6-carboxylic acid and 7-Methyl-4-(6-carboxy)benzothiophenoxyacetic acid

MAHESH ATTIMARAD
Dept. of Pharmaceutical Chemistry, Al-Ameen College of Pharmacy, Bangalore - 560 027

7-Methyl-4-hydroxybenzothiophene-6-carboxylic acid and 7-methyl- 4-(6-carboxy)benzothiophenoxyacetic acid were synthesied via Stobbe condensation and were found to possess anti-inflammatory and analgesic activities.

ANTI-INFLAMMATORY activity has been observed in benzothiophenes[1], compounds with a glycolic acid moiety[2] and with two acidic sities[3]. Therefore, it appeared interesting to synthesise 7-methyl-4- hydroxybenzothiophene-6-carboxylic acid(3) and 7-methyl-4-(6- carboxy) benzothiophenoxyacetic acid (4) possessing a benzothiophene ring and acidic sites; and to evaluate their anti- inflammatory activity.

The condensation of acetylthiphene with dimethyl succinate in the presence of potassium tert butoxide gave predominantly E-3- methoxycarbonyl-4-(2-thienyl) pent-3-enoic acid. This configuration was revealed by its cyclisation with sodium acetate in acetic anhydride to the corresponding benzothiophene (2), which was hydrolysed to the 7-methyl-4-hydroxybenzothiophene-6- carboxylic acid(3). This was extended to the preparation of the benzothiophenoxyacetic acid (4). From the intermediate methyl 7- methyl-4-acetoxy benzothiophene-6-carboxylate (2), the hydroxamic acid (6) and carboxyhydrazide (7) were also prepared.

EXPERIMENTAL

E-3-Methoxycarbonyl-4-(2-thienyl) pent-3-enoic acid (1)

A mixture of acetyl-2-thiophene (12.6 g, 10.8 ml, 0.1 mole) and dimethyl succinate (14.6 g, 13 ml, 0.1 mole) in dry tert. butanol (15 ml) was added gradually during 1 h to a solution of potassium tert. butoxide [from potassium (4.4 g, 0.11 mole) and tert. butanol (75 ml)] at 0-5°. The mixture was stirred at room temperature for 4 h. Then it was acidified with 4 N HCl (to congo red), 50-70 ml of distilled water was added and tert. butanol was distilled under reduced pressure. A reddish oil separated on cooling and this was extracted with ether. The acidic portion was extracted into sodium bicarbonate solution from the ether phase. The sodium bicarbonate layer was acidified with 4N HCl and the orange to red viscous oil was re-extracted into ether. The ether was removed by distillation and acid-ester(1) separated out, yield : 16.3 g (68%), eq. wt-240.1 (Calc. 240.3). Found : S, 13.10 $C_{11}H_{12}O_4S$ requires S, 13.34%.

Methyl 7-methyl-4-acetoxybenzothiophene-6-carboxylate (2)

The above acid ester (1) (6 g) was added to a mixture of anhydrous sodium acetate (3 g) and acetic anhydride (30 ml) and left overnight at room temperature with occasional shaking; the temperature was then gradually raised to 70- 80° during 2 h and maintained there for a further 4 h, then poured into water, and the oily material (5 g) obtained was crystallized from light petroleum (b.p. 60-80°) to given the acetoxy ester (2) as yellow needles yield: 5 g

(76%) m.p. 92°. Found: C, 58.82; H, 4.30; S, 12.18. $C_{13}H_{12}O_4$ S requires C, 59.08; H, 4.58 S, 12.13%.

IR (KBr) cm^{-1} : 3100 (w, Ar-H); 1750 (s, $OCOCH_3$); 1720 (s, $CO.OCH_3$); 1600 (m, Ar C=C); 1230-1190 (s, O-C=O). PMR ($CDCl_3$) δ : 2.40, s, 3H, $O-COCH_3$; 2.85, s, 3H, $Ar-CH_3$; 3.9, s, 3H, $COOCH_3$; 7.23, d, 1H, Ar-H (C_3); 7.56, d, 1H, Ar-H(C_2); 7.70, s, 1H, Ar- H(C_5).

Mass spectra: 264, (M^+ peak) other peaks were located at 222 (Base peak), 190, 163, 162, 134, 89 and 43.

7-Methyl-4-hydroxybenzothiophene-6-carboxylic acid (3)

The acetoxy ester (2) (3.2 g) was added to a solution of potassium hydroxide (10 g) in 20 ml water and 80 ml ethanol. It was refluxed for 2 h and cooled. Acidification precipitated the hydroxy acid which was filtered dried and recrystallized from acetone-benzene; yield: 1.9 g (71%), m.p.: 233-234°. Found : C, 57.51 H, 3.80; S, 15.5 $C_{10}H_8O_3S$. requires C, 57.68; H, 3.87; S, 15.40%.

IR (KBr) cm^{-1} : 3329 (m, O-H); 1670 (m, Ar-C=O); 1597 (m, Ar C=C). PMR ($CDCl_3$) δ : 2.65, s, 3H, Ar- CH_3: 7.28, s, 1H, Ar-H(C_5); 7.53, d, J: 5.59 cps; 1H, Ar-H(C_2); 7.79, d, J : 5.44 cps; 1H, Ar-H(C_3) 10.05, s, 1H, phenolic OH; 12.74, s, 1H, COOH.

^{13}C NMR : Found : C_2, 128.096; C_3, 121.544; C_{3a}, 137.556 C_4, 150.472; C_5, 110.561; C_6 126.481, C_7, 124.156; C_{7a}, 142.932; C_8, 169.822; C_9, 18.832.

7-Methyl-4-(6-carboxy) benzothiophenoxyacetic acid (4)

To a solution of hydroxyacid (3) (1g) and 33% NaOH solution (3.5 ml), a 50% aq. chloroacetic acid solution (2.5 ml) was added[4]. The test tube was stoppered loosely and warmed gently on a boiling water bath for 1 h. After cooling it was acidified with HCl and the precipitated aryloxyacetic acid (4) was filtered, dried and recrystallized from aq. ethanol yield: 0.65 g, (71%) m.p. : 276°. eq wt : 125.8 (Calc. for $C_{12}H_{10}O_5S$ 126.1). Found : C, 54.60; H 3.86; S, 15.28, $C_{12}H_{10}O_5S$ requires C, 54.14; H, 3.78; S, 12.04%.

IR (KBr) cm^{-1} 3090-2900 (br, C00-H); 1670 (s, C=O); 1620 (w) and 1550 (m, Ar C=C).

Methyl 7-methyl-4-hydroxybenzothiophene-6-carboxylate (5)

Methyl 7-methyl-4-hydroxybenzothiophene-6-carboxylate (5) was prepared by esterifying the acid (3) with diazomethane; yield: 90%, m.p : 167-68°. Found : S, 14.30 $C_{11}H_{10}O_3S$ requires S, 14.42%.

IR (KBr) cm^{-1} : 3290 (s, O-H); 1675 (s, C=O); 1590 (ArC=C). PMR ($CDCl_3$ + DMSO) δ : 2.65, s, 3H, Ar- CH_3; 3.29, s, 3H, $COOCH_3$; 7.23, s, 1H, Ar-H(C_5); 7.35-7.47, d, 2H, Ar-H(C_2 & C_3); 9.12 s, 1H, phenolic OH.

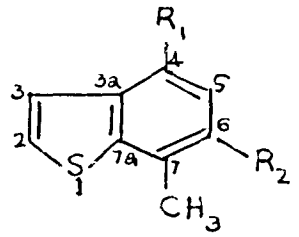
Mass spectra : 222 (M^+ peak and base peak) other peaks where located at 207, 191, 190, 163, 162, 134 and 45.

The same compound was also obtained by mild hydrolysis of acetoxy ester (2) with 10% Na_2CO_3 solution.

7-Methyl-4-hydroxybenzothiophene-6-hydroxamic acid (6)

Hydroxylamine hydrochloride (2 g) and sodium acetate trihydrate (4 g) were dissolved in distilled water and to this solution acetoxy ester 2 (1g) was added. Ethanol was added dropwise to get a clear solution. The mixture was stirred with warming for 10 min on a water bath and then cooled in ice[5]. The precipitated solid was filtered by suction and recrystallized from dilute ethanol; yield 1.4 g (82%) m.p.: 173-174°. Found : C, 53.60; H, 4.22; S, 14.28; N, 6.18. $C_{10}H_9O_3NS$ requires C, 53.82; H, 4.06; S, 14.36; N, 6.27%.

Table showing melting points, yields and pharmacological activity of compounds



Compound No.	Substitution		M.P. (°C)	% yield	Mean Vol of paw oedema ± SEM	% Anti-inflmatory activity	Mean No of WE±SEM	Percent Analgesic activity
	R^1	R^2						
2..	$OCOCH_3$	$COOCH_3$	92	76	0.42 ± 0.04	39.20*	37.50 ± 1.40	28.2
3.	OH	COOH	233-34	71	0.35 ± 0.06	49.30*	33.25 ± 2.78	36.4*
4.	OCH_2COOH	COOH	276	71	0.32 ± 0.03	53.70*	34.00 ± 2.17	34.9*
5.	OH	$COOCH_3$	167-68	90	0.41 ± 0.05	40.30*	NS	
6.	OH	CONHOH	173-74	82	0.39 ± 0.05	43.50*	35.60 ± 2.18	31.9*
7.	OH	$CONHNH_2$	256-58	72	0.40 ± 0.07	42.10*	NS	
Control					0.69 ± 0.05	–	52.20 ± 5.02	–
Ibuprofen					0.20 ± 0.03	68.20**	–	–
Paracetamol					–	–	29.10 ± 3.76	44.3*

Route : Oral, Dose : 100 mg/kg body weight, NS: Not Screened, SEM : Standard Error of Mean, WE: Writhing Episodes
* Significant ($p<0.05$, observed $p<0.01$ - $p<0.02$) ** Highly significant ($p<0.001$).

IR (KBr) cm^{-1} : 3350(m, N-H, O-H); 3100 (w, Ar-H); 1660 (s, C=O); 1510 (m, Ar C=C).

7-Methyl-4-hydroxybenzothiophene-6-carboxyhydrazide (7)

To hydrazine hydrate (1ml), acetoxy ester (2) (1 g) was added in small portions and the mixture was heated gently under reflux for 10 min. A small quantity of absolute alcohol was added and refluxed for 2 h. Alcohol was distilled off and the residual carboxyhydrazide was crystallised from ethanol, yield 0.6 g (72%); m.p.: 256-258°. Found : N, 12.68 $C_{10}H_{10}O_2N_2S$ requires N, 12.60.

IR (KBr) cm^{-1} : 3290 (w, O-H); 3240 (m, NH2); 1620 (s, CO- NH).

Pharmacological Studies

The suspensions of the test compounds were prepared in 10% v/v tween 80 solution. In all the cases the control received the same quantity of tween 80 solution. The standard error and the statistical significance (t-test) were computed by usual method.

Anti-inflammatory Activity was evaluated by carrageenin-induced rat hind paw oedema method[6].

Albino rats of either sex weighing between 150-200 g were distributed into control, standard and test (6-animals each) groups. At zero hour, the test compounds and ibuprofen were administered orally at the dose of 100 mg/kg body weight. After 30 min of this treatment an inflammatory oedema was

induced in the hind paw by injection of 0.05 ml of 1% carrageenin into the plantar tissue of the paw. The initial volume of the paw was measured plethysmographically within 30 sec of this injection. The relative increase in the paw volumes were found by measuring the paw volumes after 4 h of the carrageenin injection. The percentage inhibition of the inflammation was calculated by the formula, % inhibition = (1-Vt/Vc) 100, where Vt and Vc are the mean relative changes in the paw volume in test and control respectively.

Analgesic Activity : Acetic acid-induced writhing method[7] was adopted for evaluation of analgesic activity. Albino mice of either sex weighing between 20-25 g were randomly distributed in groups each containing six animals. The test compounds and paracetamol were administered orally at the dose of 100 mg/kg body weight. After 30 min of this administration, writhing was induced by intraperitoneal injection of 0.5% acetic acid in the volume of 0.1 ml/10 g body weight and the writhing episodes were recorded for 20 min. The percentage protection against writhing episodes was calculated by the following formula, % protection = (1-Wt/Wc) 100, were Wt and Wc are the means of the writhing episodes in test and control respectively.

RESULTS AND DISCUSSION

The melting points, yields and pharmacological activities of compounds have been tabulated (Table). The IR spectra had an absence of the acetoxy group absorbance at 1755 cm^{1} in hydroxamic acid (6) and carboxhydrazide (7) indicating that the acetoxy group of acetoxy ester (2) hydrolysed to phenolic group in the reaction.

In the PMR spectra it was observed that proton of C_5 having a chemical shift at 7.70 δ in acetoxy ester (2) gets shifted up field to 7.2-7.35 δ in case of hydroxy acid (3) and hydroxy ester (5) evidently due to the electronic neighborhood of phenol/acid function.

The compounds of this type do show moderately strong anti-inflammatory and analgestic activity compared to ibuprofen and paracetamol.

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REFERENCES

1. Guerrera, F., Salerno, L., Sirawsa, M.A., Ransisuaue, G. and Felica, A., **Formaco.,** 1990, 45, 29.
2. Stefan, A.L. Jan, A., Gerd, D.P. and Werner, K., **J. Med. Chem.**, 1994, 37, 1897.
3. Yong, R.N., **Adv. Prostaglandin, Thromboxane, Leukotriene, Res.**, 1989, 19, 643.
4. Harwood, L.M. and Moody, C.J. **Expt. Organic Chemistry**, 2nd Ed. Principles and Practice Blackwell scientific publication, London, 1992, 260.
5. Harwood, L.M. and Moody, **C.J. Expt. Organic Chemistry.**, 2nd Ed. Principles and Practice , Black well Scientific Publication, London, 1992, 260.
6. Turner, A.R., **Screening Methods in Pharmacology**, Academic Press, London and New York, 1965, 152.
7. Prem, P.S., Ajit Y.S., Seshagiri Rao, Ravindra, K.V. and Dilbag, R.S., **Expt. Clin. Pharmacol.**, 1985, 5, 601.