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Synthesis and Biological Evaluation of Some Novel Analogue of *p*-Hydroxyaniline

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Abstract

In the present study, eight new derivatives (R1 to R8) of *p*-hydroxyaniline were synthesized to investigate their analgesic, antipyretic and anti-inflammatory activities. *p*-Hydroxyaniline on reaction with appropriate dicarboxylic acid chloride (oxalyl chloride, malonyl chloride, succinyl chloride and glutaryl chloride) yielded four compounds (R1 to R4). Compounds R5 and R6 were synthesized by reacting *p*-hydroxyaniline with acid anhydride (succinic anhydride and glutaric anhydride). Cyclization of these compounds (R5 and R6) yielded compounds R7 and R 8, respectively. All the synthesized compounds were identified by IR, NMR and elemental analysis and spectral data of. The analgesic, antipyretic and anti-inflammatory activity were investigated *in-vivo* and appreciable activity were observed.

Keywords: p- hydroxyaniline, analgesic, antipyretic, anti-inflammatory.

Instruction

Perusal of literature on pharmacological studies are reported for aniline and its derivative such as acetanilide and acetaminophen analgesic and antipyretic activities with cytotoxicity and gastro toxicity as main side effect (Bessem *et al.* 1995, Sicardi *et al.* 1991, Singh and Hingorani 1990, Straat *et al.* 1987).

Significant amount of work has been reported on aniline derivatives in past and evaluated for activities but the amide derivative acetaminophen is found most effective as antipyretic and analgesic compounds with little anti-inflammatory activity (William 2006). Toxicity comparison of acetaminophen with acetanilide reveals that the former is less toxic than the later. Keeping this in view, new derivatives of *p*-hydroxyaniline were synthesized.

Material and Methods

Melting points of all the synthesized compounds were determined using open capillary tube and were uncorrected. IR data were recorded in KBr disks on Perkin Elmer R-IX FTIR

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Melting points of all the synthesized compounds were determined using open capillary tube and were uncorrected. IR data were recorded in KBr disks on Perkin Elmer R-IX FTIR spectrophotometer and H^1 NMR spectra on Bruker Avance-II 400 spectometer. Elemental analysis was done using Carlo Erba 1106 CHN analyzer. Dicarboxylic acid chlorides were obtained by reacting appropriate acids with thionyl chloride in accordance with procedure as reported in the literature (Furniss *et al.* 1998).

Chemistry

N, N-Bis- (4-hydroxyphenyl)- oxalamide (R1-R4)

To a solution of *p*- hydroxyaniline (0.02 Mol) in acetone, dicarboxylic acid chloride (0.01 Mol) was added and shook vigorously for 30 minute. The amide (R 1) so precipitated was filtered and recrystallised. Similarly, *N, N*-Bis (4-hydroxyphenyl)-malonamide (R2); *N, N*-Bis (4-hydroxy phenyl)-succinimide (R3); and Bis- (4-hydroxyphenyl)-pantanediamide (R4) were synthesized (Mann and Saunder 2003).

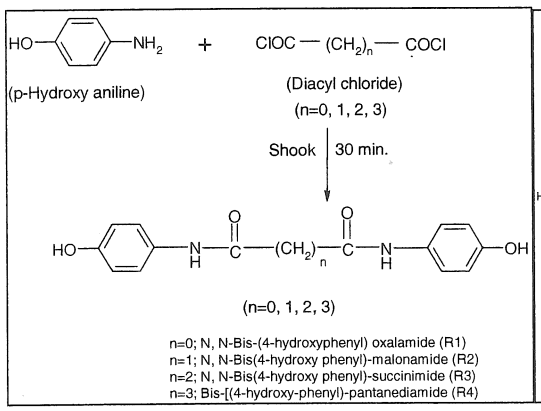
N- (4-Hydroxyphenyl)-succinamic acid (R5 and R6)

To a solution of succinic anhydride (0.01 Mol) in 30 ml benzene was added all at once the boiling solution of *p*-hydroxy aniline in about 5 ml benzene. The white crystalline solid (R5) thus obtained was crystallized with 10ml of benzene. Similarly, 4- (4-hydroxy-phenylcarbamoyl)-butyric acid (R6) was synthesized by reacting glutaric anhydride with *p*-hydroxy aniline (Vogel 2000).

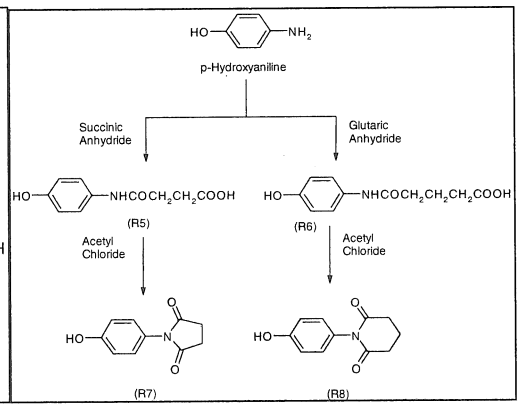
1-(4-hydroxyphenyl) pyrrolidine-2, 6-dione (R7 and R8)

A mixture of 0.01mole of compound R5 and 0.09 mole of redistilled acetyl chloride was heated on water bath for 10 minute in a round bottom flask provided with loose cotton plug. The mixture was cooled in ice. The white crystalline solid (R7) thus obtained was filtered off, washed with little ether and dried in an oven. Similarly, 4-(4-hydroxyphenyl) piperidine-2, 6-dione (R8) was synthesized from 4-(4-hydroxy-phenylcarbamoyl)-butyric acid (R6) (Vogel 2000).

Scheme-I



Scheme-II



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

1. FTIR (cm^{-1}) 3256.2 (N-substituted amide, N-H *str*), 3026.2 (Aromatic, C-H *str*), 1683.6 (N-H *def* or C=O *str*), 1611.5(Aromatic, C=C *str*), 1354.2 (C-O *str* or O-H *def*). ^{1}HNMR (ppm): 4.78 (2H, *s*, Ar-OH), 6.75- 7.47 (8H, *d*, Ar-H), 7.84 (2H, *s*, 2-NHCO-).

2.FTIR (cm^{-1}) 3226.1 (N-substituted amide, N-H *str*), 3012.8 (Aromatic, C-H *str*), 1681.4 (N-H *def* or C=O *str*), 1602.7 (Aromatic, C=C *str*), 1351.8 (C-O *str* or O-H *def*).

3: FTIR (cm^{-1}) 3241.2 (N-substituted amide, N-H *str*), 3014.3 (Aromatic, C-H *str*), 1678.9 (N-H *def* or C=O *str*), 1607.4 (Aromatic, C=C *str*), 1353.5 (C-O *str* or O-H *def*).

4: FTIR (cm^{-1}) 3239.7 (N-substituted amide, N-H *str*), 3014.9 (Aromatic, C-H *str*), 1678.2 (N-H *def* or C=O *str*), 1609.5 (Aromatic, C=C *str*), 1354.8 (C-O *str* or O-H *def*). ^{1}HNMR: 2.07 (2H, *m*, -CH_2-), 2.21 (4H, *t*, 2-CH_2-), 4.77 (2H, *s*, Ar-OH), 6.73 - 7.47 (8H, *d*, Ar-H), 7.81 (2H, *s*, 2-NHCO).

5: FTIR (cm^{-1}) 3208.2 (N-substituted amide, N-H *str*), 3021.8 (Aromatic, C-H *str*), 1708.1 (C=O *str*, -COOH), 1680.2 (C=O *str* or N-H *def*). ^{1}HNMR: 2.50 (2H, *t*, -$COCH_2$), 2.61 (2H, *t*, -CH_2 COOH), 4.79 (1H, *s*, Ar-OH), 6.74- 7.47 (4H, *d*, 4-CH-), 7.79 (1H, *s*, -NHCO-), 11.06 (1H, *s*, -COOH).

6: FTIR (cm^{-1}) 3209.6 (N-substituted amide, N-H *str*), 3023.4 (Aromatic, C-H *str*), 2990.7(C-H *str*), 1708.1 (C=O *str*, -COOH), 1681.3 (C=O *str* or N-H *def*).

7: FTIR (cm^{-1}) 3084.1 (Aromatic C-H *str*), 1704.9 (Five membered ketone, C=O *str*), 1617.2 (Aromatic, C=C *str*), 1402.4 (C-O *str* or O-H *def*), 1299.9 (C-N *str*), 910.9 (*p*-substituted benzene, OOP, C-H *def*). ^{1}HNMR: 2.74 (4H, *t*, 2-CH_2), 4.98 (1H, *s*, -OH), 6.73 -727 (4H, *d*, -CH).

8: FTIR (cm^{-1}) 3056.3(Aromatic C-H *str*), 1680.5 (six membered ketone, C=O *str*), 1601.4 (Aromatic, C=C *str*), 1370.6 (C-O *str* or O-H *def*), 1311.2 (C-N *str*), 911.6 (*p*-substituted benzene, OOP, C-H *def*).

Pharmacology

Analgesic activity

Analgesic activity was carried out by tail flick method (Vogel 2002; Fadeyi *et al.* 2004). Healthy albino mice weighing 20-30 g were divided into different group of six animals each. The control group received 0.5%w/v CMC solution; treated group was given, orally, a dose of 132 µmol/ kg of the compounds R1, R8. Reaction times were noted at 2 h and 4 h interval after the drug administration. The analgesic activity (%) was calculated by the following formula:

$$\text{Percentage of analgesic activity} = \frac{(T_2-T_1)}{T_1} * 100$$

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

Antipyretic activity

Healthy wistar rats weighting 150-200 g were given s.c. 10 ml /kg of a 20% aqueous suspension of sterilised brewers yeast powder. (Vogel 2002, Fadeyi *et al.* 2004). After 18 h, animals showing an increase higher than 0.5°C in rectal temperature were selected. Control group received 0.5% carboxy methylcellulose solution; treated group received a dose of 132 µmol/ kg of compounds R1 to R8. Rectal temperatures were noted by digital thermometer 30 min before (pre-treated) and at 1, 2 and 4 h after administration of the dose.

Anti-inflammatory activity

Anti-inflammatory activity was carried using hind paw edema method (Vogel 2002, Fadeyi et al. 2004) on albino rat of either sex. A freshly prepared solution of carrageenan (0.1 ml, 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 compounds R1 to R8, respectively 30 min before carrageenan. The volume of each paw was measured by plethysmometer after 2 and 4 h interval of carrageenan injection. The percentage of inhibition of edema was calculated by the following formula:

$$\text{Percentage of inhibition of edema} = \frac{(V_c - V_t)}{V_c} * 100$$

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

Table 1. Analgesic activity of the compounds for a dose of 132 µmol/kg

Compound	Normal reaction time	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
R1	2.18±0.15	1.55±0.10	2.31±0.16	71.05±1.19	106.07±1.88***
R2	2.9±0.05	4.38±0.07	3.08±0.06	151.19±2.33	106.33±1.45***
R3	2.23±0.06	3.86±0.11	2.08±0.04	173.15±1.78	93.01±1.85***
R4	2.85±0.07	3.55±0.09	2.08±0.04	124.56±1.59	73.14±1.38***
R5	2.41±0.06	3.08±0.06	2.3±0.05	127.66±2.68	103.83±1.90*
R6	1.98±0.06	3.0±0.08	2.3±0.07	151.27±1.39	115.95±135*
R7	2.98±0.09	3.78±0.09	2.66±0.06	126.94±1.16	89.48±1.07**
R8	2.15±0.07	3.5±0.13	2.03±0.07	162.73±1.75	94.59±1.65**
Standard (Paracetamol)	3.05±0.07	2.5±0.11	2.88±0.07	81.79±4.6	93.99±1.30***

Note: Value of reaction time are mean ± SEM, n = 6. Statistical analysis was done by student's unpaired *t*-test (Kulkarni 2003).

* $p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Table 2. Antipyretic activity of the title compound

Compound	Before drug		After drug		
	-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
R1	37.43±0.04	38.4±0.03	38.11±0.03	37.75±0.04	37.51±0.01***
R2	37.2±0.05	38.13±0.05	38.01±0.04	37.7±0.05	37.28±0.04***
R3	37.28±0.07	38.01±0.05	37.9±0.07	37.66±0.06	37.4±0.05***
R4	37.35±0.03	38.1±0.03	37.95±0.04	37.66±0.04	37.51±0.04***
R5	37.1±0.05	37.88±0.07	37.7±0.07	37.46±0.06	37.33±0.05*
R6	37.48±0.04	38.45±0.03	38.31±0.04	38.13±0.04	37.83±0.04
R7	36.96±0.05	37.88±0.04	37.78±0.03	37.66±0.042	37.53±0.05**
R8	37.31±0.05	38.11±0.05	38.0±0.03	37.73±0.04	37.48±0.03***
Standard (Paracetamol)	37.1±0.05	37.9±0.04	37.65±0.04	37.38±0.03	37.16±0.05***

Note: Value of reaction time are mean ± SEM, n = 6. Statistical analysis was done by student's unpaired *t*-test (Kulkarni 2003). $p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Table 3. Anti-inflammatory activity of the title compound Dose 132 μmol/kg

Compound	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		
R1	0.82±0.02	0.80±0.2	31.60 ± 6.22	38.88 ± 4.20*
R2	0.82±0.0	0.81±0.08	32.16±2.83	38.14±1.12*
R3	0.83±0.0	0.82±0.01	31.07±2.44	37.01±1.54*
R4	0.86±0.01	0.84±0.02	28.66±2.85	35.62±3.71**
R5	0.84±0.02	0.83±0.01	30.27±4.1	36.75±2.36
R6	0.82±0.0	0.81±0.0	32.18±2.20	38.39±1.45
R7	0.81±0.2	0.80±0.02	32.70±3.44	39.02±2.26
R8	0.83±0.01	0.82±0.02	31.11±3.45	37.51±1.76
Standard (Paracetamol)	0.86±0.01	0.85±0.0	28.78±3.02	34.84±1.35***

Note: Value of reaction time are mean ± SEM, n = 6. Statistical analysis was done by student's unpaired *t*-test (Kulkarni 2003). *p < 0.05, **p < 0.01, ***p < 0.001

Table 4. Some characterisation of the synthesized compound.

Compound	Yield (%)	Formula M.W. (g/mol)	Analyses C,H,N,O (%)
R1	87	C14H12N2O4 272.26	61.76, 4.44, 10.29, 23.51 62.068, 4.40, 10.238, 23.392
R2	79	C15H14N2O4 286.28	62.93, 4.93, 9.79, 22.35 63.244, 4.954, 9.741, 22.238
R3	89	C16H16N2O4 300.31	63.99, 5.37, 9.33, 21.31 63.670, 5.396, 9.376, 21.203
R4	83	C17H18N2O4 314.31	64.96, 5.77, 8.91, 20.3 64.635, 5.79, 8.954, 20.401
R5	91	C10H11NO5 209.20	57.41, 5.31, 6.70, 30.59 57.697, 5.336, 6.733, 30.742
R6	88	C11H13NO4 223,23	59.19, 5.87, 6.27, 28.67 59.894, 5.840, 6.238, 28.526
R7	73	C10H9NO3 191.18	62.82, 4.74, 7.33, 25.11 62.5059, 4.763, 7.366, 24.984
R8	76	C11H11NO3 205.21	64.38, 5.40, 6.83, 23.39 64.70, 5.373, 6.795, 23.506

Results and Discussion

p-Hydroxy aniline reacted with appropriate diacyl chloride (oxylyl, succinyl, malonyl, and glutryl chloride) the compounds R1 to R4 were obtained by a single step synthesis as depicated in scheme-I (Furniss *et al.*, 1998). N- (4-hydroxyphenyl)-succinamic acid (R4) and 4-(4-hydroxyphenylcarbomyl) butyric acid (R5) were synthesized by reacting *p*-hydroxy aniline with succinic and glutyric anhydride. Cyclization of the above-synthesized compounds with acetyl chlorides gives 1-(4-hydroxyphenyl) piperidine-2, 6-dione (R7) and 1-(4-hydroxyphenyl) pyrrolidone-2, 6-dione (R8) respectively as depicted on scheme-II

(Furniss *et al.* 1998). Physical data of the synthesized compounds are given in Table 4. Structures of the synthesised compounds were assigned by spectroscopic methods (IR, H^1 NMR). Spectral data of the compounds representative are given in the experimental part. In IR spectra N-H, C=O, C=C, C=N, N=O stretching were observed at expected frequencies on. H^1-NMR spectra, phenolic proton, amide proton and carboxylic acid proton are obtained at about 4.78, 7.81 and 11.06 ppm, respectively. In the introduction it was denoted that *p*-hydroxy aniline and its derivative are well known for antipyretic and analgesic activity. Therefore, analgesic, antipyretic and anti-inflammatory activities of the compounds in Table 1, 2 and 3 were taken into consideration. We may conclude that analgesic and antipyretic activities of these compounds is more appreciable than their anti-inflammatory activity.

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