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Synthesis and evaluation of acetaminophen derivatives as analgesic, antipyretic and anti-inflammatory agents

[1]Ramesh Kumar*, [2]Sandeep Jain and [3]Neelam Jain

[1]Lord Shiva College of Pharmacy, Sirsa, Haryana, India
[2]Department of Pharmaceutical Science, Guru Jambheswar University of Science and Technology, Hisar
[3]Department of Education and Research, BPS MV, Khanpur Kalan, Sonepat

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ABSTRACT

In the present study, Dicarboxylic acid bis (4-acetylaminophenyl) ester (AP1-AP4) and (4-acetylaminophenoxy) acetic acid (AP-5) have been synthesized by the reaction of acetaminophen with dicarboxylic acid chlorides and chloroacetic acid, respectively. The esterification of (4-acetylaminophenoxy) acetic acid with different alcohols yielded rest of the compounds (AP6-AP13). The characterization of the compounds was done by IR, ^1H NMR and elemental analysis. All the synthesized compounds were screened in vivo for analgesic, antipyretic and anti-inflammatory activities. Appreciable activities were observed for many compounds.

Key words: Acetaminophen, analgesic, antipyretic, anti-inflammatory.

INTRODUCTION

Despite the large efforts expanded in past exploring the “coal tar analgesic” it was found that acetaminophen is the only derivative that has analgesic, antipyretic effect with little or no anti-inflammatory activity [1]. Lack of anti-inflammatory activity of acetaminophen provides a room to synthesize new analogues. Moreover, our early studies on aniline derivatives (acetaminophen is also an aniline derivative) have shown significant analgesic, antipyretic and anti-inflammatory activity [5], [7]. The already discovered aniline derivatives like acetanilide, acetaminophen, phenacetin, anisidine, phenitidine, pertonal etc., were also having powerful analgesic and antipyretic effect. But because of serious toxicities these are no longer in use except acetaminophen [1]. Therefore, authors decided to undertake the synthesis of carboxylic acid derivatives of acetaminophen for analgesic, antipyretic and anti-inflammatory activity.

MATERIALS AND METHODS

Melting points of all the synthesized compounds were determined using open capillary tube and were uncorrected. IR data were recorded using KBr disks on Perkin Elmer R-IX FTIR spectrophotometer and H^1 NMR spectra on Bruker Avance-II 400 spectometer. Elemental analyses were carried out on Carlo Erba 1106 CHN analyzer (Table 1). Dicarboxylic acid chlorides were obtained by reacting appropriate dicarboxylic acids with thionyl chloride in accordance with procedure reported in the literature [3].

Chemistry

Dicarboxylic acid bis- (4-acetylaminophenyl) ester:

To a solution of acetaminophen (0.02 Mol) in acetone, oxylyl chloride (0.01Mol) was added and refluxed for 2-3 hr. The compound Bis- (4-acetylaminophenyl) oxalic acid (AP-1) so prepared was filtered and recrystallised. Similarly, compound Bis- (4-acetylaminophenyl) malonic acid ester (AP-2), Bis- (4-acetylaminophenyl) succinic acid ester (AP-3), Bis- (4-acetylaminophenyl) glutaric acid (AP-4) were synthesized using appropriate dicarboxylic acid [3].

Bis- (4-acetylaminophenoxy) acetic acid:

To a solution of acetaminophen (0.025 Mol) in (0.112 Mol) sodium hydroxide, added 0.02M of chloroacetic acid. Loosely stoppered, round bottom flask (RBF) was heated on water bath for an hour. After cooling diluted the content with 10 ml of water and acidified with HCl. The content was now extracted with 30 ml ether. Washed the ethereal extract with 10 ml water, dry with magnesium sulphate. Distil off the ether on a rotatory evaporator. The compound Bis- (4-acetylaminophenoxy) acetic acid (AP-5) so prepared was collected and recrystallized [3].

Bis- (4-acetylaminophenoxy) acetic acid esters:

0.02 Mol of the synthesized compound Bis- (4-acetylaminophenoxy) acetic acid was taken and to this thionyl chloride (0.06 Mol) was added. The content was refluxed for 2-3 hr using guard tube charged with anhydrous calcium chloride. Excess of thionyl chloride was distilled off. The resultant acid chloride derivative was reacted with excess of different alcohols for 3-4 hours. The solid separated was filtered and recrystallized [6].

IR (KBr ν cm^{-1}) and ^{1}H NMR (DMSO, δ ppm)

Bis- (4-acetylaminophenyl) oxalic acid ester

IR (KBr), ν cm^{-1}: 3324.5 (N-substituted amide, N-H *str*), 3158.6 (Aromatic, C-H *str*), 1877.5-1851.9 (Ester, C=O *str*.), 1656.4-1441.8 (Aromatic, C=C *str*), 1370.0-1107.5 (Ester, C-O *str*), 857.0-603.4 (Aromatic, C-H *def*).

^{1}H NMR (ppm): 7.91 (2H, *s*, -NHCO-); 7.62-7.05 (8H, *d*, CH, Ar-H); 1.85 (6H, *s*, methyl).

Bis- (4-acetylaminophenyl)-malonic acid ester

IR (KBr), ν (cm^{-1}): 3367.3 (N-substituted amide, N-H *str*), 3059.1 (Aromatic, C-H *str*), 1668.9-1449.4 (Aromatic, C=C *str*), 1370.0-1347.0 (Methyl, C-H *def*), 1227.8-1195.1 (Ester, C-O str), 870.5-811.8 (Aroamtic, C-H *def*).

^{1}H NMR (ppm): 7.90 (2H, *s*, -NHCO-), 7.61-7.05 (8H, *d*, CH, Ar-H), 1.85 (6H, *s*, Alkyl), 3.15 (2H, *s*, -CH_2-COOR).

(4-Acetylaminophenoxy) acetic acid

IR (KBr), ν cm^{-1}: 3325.8 (Aromatic C-H *str*), 3160.8 (N-substituted amide, N-H *str*), 1655.8-1440.9 (Aromatic, C=C *str*), 1369.6-1325.8 and 1170.7-1013.8 (Alkyl aryl ether, C-O *str*), 1258.5-1226.0 (Saturated aliphatic acid, C-O *str* or O-H *def*), 856.1-807.6 (Aromatic, C-H *def*).

^{1}H NMR (ppm): 10.78 (1H, *s*, -COOH), 7.90 (1, *s*, -CONH or –COOH), 7.53-6.75 (4H, *d*, Ar-H), 4.67 (2H, *s*, -CH_2-COOR), 1.85 (3H, *s*, CH_3-CONH-).

(4-Acetylaminophenoxy) acetic acid methyl ester

IR (KBr), cm^{-1}: 3627.7 (N-substituted amide, N-H *str*), 3254.2 (Aromatic, C-H *str*), 1721.9 (esters, C=O *str*), 1611.2-1454.7 (Aromatic, C=C *str*), 1353.3 (Ethyl, C-H *def*), 1274.3-1055.9 (Alkyl aryl ether, C-O *str*), 962.1-602.1 (Aromatic, OOP, C-H *def*).

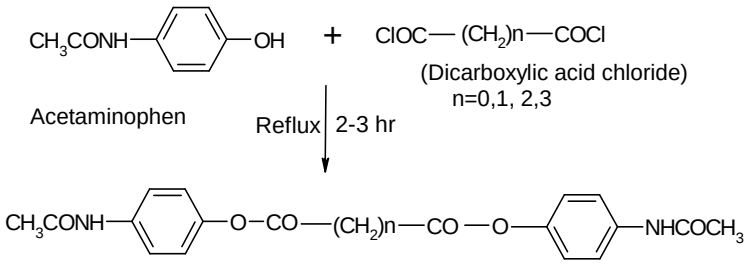
^{1}H NMR (ppm): 7.91 (1H, *s*, -CONH-), 7.53-6.75 (4H, *d*, Ar-H), 4.71 (2H, *s*, -CH_2-COOR), 3.36(3H, *s*, -$COOCH_3$), 1.85 (3H, *s*, CH_3-CONH-).

(4-Acetylaminophenoxy) acetic acid isopropyl ester

IR (KBr), cm^{-1}: 3627.7 (N-substituted amide, N-H *str*), 3254.2 (Aromatic, C-H *str*), 1721.9 (esters, C=O *str*), 1611.2-1454.7 (Aromatic, C=C *str*), 1353.3 (Ethyl, C-H *def*), 1274.3-1055.9 (Alkyl aryl ether, C-O *str*), 962.1-602.1 (Aromatic, OOP, C-H *def*).

^{1}H NMR (ppm): 7.94 (1H, s, -CONH-), 7.53-6.75 (4H, *d*, Ar-H), 4.90 (2H, *s*, -OCH_2-), 4.31(1H, *m*, CH-isopropyl), 2.02 (3H, *s*, -$COCH_3$), 1.35 (6H, *d*, CH_3- isopropyl)

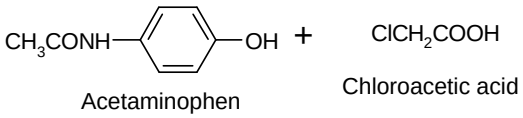
Scheme-1

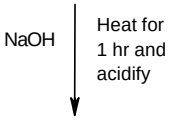


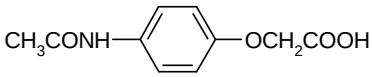
n=0,1, 2,3

AP1=(n=0) =Bis-(4-acetylaminophenyl) oxalic acid ester
AP2=(n=1) =Bis-(4-acetylaminophenyl) malonic acid ester
AP3=(n=2) =Bis-(4-acetylaminophenyl) succinic acid ester
AP4=(n=3) =Bis-(4-acetylaminophenyl) glutaric acid ester

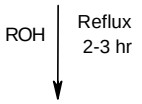
Scheme-2

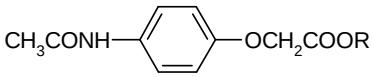






AP5= (4-Acetylaminophenoxy) acetic acid





AP6-AP13 = (4-Acetylaminophenoxy) acetic acid ester

Where, R is

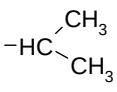
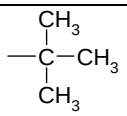
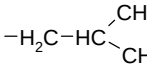
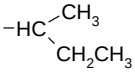
AP6	—CH_3	AP9		AP12	
AP7	—C_2H_5	AP10	—$CH_2CH_2CH_2CH_3$	AP13	
AP8	—$CH_2CH_2CH_3$	AP11			

Table 1: Characterization of the Synthesized Compounds (AP1-AP13)

Compounds	Yields (%)	Formula (M.W.)	Analyses C H N O (Calculated %) Found %
AP1	74	$C_{18}H_{16}N_2O_4$ 324.33	(66.66, 4.97, 8.64, 19.73) 66.58, 4.93, 8.61, 19.71
AP2	76	$C_{19}H_{18}N_2O_4$ 338.36	(67.44, 5.36, 8.28, 18.91) 66.46, 5.32, 8.24, 18.87
AP3	68	$C_{20}H_{20}N_2O_4$ 352.38	(68.17, 5.72, 7.95, 18.16) 68.13, 5.68, 7.91, 18.12
AP4	75	$C_{21}H_{23}N_2O_4$ 366.41	(68.84, 6.05, 7.65, 17.47) 68.80, 6.07, 7.63, 17.45
AP5	77	$C_{10}H_{11}NO_4$ 209.20	(57.41, 5.30, 6.70, 30.59) 57.39, 5.32, 6.71, 30.57
AP6	71	$C_{11}H_{13}NO_4$ 223.23	(59.19, 5.87, 6.27, 28.67) 59.21, 5.84, 6.24, 28.66
AP7	68	$C_{12}H_{15}NO_4$ 237.25	(60.75, 6.37, 5.90, 26.97) 60.74, 6.33, 5.89, 26.92
AP8	65	$C_{13}H_{17}NO_4$ 251.28	(62.14, 6.82, 5.57, 25.47) 62.17, 6.81, 5.55, 25.46
AP9	67	$C_{13}H_{17}NO_4$ 251.28	(62.14, 6.82, 5.57, 25.47) 62.15, 6.80, 5.54, 25.45
AP10	64	$C_{14}H_{19}NO_4$ 265.31	(63.38, 7.22, 5.28, 24.12) 63.35, 7.19, 5.25, 24.11
AP11	68	$C_{14}H_{19}NO_4$ 265.31	(63.38, 7.22, 5.28, 24.12) 63.35, 7.20, 5.26, 24.10
AP12	63	$C_{14}H_{19}NO_4$ 265.31	(63.38, 7.22, 5.28, 24.12) 63.33, 7.19, 5.21, 24.11
AP13	65	$C_{14}H_{19}NO_4$ 265.31	(63.38, 7.22, 5.28, 24.12) 63.37, 7.20, 5.26, 24.11

Pharmacology

Analgesic Activity

Analgesic activity was carried out by tail flick method [2], [8]. Healthy albino mice weighing 20-30 g were divided in to different group of six animals each. The control group received 0.5% w/v carboxymethylcellulose (CMC) solution; treated group was given, orally, a dose of 132 µmol/kg of the compound AP1-AP13**.** Reaction times were noted at 2 hr and 4 hr interval after the drug administration. The percentage analgesic activity was calculated by following formula:

Percentage analgesic activity= T_2-T_1/T_1×100

T_1=Normal reaction time
T_2=Reaction time after treatment.

Table: 2 Analgesic activity of the title Compounds

Compounds	Normal reaction time (sec)	Change in reaction time (sec) ± SEM		% Analgesic activity ± SD	
		2 h	4 h	2 h	4 h
Control	2.9±0.13	0.18±0.016	0.23±0.021	7.04±1.45	8.91±0.97
AP-1	2.20±0.08	2.4±0.03	2.05±0.04	109.10±0.37	93.17±2.5***
AP-2	2.35±0.10	2.95±0.04	2.25±0.042	125.57±1.14	95.73±0.19***
AP-3	2.55±0.10	3.0±0.03	2.36±0.03	115.09±2.0	92.86±2.72***
AP-4	2.53±0.08	2.95±0.042	2.38±0.04	116.44±1.44	94.07±2.16***
AP-5	2.33±0.08	2.95±0.04	2.25±0.04	126.0±1.58	96.40±1.76***
AP-6	2.51±0.07	2.93±0.03	2.36±0.03	116.57±1.74	94.04±2.13***
AP-7	2.43±0.12	3.05±0.05	2.28±0.03	125.0±1.74	93.90±1.98**
AP-8	2.48±0.07	3.55±0.09	2.35±0.07	143.04±1.63	94.66±1.85**
AP-9	2.11±0.06	2.56±0.07	1.96±0.04	121.25±1.99	93.04±3.43
AP-10	2.31±0.06	3.48±0.070	2.18±0.05	150.48±3.19	94.27±2.03**
AP-11	2.13±0.07	3.11±0.079	2.21±0.08	146.37±1.79	103.83±1.90
AP-12	3.2±0.07	3.15±0.12	1.98±0.07	151.16±2.44	95.16±0.47
AP-13	2.55±0.10	3.21±0.15	2.05±0.08	126.01±2.7	80.0±1.75*
Standard (Acetam-inophen)	2.45±0.10	3.10±0.05	2.5±0.03	129.94±1.95	103.4±1.54***

Note: Value of reaction time are mean ± SEM, n = 6. Statistical analysis was done by student's unpaired t-test [4].
** p < 0.05, **p < 0.01, ***p < 0.001, Dose 132 µm/kg*

Antipyretic Activity

Healthy Wistar rats weighting 150-200 gm were given s.c. 10 ml/kg of a 20% aqueous suspension of sterilised brewers yeast powder [2] [8]. After 18 hr, animals showing an increase in rectal temperature more than 0.5°C were selected. Control group received 0.5% w/v carboxymethylcellulose solution; treated group received a dose of 132 µmol/kg of compound AP1-AP13. Rectal temperatures were noted by digital thermometer 30 minute before (pre-treated) and at 1 hr, 2 hr and 4 hr after administration of the dose.

Table: 3 Antipyretic activity of the title Compounds

Compounds	Before drug (°C)		After drug (°C)		
	-18 h	0 h	1 h	2 h	4 h
Control	37.48±6.24	38.3±0.05	38.13±0.09	38.08±0.05	37.88±0.3
AP-1	37.49±0.05	38.45±0.04	38.13±0.03	37.78±0.05	37.54±0.02***
AP-2	37.36±0.05	38.37±0.05	38.12±0.04	37.71±0.05	37.31±0.04***
AP-3	37.57±0.03	38.44±0.04	38.29±0.03	38.09±0.04	37.41±0.04***
AP-4	37.35±0.07	38.31±0.05	37.93±0.06	37.68±0.05	37.29±0.05***
AP-5	37.21±0.04	38.92±0.07	37.65±0.06	37.48±0.05	37.14±0.03***
AP-6	37.44±0.04	38.41±0.03	38.27±0.04	38.09±0.04	37.78±0.04***
AP-7	37.35±0.03	38.22±0.03	37.92±0.04	37.62±0.04	37.47±0.04**
AP-8	37.33±0.05	38.30±0.05	38.03±0.05	37.74±0.04	37.42±0.03**
AP-9	37.40±0.05	38.31±0.06	38.18±0.03	37.94±0.05	37.61±0.042
AP-10	37.41±0.05	38.48±0.03	38.29±0.05	38.13±0.06	37.71±0.041**
AP-11	37.33±0.04	38.38±0.03	38.27±0.05	38.10±0.04	37.74±0.04
AP-12	37.28±0.04	38.01±0.54	37.81±0.045	37.51±0.06	37.41±0.03
AP-13	37.04±0.06	37.96±0.04	37.71±0.03	37.58±0.04	37.46±0.051*
Standard (Acetaminophen)	37.11±0.05	37.96±0.04	37.71±0.03	37.38±0.03	37.19±0.05***

Note: Value of reaction time are mean ± SEM, n = 6. Statistical analysis was done by student's unpaired t-test [4].
** p < 0.05, **p < 0.01, ***p < 0.001*
Dose 132 µmol/kg

Table: 4 Anti-inflammatory Activity of the title Compounds

Compounds	Change in reaction time (sec)±SEM		% Anti-inflammatory Activity±SD	
	2 h	4 h	2 h	4 h
Control	1.27±0.01	1.31±0.008		
AP-1	0.81±0.01	0.86±0.01	35.37 ± 2.21	36.41 ± 2.18**
AP-2	0.83±0.01	0.84±0.02	34.04 ± 2.91	36.47 ± 2.09**
AP-3	0.86±0.02	0.84±0.02	29.04 ± 3.01	31.58 ± 3.54**
AP-4	0.87±0.02	0.83±0.02	29.65 ± 3.42	33.22 ± 3.39**
AP-5	0.83±0.03	0.80±0.01	33.21 ± 2.81	37.82 ± 3.40***
AP-6	0.84±0.02	0.81±0.02	32.64±3.05	38.12±2.36**
AP-7	0.81±0.0	0.79±0.01	31.14±2.84	34.75±1.27*
AP-8	0.86±0.01	0.84±0.02	29.76±2.35	32.69±3.02**
AP-9	0.83±0.02	0.85±0.02	28.70±2.40	31.38±1.89
AP-10	0.81±0.00	0.83±0.01	30.27±3.51	35.60±1.88**
AP-11	0.79±0.01	0.82±0.02	34.36±3.42	34.27±2.91
AP-12	0.82±0.0	0.85±0.02	28.69±2.61	32.58±1.75
AP-13	0.81±0.1	0.84±0.02	30.60±3.24	34.46±1.97*
Standard (Acetaminophen)	0.82±0.01	0.83±0.0	28.77±3.04	31.84±1.33*

Note: Value of reaction time are mean ± SEM, n = 6. Statistical analysis was done by student's unpaired t-test [4].
** p < 0.05, **p < 0.01, ***p < 0.001*
Dose 132 µm/kg

Anti-inflammatory Activity

Anti-inflammatory activity was carried out using hind paw edema method on albino rat of either sex [2], [8]. A freshly prepared solution of carrageenan (0.1ml, 1%w/v) was injected in to the sub-plantar surface of the right hind limb of each animal. The control group received 0.5% w/v CMC solution; treated group was given, orally, a dose of 132 µmol/kg of the compound AP1-AP13, respectively 30 minute before carrageenan. The volume of each paw was measured by plethysmometer after 2 hr and 4 hr interval of carrageenan injection. The percentage inhibition of edema was calculated by formula:

Percentage inhibition of edema: V_C-V_T/V_C×100

V_C=Paw volume of control animal
V_T=Paw volume of treated animals (standard / test compound)

RESULTS AND DISCUSSION

The reaction of the acetaminophen with appropriate diacyl chlorides resulted in four compounds AP-1 to AP-4 as shown in scheme-I [3]. (4-Acetylaminophenoxy) acetic acid (AP-5) was synthesised by the treatment of acetaminophen with chloroacetic acid. Reaction of the compound AP 5 with different alcohols resulted in a series of (4-Acetylaminophenoxy) acetic acid esters derivatives i.e. compounds AP6-AP13 [6]. Examination of the analytical and spectral data of the synthesized compounds are in good agreement with calculated values based on proposed structures shown in scheme-I and scheme –II. The predicted structures of the synthesized compounds were confirmed by spectroscopic methods (IR, ^{1}H NMR**)**. In IR spectra N-H, C=O, C=C, C=N, N=O stretching were observed at expected frequencies. All the synthesized compounds were evaluated for the *in vivo* analgesic, antipyretic and anti-inflammatory activities. Acetaminophen was used as standard drug. Evaluation of the analgesic and antipyretic activity shows that compounds AP1-AP6 are having highly significant, compound AP7, AP8, AP10 moderate and compound AP13 little analgesic and antipyretic activity. Other compounds (AP9, AP11, and AP12) are having no significant analgesic and antipyretic activity (Table 2 and Table 3).

Result of the anti-inflammatory activity reveals that compound AP5 have highly significant activity while compounds AP1, AP2, AP3, AP4, AP6, AP8, and AP10 showed moderate activity. Other compounds were either having little or no activity (Table 4).

CONCLUSION

From above study it may be concluded that many synthesized compounds have shown analgesic and antipyretic activity similar to that of acetaminophen (compound AP1-AP6), while their anti-inflammatory activity (compound AP5) is more appreciable than the reference drug i.e. acetaminophen.

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