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Synthesis and Pharmacological Activities of *N*-(3-Hydroxyphenyl)Benzamide and its 3-*O*-Derivatives

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ABSTRACT
N-(3-hydroxyphenyl) benzamide (**3**) was synthesized by the condensation of 3-hydroxyaniline (**1**) with benzoyl chloride (**2**) in aqueous medium. From this parent molecule **3**, various 3-*O*-derivatives, **5a-f**, were prepared *via O*-alkylation, by reacting it with different alkyl halides, **4a-f**, for 2 h under reflux conditions in the presence of mixture of Na-ethoxide and C_2H_5OH as solvent. The synthesized compounds were characterized by using different spectroscopic techniques and were subjected to enzyme inhibition activity against butylcholinesterase, acetylcholinesterase, and lipoxygenase enzymes.

Key words: Benzamides, 3-Hydroxyaniline, Butylcholinesterase, Acetylcholinesterase, and Lipoxygenase.

1. INTRODUCTION
Benzamides are important class of organic compound which are derived from benzoic acid. They are biological active molecules with a large number of applications; their most profound use is inhibitors in medicine. A variety of benzamide derivatives inhibit Histone deacetylases, or they act as HDAC enzyme inhibitors. Benzamides have been reported as relaxant for smooth muscle and activators of potassium channel. Some synthetic benzamides are antihelmintic agent[1], while some others are anti-inflammatory and analgesic[2]. Pharmacologically active benzamide[3] derivatives have anti-bacterial and anti-fungal activities[4]. Benzamide derivatives are also very importance in medicinal chemistry[5]. The Non-biaryl benzamides are used as ligands and suitable for asymsmetric catalysis[6]. Therapeutically active compounds are listed under Heterocyclic benzamides, which show activity in central nervous system. These heterocyclic compounds act as antipsychotics, antiemetics and gastric motility stimulants[7, 8, 9]. In this project we synthesized various *O*-substituted derivatives, **5a-f**, of *N*-(3-hydroxyphenyl) benzamide (**3**) and their biological screening against three enzymes namely, butyryl cholinesterase, acetyl cholinesterase, and lipoxygenase. The synthesized compounds (**3, 5a, 5b**) have already been reported in literature[10-12] whereas compounds (**5c, 5d, 5e, 5f**) have been synthesized for the first time.

2. RESULTS AND DISCUSSION

2.1 *Chemistry*
In the present study, first, *N*-(3-hydroxyphenyl)benzamide (**3**) was synthesized on reacting 3-hydroxyaniline (**1**) with benzoyl chloride (**2**) in aqueous medium. Then compound **3** was treated with different alkyl halides **4a-f** to synthesis new 3-*O*-derivatives *via O*-alkylation (Scheme-1). The molecular formula $C_{13}H_{11}NO_2$ of *N*-(3-hydroxyphenyl)benzamide (**3**) was inferred with the help of EIMS with molecular ion peak at *m/z* 213 and integration of protons in its PMR data. The EIMS of this new compound illustrate base peak at *m/z* 105 due to benzoyl $[C_6H_5CO]^+$ group. In IR spectrum peaks at (3420 cm^{-1}), (1620 cm^{-1}) and (3230 cm^{-1}) explained the existence of hydroxyl, carbonyl and -NH- groups respectively in the molecule. In its ^{1}H-NMR spectrum, the signals in aromatic region at δ 7.89 (dd, *J* = 1.5, 7.0 Hz, 2H, H-2′ and H-6′), 7.56 (br t, *J* = 7.5 Hz, 1H, H-4′) and δ 7.49 (br t, *J* = 7.0 Hz, 2H, H-3′ and H-5′) were characteristics of a benzoyl group[13] while the signals at δ 7.29 (t, *J* = 2.0 Hz, 1H, H-2), 7.14 (t, *J* = 8.0 Hz, 1H, H-5), 7.08 (m, 1H, H-6) and 6.58 (ddd, *J* = 1.0, 2.5, 8.0 Hz, 1H, H-4) were attributed to the protons of the *N*-substituted 3-hydroxyaniline moiety in the molecule. Keeping in view these spectroscopic studies, the structure of **3** was established as *N*-(3-hydroxyphenyl)benzamide. In the same way, the structures of other derivatives, **5a-f**, were established by interpreting IR, ^{1}H-NMR and EI-MS spectra, as given in experimental section.

2.2 *Bioactivity Studies*
The screening of all the synthesized derivatives of **3** rendered them as good inhibitors of butyryl cholinesterase (BChE) and acetyl cholinesterase (AChE), affirmed by their IC_{50} values (Table-1). Our results revealed that due to the substitution of ethyl group *N*-(3-ethoxyphenyl)benzamide (**5b**) was found to be more potent inhibitor against butyrylcholinesterase having IC_{50} value of 20.90 ± 0.11 μmoles comparative to a reference standard (eserine) having

IC_{50} value of 0.85±0.001 μmoles. However, for acetylcholinesterase inhibitory test the substitution of five carbon chain in *N*-[3-(pentoxy)phenyl]benzamide (**5f**) resulted in an increase in its inhibitory potential with IC_{50} value of

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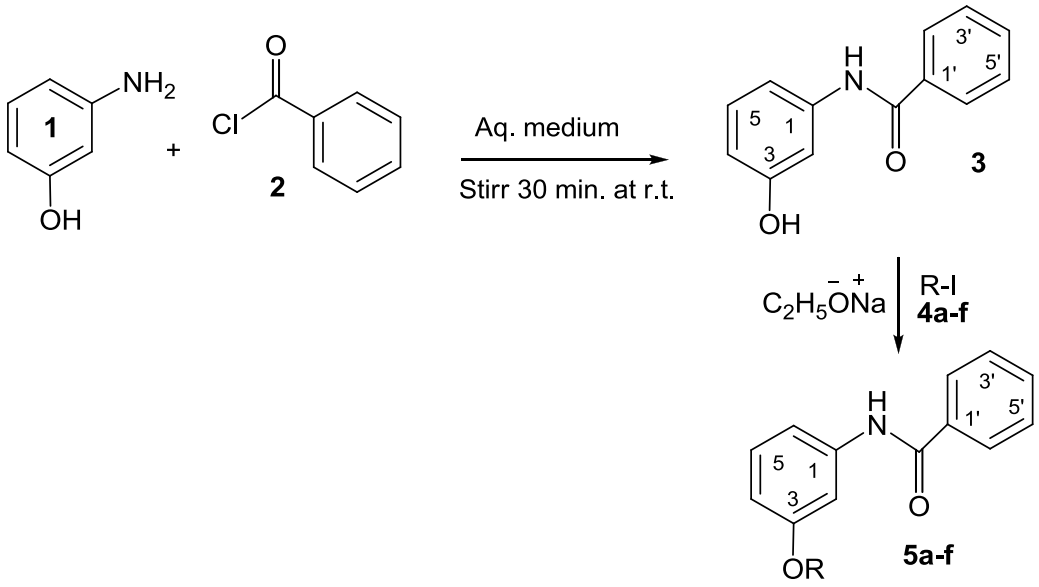
158.81±0.12 µmoles relative to eserine having IC_{50} value of 0.04±0.001µmoles against this enzyme. Against lipoxygenase **5a**, **5b** and **5c** were not found to be active. On these molecules Antioxidant activity by DPPH method[14] was also performed. Table-1 illustrates the results of DPPH assay. These outcomes showed that only **3** demonstrated good scavenging of DPPH radical. This scavenging was due to free phenolic group. From the plotted curves, for each sample the IC_{50} value was premeditated.



Compound	R	Compound	R
5a	$-CH_3$ (1")	**5d**	$-CH_2-CH_2-CH_3$ (1", 2", 3")
5b	$-CH_2\,CH_3$ (1", 2")	**5e**	$-CH_2-CH_2-CH_2-CH_3$ (1", 2", 3", 4")
5c	$-CH(CH_3)_2$ (1", 2", 3")	**5f**	$-CH_2-CH_2-CH_2-CH_2-CH_3$ (1", 2", 3", 4", 5")

Scheme-1: Synthesis of *N*-(3-hydroxyphenyl)benzamide (3) and its derivatives 5a-f.

Table-1: Bioactivity studies on *N*-(3-hydroxyphenyl)benzamide (**3**) and its derivatives **5a-f**

Sample No.	DPPH (% inhibition) at 0.5mM	DPPH (IC_{50}) µM	BChE (% inhibition) at 0.5mM	BChE (IC_{50}) µM	AChE (% inhibition) at 0.5mM	AChE (IC_{50}) µM	LOX (% inhibition) at 0.5mM	LOX (IC_{50}) µM
3	66.61±0.11	283.21±0.25	64.49±0.11	84.21±0.35	62.38±0.81	411.37±0.35	92.03±0.25	185.61±0.32
5a	20.24±0.62	Nil	71.10±0.52	201.11±0.45	78.48±0.69	143.51±0.45	53.25±0.32	Nil
5b	22.24±0.53	Nil	95.34±0.35	20.90±0.11	52.75±0.22	403.24±0.75	40.08±0.11	Nil
5c	19.55±0.95	Nil	84.15±0.59	98.71±0.34	61.87±0.95	408.11±0.41	68.52±0.87	<400
5d	13.66±0.31	Nil	97.62±0.13	20.11±0.22	57.31±0.62	246.11±0.38	81.36±0.54	145.11±0.39
5e	19.44±0.11	Nil	87.03±0.01	22.31±0.15	68.07±0.56	169.30±0.25	68.97±0.33	319.51±0.63
5f	17.84±0.25	Nil	77.03±0.46	134.41±0.23	54.50±0.35	158.81±0.12	77.91±0.69	134.91±0.01
Control	Quercetin	16.96±0.14	Eserine	0.85±0.001	Eserine	0.04±0.001	Baicalein	22.4±1.3

Note: IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ-Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA)

DPPH= 1,1-Diphenyl-2-picrylhydrazyl radical; BChE = Butyryl cholinesterase; AChE= Acetyl cholinesterase; LOX= Lipoxygenase.

3. EXPERIMENTAL

3.1 *General*

Purity was checked on TLC cards. They were formed from already coated silica gel G-25-UV_{254} plates. TLC was visualized at 254 nm and developed by ceric sulphate reagent with single spot. Mobile phase consisting of EtOAc and

n-C_6H_{12}. The IR spectra were computed in Pot. bromide on a Jasco-320-A spectrophotometer with wave number in percentimeter. ^{1}H-NMR spectra were computed using $CDCl_3$ on a Bruker spectrometers working at 400 MHz. δ–values are specified in ppm and *J*-values in hertz. Mass spectra (EIMS) were done on Finnigan MAT-112 instrument with a data system.

3.2 *Procedure for the Synthesis of N-(3-Hydroxyphenyl)benzamide* (3)

3-Hydroxy aniline (2.0 mmol; 5.0 g, **1**) was reacted with benzoyl chloride (2.0 mmol; 6.44g, **2**) in aqueous medium by vigorous shaking for 30 min. The pH of the contents was lowered during reaction due to the evolution of HCl gas. On TLC conformation, the reaction contents were diluted by cold water till the formation of white precipitates. The pure precipitates were obtained by filtration and washing with dist. H_2O. On drying the title compound *N*-(3-hydroxyphenyl)benzamide (**3**) was obtained. Recrystallization was done with methanol; white powdered product was acquired on evaporating CH_3OH. Yield: 88.6% [10-12].

3.3 *Procedure for Synthesis of O-Alkylated Derivatives* (5a-f) *of N-(3-Hydroxyphenyl)benzamide*

0.4g sodium metal was completely dissolved in 10.0 ml absolute ethanol in a 250 ml round bottom flask till no evolution of hydrogen gas. On cooling, calculated amount of **3** (2 g) was added followed by pouring alkyl halides, **4a-f**. Reaction contents were refluxed for about 2 hrs, followed by slow addition of water to avoid the formation of lumpy material. The temperature of reaction was maintained at 0-4 ^oC on ice bath. The products were obtained through filtration or in some cases liquid product was extracted with chloroform, depending upon the nature of product[10-12].

3.4 *Cholinesterase Assays*

The AChE and BChE inhibitory assays were performed by the reported method[15,16] with minor modifications. 100 µL volume was made by 60 µL Na_2HPO_4 buffer (50 mM; pH 7.7), 10 µL test compound (0.5 mM well^{-1}) and 10 µL (0.005 unit well^{-1}) enzyme. These 100 µL assay's volume was mixed together. After that its reading was taken at 405 nm followed by pre-incubation for 10 mins at 37 ºC. The initiation of reaction was performed by the 10 µL of 0.5 mM well^{-1} substrate (acetylthiocholine iodide for AChE and butyrylthiocholine chloride for BChE) followed by 10 µL DTNB (0.5 mM well^{-1}). After incubating for 15 mins at 37 ºC, Again absorbance was taken at 450 nm. All conducted tests were performed in three folds with the particular controls. Eserine (0.5 mM well^{-1}) was employed as a +tive control. The % age inhibition was accounted as,

$$\% \, Inhibition = \frac{Control - Test}{Control} \text{X } 100$$

By using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA), IC_{50} values of compounds were intended, it is the concentration at which there is 50% of enzyme is inhibited.

3.5 *Lipoxygenase Assay*

Lipoxygenase activity was assayed using the reported method[17-19] with some modifications. 200 µL assay mixture was prepared from 150 µL Na_3PO_4 buffer (100 mM; pH 8.0), 10 µL test compound and 15 µL enzyme followed by mixing, pre reading (at 234 nm) and pre incubation (10 minutes at 25 °C). Reaction was started at 25 µL of substrate concentration, followed by absorbance at 234 nm repeated after every 6 min. All reading were taken thrice using +tive and -tive controls. Quercetin (0.5 mM well^{-1}) was used as a +tive control. All readings were taken thrice using Baicalein as control.

3.6 *DPPH Assay*

The DPPH radical scavenging activities of synthesized compounds were observed by comparing with butylated hydroxytoluene (BHT) using the reported method[14]. The different amounts of samples were added to the DPPH solution in methanol (3 ml; 0.1mM). Solution was shaked vigorously for about 1 hour at RT. Absorbance of each sample was measured at 517 nm against blank. Methanol was used as a blank. Smaller the absorbance, higher will be the free radical scavenging activity of respective compound. For each sample %age of DPPH decolouration was calculated. Calculations were made by the method as given for cholinesterase assays.

4. SPECTRAL CHARACTERIZATION OF SYNTHESIZED BENZAMIDES

4.1 *N-(3-Hydroxyphenyl)benzamide* (3)

Yield: 88.6%; M.P: 152-154 ^oC; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.89 (dd, *J* = 1.5, 7.0 Hz, 2H, H-2′& H-6′), 7.56 (br t, *J* = 7.5 Hz, 1H, H-4′), 7.49 (br t, *J* = 7.0 Hz, 2H, H-3′& H-5′), 7.29 (t, *J* = 2.0 Hz, 1H, H-2), 7.145 (t, *J* = 8.0 Hz, 1H, H-5), 7.08 (m, 1H, H-6), 6.58 (ddd, *J* = 1.0, 2.5, 8.0 Hz, 1H, H-4); IR (KBr): ν_{max} (cm^{-1}): 3420 (O-H), 3230 (N-H), 3056 (Ar-H), 1620 (C=O), 1600-1517 (Ar (C=C)); EIMS: *m/z* 213 $[M]^+$, 105 $[C_6H_5CO]^+$, 77 $[C_6H_5]^+$.

4.2 *N-(3-Methoxyphenyl)benzamide* (5a)

Yield 70.42%; M.P: 150-152 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.90 (dd, *J* = 1.5, 7.5 Hz, 2H, H-2′ & H-6′), 7.56 (br t, *J* = 7.5 Hz, 1H, H-4′), 7.49 (br t, *J* = 7.5 Hz, 2H, H-3′ & H-5′), 7.40 (br d, *J* = 2.0 Hz, 1H, H-2), 7.24 (t, *J* = 8.0 Hz, 1H, H-5), 7.22 (m, 1H, H-6), 6.71 (dt, *J* = 2.0, 7.0 Hz, 1H, H-4), 3.80 (s, 3H, CH_3-1″); IR (KBr): ν_{max} (cm^{-1}): 3300 (N-H), 3040 (Ar-H), 1640 (C=O), 1600-1517 (Ar, C=C), 1230 (C-O); EIMS: *m/z* 227 $[M]^+$, 105 $[C_6H_5CO]^+$, 77 $[C_6H_5]^+$.

4.3 *N-(3-Ethoxyphenyl)benzamide* (5b)

Yield: 78.76%; M.P: 155-157 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.90 (dd, *J* = 1.5, 7.0 Hz, 2H, H-2′ & H-6′), 7.56 (br t, *J* = 7.5 Hz, 1H, H-4′), 7.49 (t, *J* = 7.5 Hz, 2H, H-3′ & H-5′), 7.38 (d, *J* = 1.5 Hz, 1H, H-2), 7.23 (t, *J* = 7.0 Hz, 1H, H-5), 7.21 (m, 1H, H-6), 6.69 (dt, *J* = 2.0, 7.0 Hz, 1H, H-4), 4.04 (q, *J* = 7.0 Hz, 2H, CH_2-1″), 1.38 (t, *J* = 7.0 Hz, 3H, CH_3-2″); IR (KBr): ν_{max} (cm^{-1}): 3330 (N-H), 3000 (Ar-H), 1650 (C=O), 1600-1517 (Ar, C=C), 1245 (C-O); EIMS: *m/z* 241 $[M]^+$, 213 $[M\text{-}CO]^+$, 105 $[C_6H_5CO]^+$, 77 $[C_6H_5]^+$.

4.4 *N-[3-(Propan-2-yloxy)phenyl]benzamide* (5c)

Yield: 81.58%; M.P: 159-161 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.90 (dd, *J* = 1.5, 7.0 Hz, 2H, H-2′ & H-6′), 7.56 (t, *J* = 7.0 Hz, 1H, H-4′), 7.49 (t, *J* = 7.0 Hz, 2H, H-3′ & H-5′), 7.38 (d, *J* = 1.5 Hz, 1H, H-2), 7.22 (t, *J* = 8.0 Hz, 1H, H-5), 7.19 (m, 1H, H-6), 6.68 (dt, *J* = 2.0, 7.5 Hz, 1H, H-4), 4.59 (heptet, *J* = 6.5 Hz, 1H, H-1″), 1.3 (d, *J* = 6.5 Hz, 6H, CH_3-2″ & CH_3-3″); IR (KBr): ν_{max} (cm^{-1}): 3290 (N-H), 3070 (Ar-H), 1630 (C=O), 1600-1517 (Ar, C=C), 1225 (C-O); EIMS: *m/z* 255 $[M]^+$, 105 $[C_6H_5CO]^+$, 77 $[C_6H_5]^+$.

4.5 *N-(3-Propoxyphenyl)benzamide* (5d)

Yield: 73.6%; M.P: 164-166 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.90 (dd, *J* = 1.0, 7.5 Hz, 2H, H-2′ & H-6′), 7.56 (br t, *J* = 7.5 Hz, 1H, H-4′), 7.49 (t, *J* = 7.5 Hz, 2H, H-3′ & H-5′), 7.38 (d, *J* = 2.0 Hz, 1H, H-2), 7.23 (t, *J* = 8.0 Hz, 1H, H-5), 7.21 (m, 1H, H-6), 6.69 (dt, *J* = 2.0, 7.5 Hz, 1H, H-4), 3.98 (t, *J* = 6.5 Hz, 2H, CH_2-1″), 1.51 (sextet, *J* = 7.5 Hz, 2H, CH_2-2″), 0.97 (t, *J* = 7.5 Hz, 3H, CH_3-3″); IR (KBr): ν_{max} (cm^{-1}): 3320 (N-H), 3050 (Ar-H), 1630 (C=O), 1600-1517 (Ar, C=C), 1240 (C-O); EIMS: *m/z* 255 $[M]^+$, 213 $[M\text{-}C_3H_6]^+$, 105 $[C_6H_5CO]^+$, 77 $[C_6H_5]^+$.

4.6 *N-(3-Butoxyphenyl)benzamide* (5e)

Yield: 74.6%; M.P: 169-171 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.90 (dd, *J* = 1.5, 8.5 Hz, 2H, H-2′ & H-6′), 7.56 (br t, *J* = 7.5 Hz, 1H, H-4′), 7.50 (t, *J* = 7.5 Hz, 2H, H-3′ & H-5′), 7.39 (d, *J* = 1.5 Hz, 1H, H-2), 7.22 (t, *J* = 7.5 Hz, 1H, H-5), 7.20 (m, 1H, H-6), 6.69 (dt, *J* = 2.5, 7.5 Hz, 1H, H-4), 3.99 (t, *J* = 6.5 Hz, 2H, CH_2-1″), 1.76 (quintet, *J* = 6.5 Hz, 2H, CH_2-2″), 1.59 (sextet, *J* = 7.5 Hz, 2H, CH_2-3″), 0.99 (t, *J* = 7.5 Hz, 3H, CH_3-4″); IR (KBr): ν_{max} (cm^{-1}): 3340 (N-H), 3000 (Ar-H), 1660 (C=O), 1600-1517 (Ar, C=C), 1235 (C-O); EIMS: *m/z* 269 $[M]^+$, 105 $[C_6H_5CO]^+$, 77 $[C_6H_5]^+$.

4.7 *N-[3-(Pentoxy)phenyl]benzamide* (5f)

Yield: 88.6%; M.P: 173-175 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.90 (dd, *J* = 1.5, 7.0 Hz, 2H, H-2′ & H-6′), 7.57 (br t, *J* = 7.5 Hz, 1H, H-4′), 7.49 (t, *J* = 7.5 Hz, 2H, H-3′ & H-5′), 7.39 (d, *J* = 2.0 Hz, 1H, H-2), 7.23 (t, *J* = 8.0 Hz, 1H, H-5), 7.20 (m, 1H, H-6), 6.69 (dt, *J* = 2.5, 7.5 Hz, 1H, H-4), 3.96 (t, *J* = 6.5 Hz, 2H, CH_2-1″), 1.78 (quintet, *J* = 6.5 Hz, 2H, CH_2-2″), 1.47 (quintet, *J* = 6.5 Hz, 2H, CH_2-3″), 1.41 (sextet, *J* = 6.5 Hz, 2H, CH_2-4″), 0.95 (t, *J* = 7.0 Hz, 3H, CH_3-5″); IR (KBr): ν_{max} (cm^{-1}): 3323 (N-H), 3010 (Ar-H), 1635 (C=O), 1600-1517 (Ar, C=C), 1250 (C-O); EIMS: *m/z* 283 $[M]^+$, 105 $[C_6H_5CO]^+$, 77 $[C_6H_5]^+$.

3. CONCLUSION

It was usually concluded from such *O*-substituted *N*-[3-(alkoxy)phenyl]benzamide derivatives that these are suitable entities for the inhibition of butylcholinesterase and acetylcholinesterase enzymes. Therefore, they might serve as therapeutic agents, particularly for the treatment of Alzheimer's disease.

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