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Synthesis and 5-HT_{2A} Antagonist Activity of some 7-[3-(Substituted Amino) Propoxyl]-4-Methyl Chromen-2-Ones

M. V. L. APARNA, N. SATI, V. S. VEER, S. H. BHOSALE*, M. S. BHOSALE, S. L. BODHANKAR, K. R. MAHADIK AND S. S. KADAM
Department of Pharmaceutical Chemistry, Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune-411 038

Resorcinol on reaction with ethylacetoacetate in the presence of concentrated sulphuric acid afforded 7-hydroxy-4-methyl chromen-2-one (1), which when refluxed with 1-bromo-3-chloropropane in acetonitrile and in the presence of anhydrous potassium carbonate yielded 7-(3-chloropropoxyl)-4-methyl chromen-2-one (2). Substitution of chlorine from (2) with secondary amines in toluene and in the presence of triethylamine resulted in the title compounds (3a-e) in 50-60 % yield. Their chemical structures have been assigned by elemental analysis , IR ,and ^{1}HNMR analyses. The compounds were screened for 5-HT_{2A} antagonist activity and spontaneous motor activity. All title compounds have been found to show significant 5-HT_{2A} antagonist activity and caused reduction in spontaneous motor activity.

Since the discovery of the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) in 1948, the knowledge about its role in pathophysiological processes, both peripherally and centrally is steadily growing . The actions of 5-HT are mediated by a number of specific receptors, which have been classified into 7 classes (5-HT_{1-7}) including 15 different subtypes[1-4]. The 5-HT_2 receptor is a member of G-protein coupled receptor (GPCR) superfamily and it is positively linked to adenylcyclase in the central nervous system (CNS)[5]. Centrally acting 5-HT_2 antagonists have shown promising effects in animal models for anxiety[6] and depression[7], as well as in certain drug abuse models[8,9]. Clinical investigations with 5-HT_2 antagonist ritanserin have demonstrated efficacy in anxiety[10], dysthymic disorders[11] and improvement of sleep quality in dysthymic patients[12]. In schizophrenic patients, improvements of negative symptoms[13] and extra-pyramidal symptoms[14] have also been demonstrated. A prophylactic effect of 5-HT_2 antagonist against migraine[15] has also been hypothesized, however clinical evidences are lacking.

Some novel aminocoumarins[16] and many piperazines bearing coumarin fragments[17] have been reported to possess 5-HT_{2A} affinity. These observations lead us to the conception that a new series of 7-[3-(substituted amino)-propoxyl]-4-methylchromene-2-one would posses 5-HT_2 antagonist activity. In the present study our aim was to investigate the possibilities of further exploring the selectivity for central 5-HT_2 receptors within chromen-2-one derivatives with secondary amino groups .The inhibition of 5-hydroxytryptophan (5HTP)-induced head twitches is considered as 5-HT_{2A} antagonist activity[17], consequently, in this paper, we report the synthesis and pharmacological screening of 7-[substituted amino propoxyl]-4-methyl chromen-2-ones for their 5-HT_{2A} antagonist activity.

The reaction sequence leading to the formation of different title compounds is outlined in Scheme 1. The 7-hydroxy-4-methyl chromen-2-one (1) was synthesized using the method of Pechmann and Duisberg[18]. Intermediate (1) when refluxed with 1-bromo-3-chloropropane in acetonitrile and in presence of K_2CO_3 afforded 7-(3-chloropropoxyl)-4-methylchromen-2-one (2). Substitution of chlorine from (2) with secondary amines in toluene, in presence of triethylamine resulted in the title compounds (3a-e) in 50-60 % yield. The synthesized compounds were

*For correspondence
E-mail: drshbhosale4143@rediffmail.com

screened for 5-HT_{2A} antagonism activity (antagonism of L-5-HTP induced head twitches) and spontaneous motor activity (using an actophotometer).

MATERIALS AND METHODS

Melting points were determined by open capillary method on Campbel electronic apparatus and are uncorrected. The purity of the synthesized compounds was checked by TLC using precoated silica G_{254} plates and visualized in iodine and also by UV light. The IR spectra of synthesized compounds were recorded on a Jasco-V-5300 FTIR in potassium bromide discs. The ^1H NMR were recorded on a 300 MHz Jeol spectrophotometer in $CDCl_3$ and using tetramethylsilane as internal standard.

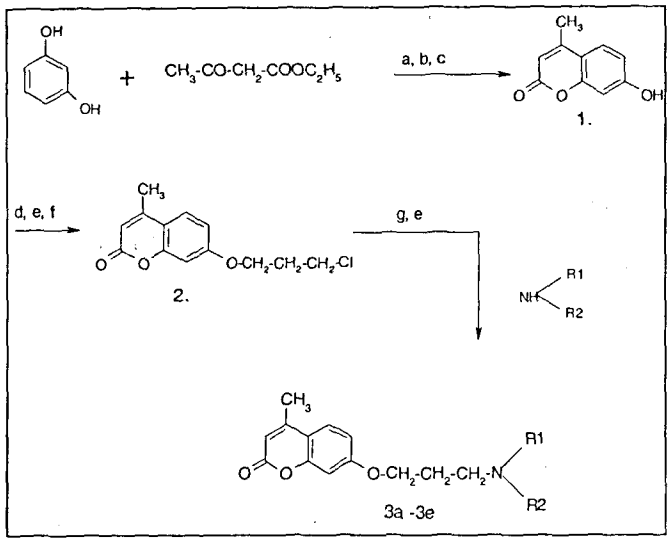
Haloperidol (Serenace) was procured from RPG Life Sciences, Mumbai. (-) 5-Hydroxytryptophan (Sigma) was dissolved in water for injection (WFI) procured from Core Healthcare Ltd. Carbidopa was added to WFI, which was solubilised using dilute hydrochloric acid. All the drugs were orally administered as a suspension in 3 % gum acacia. Only for 5-HTP, the route was intravenous. Swiss mice were procured from National Toxicological Center (Pune). The animals were housed in plastic cages under controlled experimental conditions (temperature 23±2°, humidity 50±5 %). The animals were fed with standard food pellet (Chakkan Oil-mills, Pune). Food and water were made freely available. Institutional Animal Ethics Committee, Poona College of Pharmacy, approved all the animal experiments. Only male mice weighing in the range of 20-25 g were selected for the purpose of experiment. Evaluation of spontaneous motor activity was done on actophotometer (Hiscom instruments). The dose selected was 25 mg/kg as for the lower dose tested , statistically nonsignificant results were obtained .

7-Hydroxy-4-methyl chromen-2-one (1):

The method of Pechmann and Duisberg[19] was followed for the preparation of 7-hydroxy-4-methylchromen-2-one. IR (KBr) cm^{-1}: 3500(-OH), 2957 (aromatic-C-H), 1680(C=O), 1601-1452 (C=C), 1336-1159 (-C-CO-O), 1215 (-C-O phenol) and 746 (C-H out of plane), ^1H NMR (DMSO-d_6) δ: 10.5 (b, 1H, -OH), 7.51-7.53 (d, 1H, C_5-H), 6.6-6.9 (m, 2H, C_6-H and C_8-H), 6.06 (s, 1H, C_3-H), 2. 29 (s, 3H, C_4-$C\underline{H}_3$).

7-(3-(Chloropropoxyl)-4-methyl chromen-2-one (2):

A mixture of (1) (3 g, 0.017 mol), 1-bromo-3-chloropropane (3.5 g, 0.025 mol) and anhydrous K_2CO_3 (3.5 g, 0.025 mol) in 25 ml acetonitrile was refluxed for 24 h.



Scheme 1: Synthesis of chromene-2-ones

a. Conc.H_2SO_4, b. 5 % NaOH, c.2MH_2SO_4, d. CH_3CN, e. K_2CO_3, f. 1- bromo-3- chloropropane, g. toluene, e. triethylamine

The solvent was removed under vacuum. The residue was dissolved in dichloromethane and repeatedly washed with 5% NaOH solution to remove the traces of unreacted (1). Dichloromethane layer was further washed with water ,dried over anhydrous sodium sulphate and the solvent was removed under vacuum to afford (2). The residue was recrystallized from ethanol. Yield: 73 % (3.15 g), mp: 81-82°, R_f-0.75 (benzene:ethyl acetate 4: 1). IR (KBr) cm^{-1}: 3058-2879 (C-H), 1732 (C=O), 1618-1431 (C=C), 1386 (-C-O), 1294 (-CO-O-C), 869 (-C-H out of plane): ^1H NMR ($CDCl_3$) δ: 7.49 (d ; 1H, C_5-H), 6.83-6.88 (m, 2H, C_6-H and C_8-H), 6.14 (s, 1H, C_3-H), 4.10-4.2 (m, 2H, O-$C\underline{H}_2$), 3.7-3.8 (m, 2H, O-CH_2CH_2-$C\underline{H}_2$), 2.4 (s ,3H,C_4-CH_3), 2.2-2.3 (q, 2H, O-$CH_2C\underline{H}_2$-CH_2).

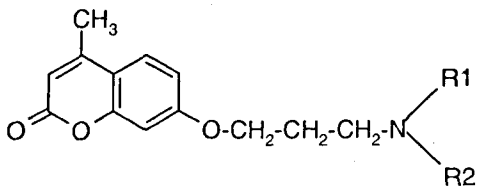
General method of synthesis of derivatives (3a-e):

A solution of (0.005 mol) 7-[3-chloropropoxyl]-4-methylchromen-2-one (2) and 0.01 mol of various amines in 50 ml toluene, in the presence of triethylamine, was refluxed for 15 h. After cooling the reaction mixture was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness under vacuum. The residue on recrystalization with ethanol afforded various 7-[3-(substituted amino) propoxyl]-4-methyl chromen-2-ones (Table 1).

7-[3-(1-Piperidinyl) propoxyl]-4-methylchromen-2-one (3a):

Yield: 50 %, mp: 100-101°, IR (KBr) cm^{-1} 2928 (-C-H),

TABLE 1: PHYSICOCHEMICAL CHARACTERIZATION OF SYNTHESIZED COMPOUNDS



Comp.	$R_1(NH)R_2$	Molecular formula	Yield %	mp	R_f*
3a	Piperidine	$C_{18}H_{23}NO_3$	50	100-101	0.35
3b	Morpholine	$C_{17}H_{21}NO_4$	60	82-83	0.30
3c	Diethylamine	$C_{17}H_{23}NO_3$	50	98-99	0.42
3d	Dibutylamine	$C_{21}H_{31}NO_3$	45	154-155	0.40
3e	Pyrrolidine	$C_{17}H_{21}O_3$	43	86-87	0.74

*Mobile phase: benzene:ethyl acetate (4: 1)

1726 (C=O), 1610 (C=C), 1390 and 1304 (-C-N), 1370 - 1250 (-CO-O-C), 1150 - 1070 (C-O-C). ^{1}H NMR ($CDCl_3$) δ: 7.4 -7.48 (d, 1H, C_5-H), 6.8 (m, 2H, C_6-H and C_8-H), 6.12 (s, 1H, C_3-H), 4-4.1 (t, 2H, -O-C$\underline{H}_2$), 2.4 -2.6 [{m, 6H, -C$\underline{H}_2$-N<, -N-2(C$\underline{H}_2$)}], 2.36 (s, 3H, C_4-C$\underline{H}_3$), 1.97-2.14 (q, 2H, -O-CH_2–C$\underline{H}_2$-CH_2), 1.6 (m, 4H, C_3-H_2 and C_5-H_2 in piperdine), 1.4 (b, 2H, C_4-H_2 in piperdine).

7-[3-(1-Morphonyl) propoxyl]-4-methyl chromen-2-one (3b):

Yield: 60 %, mp: 82-83°, IR (KBr) cm^{-1}: 2967 and 2802 (-C-H), 1716 (C=O), 1620 (C=C), 1394 (-C-N), 1267 (CO-O-C), 1145 and 1072 (C-O-C). ^{1}H NMR (δ ppm, $CDCl_3$) δ: 7.4 (d, 1H, C_5 -H), 6.8-7.0 (m, 2H, C_6 -H and C_8 -H), 6.1 (s, 1H, C_3-H), 4.1 (t, 2H, -O-C$\underline{H}_2$), 3.6-3.8 (t, 4H, -C$\underline{H}_2$-O-C$\underline{H}_2$ in morpholine), 2.4-2.6 [{m, 6H, -C$\underline{H}_2$-N, -N<2(C$\underline{H}_2$)}], 2.37 (s, 3H, C_4-C$\underline{H}_3$), 1.9-2.0(q, 2H,-O-CH_2–C$\underline{H}_2$-CH_2).

7-[3-(1-diethylamino) propoxyl]-4-methyl chromen-2-one (3c):

Yield: 50 %, mp: 98-99°, IR (KBr) cm^{-1}: 2957 (-C-H), 1716 (C=O), 1620 (C=C), 1394 (-C-N), 1346 and 1280 (CO-O-C), 1145 and 1072 (-C-O-C). ^{1}H NMR ($CDCl_3$) δ: 7.49 (d, 1H, C_5 -H), 6.8-6.9 (m, 2H, C_6-H and C_8-H), 6.1 (s, 1H, C_3H), 4.0 (t, 2H, -O-C$\underline{H}_2$), 2.6-2.8 (m, 6H, -C$\underline{H}_2$-CH_3 in diethylamino and –C$\underline{H}_2$-N<), 2.39 (s, 3H, C_4-C$\underline{H}_3$), 1.98-2.0 (q, 2H, -O-CH_2–C$\underline{H}_2$-CH_2), 1.00 [{t, 6H, 2 (-CH_2-C$\underline{H}_3$) of diethyl amino}]

7-[3-(1-dibutylamino) propoxyl]-4-methyl chromen-2-one (3d):

Yield: 45 %, mp: 154-155°, IR (KBr) cm^{-1}: 2957 and 2798 (-C-H), 1716 (C=O), 1620 (C=C), 1390 and 1304 (-C-N), 1346 and 1280 (CO-O-C), 1150 and 1020 (-C-O-C), ^{1}H NMR ($CDCl_3$) δ: 7.49 (d, 1H, C_5-H), 6.8-6.9 (m, 2H, C_6-H and C_8-H), 6.1 (s, 1H, C_3-H), 4.0 (t, 2H, -O-C$\underline{H}_2$), 2.6[{m, 6H, -C$\underline{H}_2$-N<2 (C$\underline{H}_2$)}], 2.38 (s, 3H, C_4 -C$\underline{H}_3$), 1.9 (q, 2H, -O-CH_2–C$\underline{H}_2$-CH_2), 1.4 (m, 4H, 2(-CH_2-C$\underline{H}_2$-CH_2-CH_3), 1.2 [{m, 4H, 2 (-CH_2-CH_2-C$\underline{H}_2$-CH_3)}], 0.92 (t, 6H, 2(-CH_2-CH_2-CH_2-C$\underline{H}_3$).

7-[3-(1-Pyrolidinyl) propoxyl]-4-methyl chromen-2-one (3e):

Yield: 50 %, mp: 86-87°. IR (KBr) cm^{-1}: 2952 (C-H), 1724 (C=O), 1610 (C=C), 1390 and 1304 (C-N), 1370 and 1250 (CO-O-C), 1150 and 1070 (C-O-C). ^{1}H NMR ($CDCl_3$) δ: 7.5 (d, 1H, C_5 -H), 6.8-7.2 (m, 2H, C_6-H and C_8-H), 6.12 (s, 1H , C_3-H), 4.04-4.08 (t, 2H, -O-C$\underline{H}_2$), 2.42 [{m, 6H, -N<(C$\underline{H}_2$-CH_2-CH_2-C$\underline{H}_2$) of pyrrolidine and -O-CH_2–CH_2-C$\underline{H}_2$}], 2.42 (s, 1H, C_4-C$\underline{H}_3$), 1.97-2.14 [{m, 4H, -N<(CH_2-C$\underline{H}_2$-C$\underline{H}_2$-CH_2) of pyrrolidine}], 1.59-1.62 (q, 2H, -O-CH_2–C$\underline{H}_2$-CH_2).

Antagonism of 5-HTP- induced head twitches:

Groups of healthy mice (20-25 g) were used for screening. Twelve mice were used separately for testing the synthesized compounds. The twelve mice were divided into two groups each consisting of six animals. Each animal in the control group was administered WFI p.o. Thereafter they received L-5HT (50 mg/kg, i.P) 30 min past to carbidopa (25 mg/kg, i.P). Animals were observed for head twitches for 5 min after 15 min of L-5HTP administration. Observations were recorded at an interval of 15 min until seven readings were noted down. The second group was used for test compounds, wherein the compounds were adminis-

TABLE 2: INHIBITION OF 5-HTP- INDUCED HEAD TWITCHES*

Group	Time interval (min±SD)						
	15	30	45	60	75	90	105
Control	5.66 (±0.84)	7.83 (±0.32)	6.83 (±0.16)	6.52 (±0.31)	4.66 (±0.32)	3.33 (±0.18)	0.44 (±0.11)
3a	3.83 (± 0.75)	2.50 (±0.14)	1.83 (± 0.16)	1.22 (± 0.24)	1.00 (±0.14)	0.80 (±0.08)	0.33 (± 0.08)
3b	3.66 (±0.27)	2.66 (±0.27)	1.66 (±0.17)	1.33 (±0.15)	1.33 (±0.17)	1.33 (±0.17)	0.29 (±0.02)
3c	3.86 (±0.36)	2.33 (±0.19)	2.33 (±0.17)	2.33 (±0.18)	1.66 (±0.15)	1.66 (±0.12)	0.26 (±0.02)
3d	3.33 (±0.37)	3.00 (±0.28)	2.66 (±0.22)	2.17 (±0.17)	1.76 (±0.1)	1.33 (±1.0)	0.30 (±0.02)
3e	3.66 (±0.28)	3.33 (±0.25)	2.66 (±0.25)	2.33 (±0.17)	1.33 (±0.17)	1.33 (±0.11)	0..33 (±0.03)

*n=6, p<0.05, dose=25 mg/kg

TABLE 3: REDUCTION IN SPONTANEOUS MOTOR ACTIVITY*

Group	Time interval (min±SD)			
	30	60	90	120
Control	100.0±4.33	112.0±5.66	88.00±3.66	102.0±3.66
3a	67.67 ± 2.05	72.66 ± 3.22	77.67 ± 3.01	89.66±2.29
3b	74.33 ± 4.03	77.66 ± 2.73	80.60 ± 3.36	85.00±3.71
3c	88.00 ± 5.79	81.00 ± 3.34	83.00 ± 4.16	86.33±6.23
3d	57.33 ± 2.05	42.66 ± 3.30	61.33 ± 3.91	66.00±2.03
3e	75.30 ± 4.03	78.60 ± 3.57	81.66 ± 3.36	86.00±5.71

*n=6, p<0.05, dose=25 mg/kg

tered p.o. After 20 min of test drug administration (25 mg/kg, i.p), carbidopa (25 mg/kg, i.p) was administered. L-5HTP (50 mg/kg, i.p) was administered 30 min after carbidopa. Readings were recorded as in the control group. All results were statistically evaluated using Student's t- test.

Spontaneous motor activity:

The spontaneous motor activity was measured by means of an actophotometer. The mice were used for the test. The animals were divided into four groups each containing six animals; each animal acted as its own control in the group. These six animals were again divided into three groups each with two animals. At a given time, one pair was present in the actophotometer. The movements of the mice in control group were recorded at an interval of 30 min for a period of 2 h. Then the first test drug (25 mg/kg, i.p.) was administered to these four groups p.o. The drug was suspended in gum acacia 3 % as vehicle. The movements of mice were recorded in terms of count at an interval of 30 min for 2 h.

RESULTS AND DISCUSSION

The purity and homogeneity of all the title compounds were confirmed by their sharp melting points and TLC. In all the cases these chromen-2-ones were obtained in solid state and the yields varied from maximum 60 % to minimum 40 %. The synthesized compounds were subjected to physio-chemical characterization (Table 1). The C, H and N analytical data were found within ±0.4 % to the theoretical values.

The disappearance of O-H stretching band at 3500 cm^{-1} in the IR spectra and the appearance of signals at 2.2 -2.3 (2H), 3.7-3.8 (2H) and 4.1 -4.2 (2H) d ppm for side

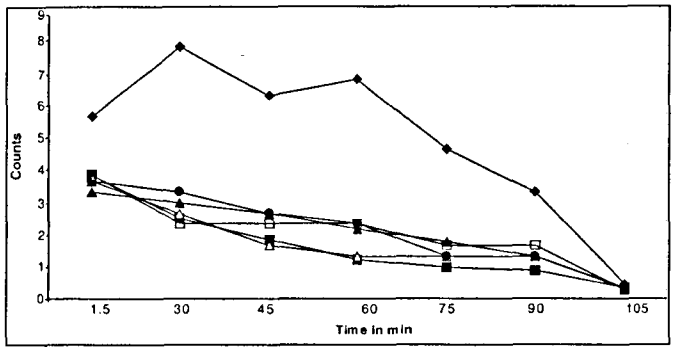


Fig. 1: Inhibition of 5-HTP-induced head twitches at the dose of 25 mg/kg

Mean score inhibition of 5-HTP-induced head twitches in the different time interval. Drugs were suspended in gum acacia 3% as a vehicle . Observation at an interval of 60 min. was taken as a measure of potency. Animals were administered p.o. with control (-♦-) and drug dose at 25mg/kg of 3a (-◘-), 3b (-△-), 3c (-□-), 3d (-▲-) and 3e (-●-)

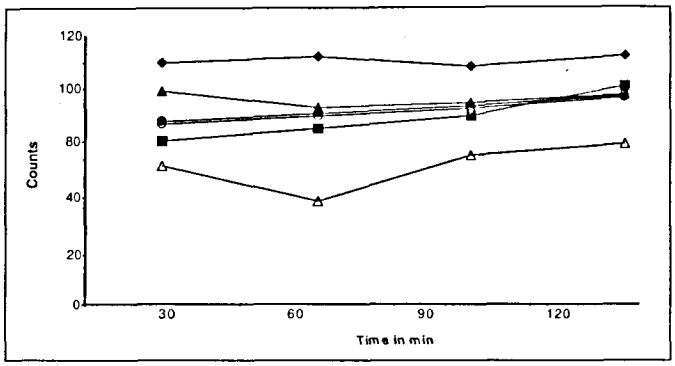


Fig. 2: Reduction in spontaneous motor activity at the dose of 25 mg/kg

Mean score reduction in the spontaneous motor activity in different time interval. animals were administered p.o. with control (-♦-), and the drug dose at 25 mg/kg of 3a (-◘-), 3b (-0-), 3c (-▲-), 3d (-△-) and 3e (-●-).

chain protons in the ^{1}H NMR spectrum of (2) confirmed its anticipated structure. Similarly structures of (3a–e) were confirmed on the basis of the comparison of their IR and ^{1}H NMR spectra with those of (1) and (2). As far as the biological property was concerned, the compounds were screened for inhibition of 5-HTP- induced head twitches, as a measure of antagonism at 5-HT_{2A} receptor, at the dose of 25 mg/kg (Table 2) as for the lower dose tested, statistically non significant result were obtained. From fig.1 it can be seen that for all the derivatives (3a- 3e) significant action starts after an interval of 30 min. Time to show the maximum action was 60 min for all the compounds (except for 3e where the time was 75 min). So observations after an interval of 60 min were taken as a measure of potency of synthesized compound for antagonism at 5–HT_{2A} receptor. The potency in decreasing order is 3a>3b>3d>3c≈3e. For studying the effect of synthesized compound on spontaneous motor activity, which is an index of wakefulness, the synthesized compounds were tested at a dose of 25 mg/kg (Table 3). From fig. 2, it can be seen that all the derivatives showed maximum reduction in motor activity after an interval of 60 min, as in the test of inhibition of 5-HTP-induced head twitches. The decreasing order of potency to cause reduction in spontaneous motor activity at a dose of 25 mg/kg is 3d>3c>3b≈3e>3a.

Thus, it can be concluded that all the synthesized compounds were potent antagonist of 5-HT_{2A} receptor. In the series of the synthesized compounds 3a was the most interesting member as it showed average propensity to cause reduction in spontaneous motor activity as well as it was the most potent antagonist of the 5-HT_{2A} receptor.

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