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SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP OF 1-(2-FUROYL)PIPERAZINE BEARING BENZAMIDES AS BUTYRYLCHOLINESTERASE INHIBITORS

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Four benzamide derivatives (**5a, 5b**, **8a, 8b**) bearing heterocyclic furan and piperazine ring have been synthesized and evaluated for enzyme inhibition and hemolytic activity. Initial 4-(chloromethyl)benzoyl chloride (**1**) and 3-(chloromethyl)benzoyl chloride (**6**) were stirred with benzyl amine (**2a**) and cyclohexyl amine (**2b**), respectively, in aqueous medium at pH 9 – 10 maintained by aqueous sodium carbonate. The resulting benzamides (**3a, 3b**, **7a, 7b**) were refluxed with 1-(2-furoyl)piperazine (**4**) in the presence of K_2CO_3 and CH_3CN to acquire target compounds (**5a, 5b**, **8a, 8b**). The spectroscopic techniques including ^{13}C NMR, ^1H NMR, IR and EI-MS corroborated the proposed molecular structures of final compounds. Among these, two compounds (**5b**, **8b**) proved to be considerable inhibitors of butyrylcholinesterase enzyme. Study of the hemolytic activity potential revealed low toxicity level of compound **5b**.

Keywords: benzamides; enzyme inhibition; furan; hemolytic activity; piperazine.

1. INTRODUCTION

The bioactive nature of heterocyclic compounds is well known to researchers. Piperazine is a six membered nitrogen-containing heterocyclic compound, which has been modified structurally and found numerous applications in engineering [1] and polymer [2] fields. Derivatives of this moiety showed inhibition potential against certain microbial strains [3], various enzymes [4], noroviruses [5] and triple reuptake [6]. The cannabinoid CB1 receptor agonists [7] and antagonists for melanocortin-4 receptor [8] have been found to include piperazine heterocycle. In synthesized compounds, another biologically potent functionality of amides or benzamides [9 – 13] is inserted to boost up the potential of piperazine. It was found that biologically significant compounds have amide bonds as a key functional group. These types of compounds include peptides or proteins. At present, about 30% known drugs [14, 15] contain moieties of this type. When carboxylic acids and amines are coupled, then amide bond is formed as a key functional group [16 – 19]. The reaction of benzyl chloride with ammonia also yielded this moiety known as benzoic acid amide. Amide ($RCO\text{-}NH_2$) is a compound which has C=O group and R substituent that may be hydrogen, linear chain or ring structures. It may also be a compound having metal replacing hydrogen in ammonia like sodium amide. Nitrogen admits various substituents which classify the amides into different classes exhibiting variable physical and chemical behavior [20].

Progress in the medicinal chemistry led to the development of many drugs for the cure and treatment of cancer. Benzamide, its derivatives and its other substituents are considered to be pharmacologically active. These compounds have interesting structures containing moieties which show diverse and remarkable activities. These compounds can be used for the treatment of different diseases including cancer [21 – 24]. Metoclopramide is an important benzamide derivative. This derivative has been reported to enhance the cisplatin effects [25, 26]. Anacardic acid has been used for the synthesis of many benzamide derivatives. In particular, cyano(acrylamido)benzamide is a benzamide derivative possessing anti-tumor activity against breast cancer cells [27]. Benzamides and their analogs exhibit different types of bio-

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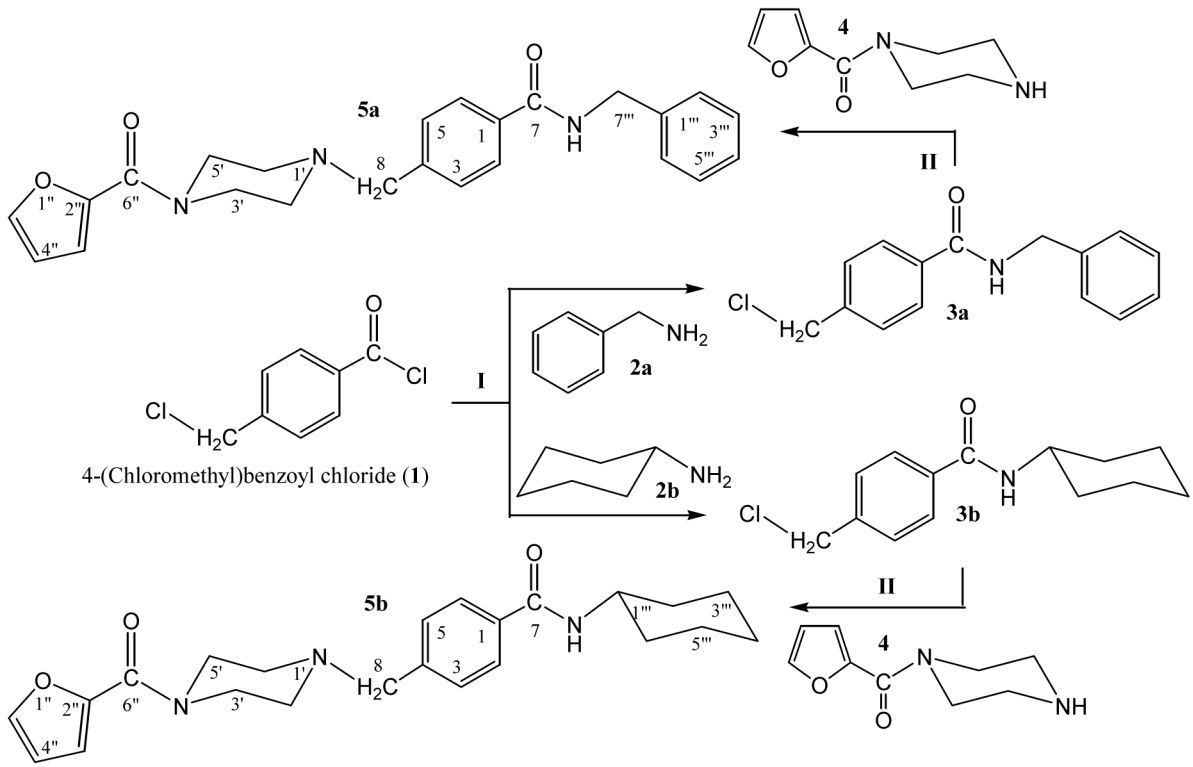


Scheme 1. Synthesis of *N*-(2-furoyl)piperazine bearing benzamides (**5a**, **5b**) from 4-(chloromethyl)benzoyl chloride (**1**). Reagents and conditions: (I) aq. Na_2CO_3 soln./pH 9 – 10/stirring at RT for 2 – 3 h; (II) acetonitrile/K_2CO_3/reflux of **4** for 0.5 h for its activation, followed by addition of respective electrophile (**3a**, **3b**) and final reflux for 3 – 4 h.

logical activities such as anti-convulsant, anti-inflammatory, analgesic, serotonin, anti-tumor, anti-microbial and anti-depressant [28, 29].

We have synthesized a series of molecules bearing piperazine, furan and benzamide and evaluated them for the inhibition potential against butyrylcholinesterase (BChE) in search of new drug candidates for treating diseases [30] caused by this enzyme. In addition, toxicity level was assessed through hemolytic activity analysis.

2. EXPERIMENTAL

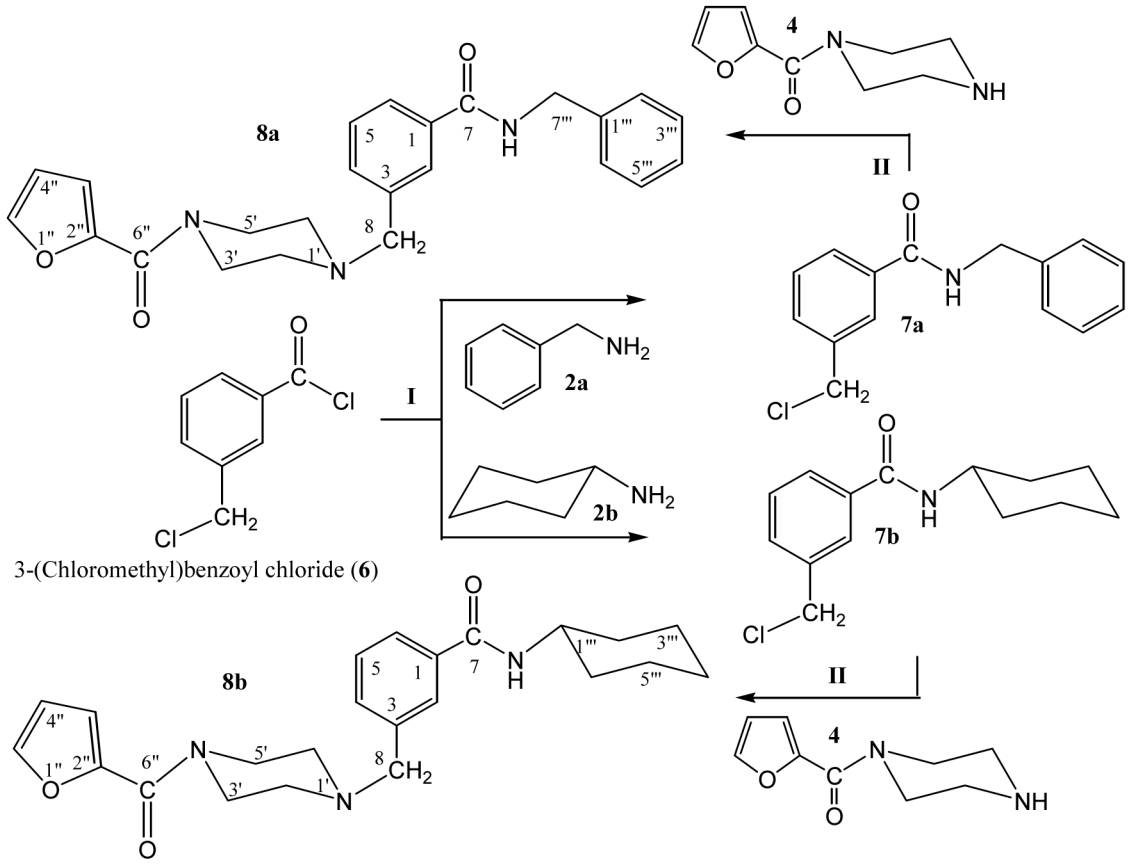
2.1. Materials and Instruments

The ^{1}H NMR spectra were measured in $CDCl_3$ using Bruker spectrometer operating at 600 MHz. The ^{13}C-NMR spectra were measured in CD_3OD using the same instrument operating at 150 MHz. The IR spectra were recorded on Jasco-320-A spectrometer using KBr pellet method. EIMS spectra were obtained on JMS-HX-110 spectrometer. Solvents (analytical grade) and chemicals (Sigma Aldrich and Alfa Aesar) were purchased through local supplier. Melting points were determining using the Griffin-George apparatus in open capillary tubes and remained uncorrected. Thin layer chromatography (TLC) was performed on silica coated aluminum plates using ethyl acetate and *n*-hexane (30:70) as solvent system, followed by detection under UV lamp at 254 nm.

2.2. Chemical Synthesis

Synthesis of *N*-(benzyl/cyclohexyl)-4/3-(chloromethyl)-benzamides 3a, 3b, 7a, and 7b (Scheme 1). Benzyl amine (**2a**) and cyclohexyl amine (**2b**) (18.0 mmol), respectively, were dispersed in distilled water (20.0 mL) taken in an iodine flask (250 mL). Then, 10% Na_2CO_3 aqueous solution was added to adjust pH at 9 – 10 for proper reaction, 4-(chloromethyl)benzoyl chloride (**1**) and 3-(chloromethyl)benzoyl chloride (**6**), respectively, were added dropwise on strong stirring, and the mixture was further stirred for 2 – 3 h until TLC ascertained the reaction completion. Finally, the precipitates of title electrophiles (**3a, 3b**, **7a, 7b**) were filtered, washed with distilled water and dried.

Synthesis of ***N*-(benzyl/cyclohexyl)-4/3-{[4-(2-furoyl)-1-piperazinyl]methyl}benzamides 5a, 5b, 8a, and 8b (Scheme 2).** 1-(2-Furoyl)piperazine (**4**) (14.0 mmol) was mixed with acetonitrile (22 mL) and solid K_2CO_3 (14.0 mmol) in a 250 mL round bottom flask. The mixture was refluxed for 0.5 hour and then electrophiles (**3a, 3b**, **7a, 7b**) were added. The reaction was completed on further reflux for 3 – 4 h. After final TLC report, the title compounds



Scheme 2. Synthesis of *N*-(2-furoyl)piperazine bearing benzamides (**8a**, **8b**) from 3-(chloromethyl)benzoyl chloride (**6**). Reagents and conditions: (I) aq. Na_2CO_3 soln./pH 9 – 10/stirring at RT for 2 – 3 h; (II) acetonitrile/K_2CO_3/reflux of **4** for 0.5 h for its activation, followed by addition of respective electrophile (**7a**, **7b**) and final reflux for 3 – 4 h.

(**5a, 5b**, **8a. 8b**) were collected through filtration or extraction by chloroform.

***N*-Benzyl-4-[4-(2-furoyl)-1-piperazinyl]methylbenzamide (5a):** brown liquid; yield: 90%; Mol. F.: $C_{24}H_{25}N_3O_3$; Mol. Mass: 403; IR (KBr, cm^{-1}) ν_{max}: 1110 (C-N-C), 1195 (C-O-C), 1588 (Ar C=C), 1665 (C=O), 2884 (R C-H), 3097 (Ar C-H), 3412 (N-H); 7.85 (d, *J* = 8.2 Hz, 2H, H-2 & H-6), 7.45 (d, *J* = 1.0 Hz, 1H, H-5″), 7.38 – 7.27 (m, 5H, H-2‴ to H-6‴), 7.21 (d, *J* = 8.4 Hz, 2H, H-3 & H-5), 7.03 (d, *J* = 2.6 Hz, 1H, H-3″), 6.46 (dd, *J* = 3.4, 1.6 Hz, 1H, H-4″), 3.84 (br.s, 4H, CH_2-3′ & CH_2-5′), 3.62 (s, 2H, CH_2-8), 2.54 (br.s, 4H, CH_2-2′ & CH_2-6′), 2.47 (s, 2H, CH_2-7‴); ^{13}C-NMR (150 MHz, CD_3OD, δ in ppm): 167.38 (C-6″), 167.27 (C-7), 159.08 (C-3 & C-5), 147.77 (C-2 & C-6), 145.10 (C-3″), 143.73 (C-2″), 141.32 (C-1‴), 138.41 (C-4″), 133.46 (C-5″), 129.20 (C-2‴ & C-6‴), 127.20 (C-4‴), 126.62 (C-3‴ & C-5‴), 116.41 (C-1), 111.28 (C-4), 64.24 (C-8), 53.07 (C-2′, C-3′, C-5′ & C-6′), 44.00 (C-7‴); ; EIMS (*m/z*): 403 $[M]^+$, 308 $[C_{19}H_{22}N_3O]^+$, 279 $[C_{18}H_{19}N_2O]^+$, 268 $[C_{16}H_{16}N_2O_2]^{\bullet+}$, 225 $[C_{15}H_{15}NO]^{\bullet+}$, 179 $[C_9H_{11}N_2O_2]^+$, 118 $[C_8H_6O]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

***N*-Cyclohexyl-4-[4-(2-furoyl)-1-piperazinyl]methylbenzamide (5b):** white crystalline solid; yield: 94%; m.p: 125 – 127°C; Mol. F.: $C_{23}H_{29}N_3O_3$; Mol. Mass: 395; IR (KBr, cm^{-1}) ν_{max}: 1109 (C-N-C), 1191 (C-O-C), 1587 (Ar C=C), 1650 (C=O), 2881 (R C-H), 3079 (Ar C-H), 3409 (N-H); ^{1}H-NMR (600 MHz, $CDCl_3$, δ / ppm): 7.86 (d, *J* = 8.2 Hz, 2H, H-2 & H-6), 7.45 (d, *J* = 0.9 Hz, 1H, H-5″), 7.26 (d, *J* = 8.4 Hz, 2H, H-3 & H-5), 7.09 (d, *J* = 2.7 Hz, 1H, H-3″), 6.41 (dd, *J* = 3.2, 1.5 Hz, 1H, H-4″), 3.99 – 3.93 (m, 1H, CH-1‴), 3.87 (br.s, 4H, CH_2-3′ & CH_2-5′), 3.62 (s, 2H, CH_2-8), 2.55 (br.s, 4H, CH_2-2′ & CH_2-6′), 1.91 – 1.64 (m, 10H, CH_2-2‴ to CH_2-6‴); ^{13}C-NMR (150 MHz, CD_3OD, δ in ppm): 169.47 (C-6″), 167.79 (C-7), 157.36 (C-3 & C-5), 149.24 (C-2 & C-6), 146.18 (C-3″), 142.53 (C-2″), 136.57 (C-4″), 132.89 (C-5″), 115.22 (C-1), 111.25 (C-4), 65.38 (C-8), 53.24 (C-2′, C-3′, C-5′ & C-6′), 37.48 (C-1‴), 36.78 (C-2‴ & C-6‴), 30.14 (C-4‴), 29.16 (C-3‴ & C-5‴); EIMS (*m/z*): 395 $[M]^+$, 300 $[C_{18}H_{26}N_3O]^+$, 271 $[C_{17}H_{23}N_2O]^+$, 268 $[C_{16}H_{16}N_2O_2]^{\bullet+}$, 217 $[C_{14}H_{19}NO]^{\bullet+}$, 179 $[C_9H_{11}N_2O_2]^+$, 118 $[C_8H_6O]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

***N*-Benzyl-3-{[4-(2-furoyl)-1-piperazinyl]methyl}benzamide (8a):** off white amorphous solid; yield: 79%; m.p: 130 – 132°C; Mol. F.: $C_{24}H_{25}N_3O_3$; Mol. Mass.: 403; IR (KBr, cm^{-1}) ν_{max}: 1118 (C-N-C), 1197 (C-O-C), 1584 (Ar C=C), 1658 (C=O), 2881 (R C-H), 3080 (Ar C-H), 3406 (N-H); ^{1}H-NMR (600 MHz, $CDCl_3$, δ in ppm): 8.20 (s, 1H, H-2), 8.13 (d, *J* = 7.6 Hz, H, H-6), 7.84 (br.s, 1H, H-5″), 7.82 (distorted d, *J* = 7.0 Hz, 1H, H-4), 7.79 (t, *J* = 7.6 Hz, 1H, H-5), 7.39 – 7.33 (m, 5H, H-2‴ to H-6‴), 7.33 (d, *J* = 3.3 Hz, 1H, H-3″), 6.85 (dd, *J* = 2.4, 4.1 Hz, 1H, H-4″), 3.91 (br.s, 4H, CH_2-3′, CH_2-5′), 3.69 (s, 2H, CH_2-8), 2.85 (br.t, *J* = 5.1 Hz, 4H, CH_2-2′, CH_2-6′), 2.47 (s, 2H, CH_2-7‴); ^{13}C-NMR (150 MHz, CD_3OD, δ in ppm): 168.04 (C-6″), 168.00 (C-7), 159.83 (C-6), 150.90 (C-2), 148.34 (C-5), 141.36 (C-3″), 139.06 (C-2″), 138.11 (C-1‴), 136.45 (C-4″), 132.55 (C-5″), 131.49 (C-2‴ & C-6‴), 130.39 (C-4‴), 129.63 (C-3‴ & C-5‴), 120.77 (C-3), 115.29 (C-4), 66.35 (C-8), 53.03 (C-2′, C-3′, C-5′ & C-6′), 44.15 (C-7‴); EIMS (*m/z*): 403 $[M]^+$, 308 $[C_{19}H_{22}N_3O]^+$, 279 $[C_{18}H_{19}N_2O]^+$, 268 $[C_{16}H_{16}N_2O_2]^{\bullet+}$, 225 $[C_{15}H_{15}NO]^{\bullet+}$, 179 $[C_9H_{11}N_2O_2]^+$, 118 $[C_8H_6O]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

***N*-Cyclohexyl-3-{[4-(2-furoyl)-1-piperazinyl]methyl} benzamide (8b):** light brown sticky solid; yield: 87%; Mol. F.: $C_{23}H_{29}N_3O_3$; Mol. Mass: 395; IR (KBr, cm^{-1}) ν_{max}: 1114 (C-N-C), 1208 (C-O-C), 1573 (Ar C=C), 1650 (C=O), 2877 (R C-H), 3063 (Ar C-H), 3421 (N-H); ^{1}H-NMR (600 MHz, $CDCl_3$, δ in ppm): 8.06 (s, 1H, H-2), 8.02 (d, *J* = 7.7 Hz, H, H-6), 7.84 (br.s, 1H, H-5″), 7.78 (d, *J* = 7.6 Hz, 1H, H-4), 7.72 (t, *J* = 7.6 Hz, 1H, H-5), 7.30 (d, *J* = 3.5 Hz, 1H, H-3″), 6.83 (dd, *J* = 2.0, 4.9 Hz, 1H, H-4″), 4.21 – 4.16 (m, 1H, H-1‴), 3.91 (br.s, 4H, CH_2-3′, CH_2-5′), 3.64 (s, 2H, CH_2-8), 2.86 (distorted t, *J* = 5.0 Hz, 4H, CH_2-2′, CH_2-6′), 1.72 – 1.59 (m, 10H, CH_2-2‴ to CH_2-6‴); ^{13}C-NMR (150 MHz, CD_3OD, δ in ppm): 171.76 (C-6″), 171.69 (C-7), 163.49 (C-6), 150.95 (C-2), 148.29 (C-5), 141.14 (C-3″), 138.92 (C-2″), 136.23 (C-4″), 132.40 (C-5″), 120.72 (C-3), 115.25 (C-4), 66.08 (C-8), 53.40 (C-2′, C-3′, C-5′, C-6′), 36.61 (C-1‴), 36.58 (C-2‴, C-6‴), 29.33 (C-4‴), 28.98 (C-3‴, C-5‴); EIMS (*m/z*): 395 $[M]^+$, 300 $[C_{18}H_{26}N_3O]^+$, 271 $[C_{17}H_{23}N_2O]^+$, 268 $[C_{16}H_{16}N_2O_2]^{\bullet+}$, 217 $[C_{14}H_{19}NO]^{\bullet+}$, 179 $[C_9H_{11}N_2O_2]^+$, 118 $[C_8H_6O]^{\bullet+}$, 95 $[C_5H_3O_2]^+$.

2.3. Enzyme Inhibition Assay

The enzyme inhibition activity was assessed using a method reported for butyrylcholinesterase enzyme [32]. Eserine was used as a positive control for cholinesterase enzymes. The inhibition (%) and IC_{50} were calculated by the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100.$$

2.4. Statistical Analysis

The results of triple experiments are expressed as mean ± SEM. Microsoft Excel 2010 was utilized for statistical analysis and 50% inhibitory concentration (IC_{50}) was calculated using EzFit Perrella Scientific Inc. (Amherst, USA) software.

3. RESULTS AND DISCUSSION

Two schemes have been used for the synthesis of four benzamides bearing heterocyclic furan and piperazine moieties (Schemes 1 and 2). The four synthesized compounds were subjected to screening for inhibition of butyrylcholinesterase enzyme (Table 1) followed by hemolytic activity analysis (Table 2).

3.1. Chemistry

N-Benzyl-4-[4-(2-furoyl)-1-piperazinyl]methylbenzami de (**5a**) was collected as brown liquid after extraction using chloroform as solvent with 90% yield. The EI-MS spectrum well supported the molecular formula of this compound as $C_{24}H_{25}N_3O_3$ with mass of *m/z* of 403. The significant mass fragments are also given for the final synthesized compounds (**5a, 5b**, **8a, 8b**). The prominent absorptions in IR spectrum were noted at 3412 (N-H), 3097 (Ar C-H), 2884 (R C-H), 1665 (C=O), 1588 (Ar C=C), 1195 (C-O-C) and 1110 (C-N-C).

In the typical ^{1}H-NMR spectrum (Fig. 1), the aromatic region well confirmed the presence of three aromatic moieties including furan ring, *p*-substituted benzoyl group and

TABLE 1. Enzyme Inhibition and Hemolytic Activity of Synthesized Compounds

Compound	BChE inhibition		Hemolytic activity
	Inhibition (%) at 0.5 µmole/L	IC_{50} (µmoles/L)	(%)
5a	58.13 ± 0.11	289.61 ± 0.09	5.5
5b	74.52 ± 0.11	54.81 ± 0.06	2.8
8a	56.37 ± 0.12	412.71 ± 0.09	55.47
8b	78.52 ± 0.08	52.36 ± 0.02	19.32
Eserine	**82.82** ± **1.09**	0.85 ± **0.0001**	-
Triton-X-100	-	-	100
PBS	-	-	0.09

(IC_{50}): 50% inhibitory concentration calculated through EzFit Perrella Scientific Inc. (Amherst, USA) software from results of triplicate experiments and expressed as mean ± SEM.

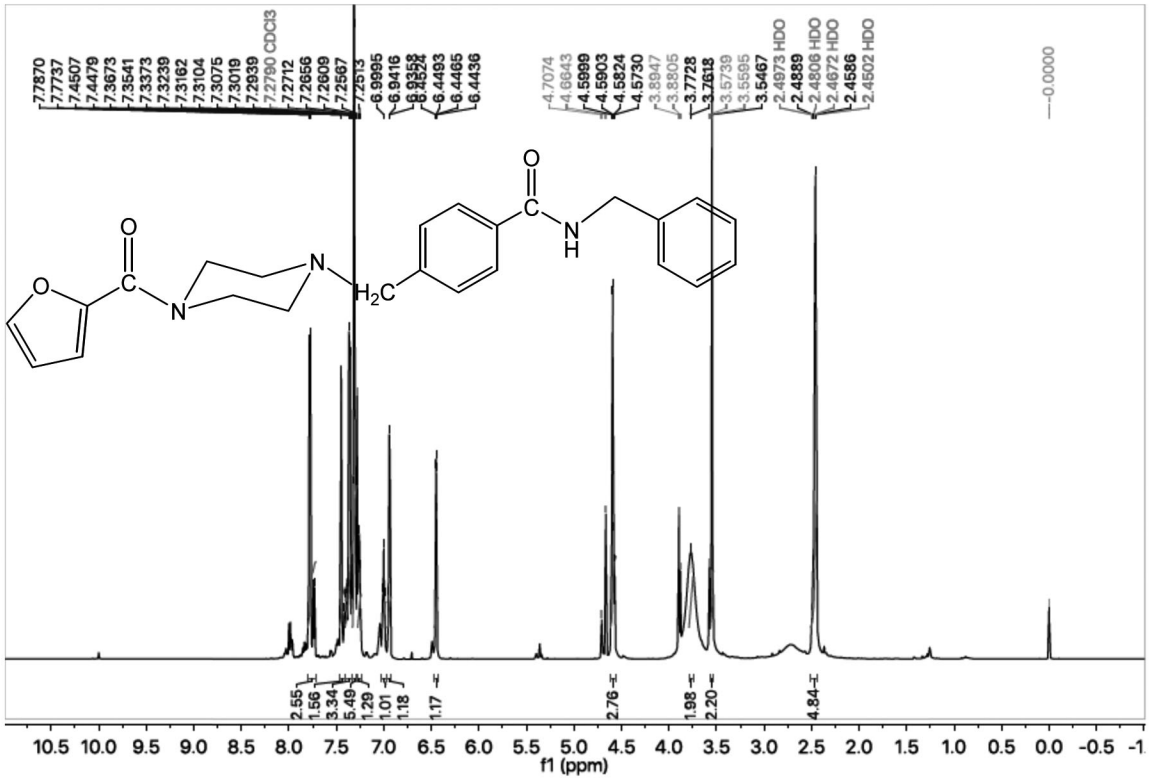


Fig. 1. ^{1}H-NMR spectrum of *N*-benzyl-4-{[4-(2-furoyl)-1-piperazinyl]methyl}benzamide (**5a**).

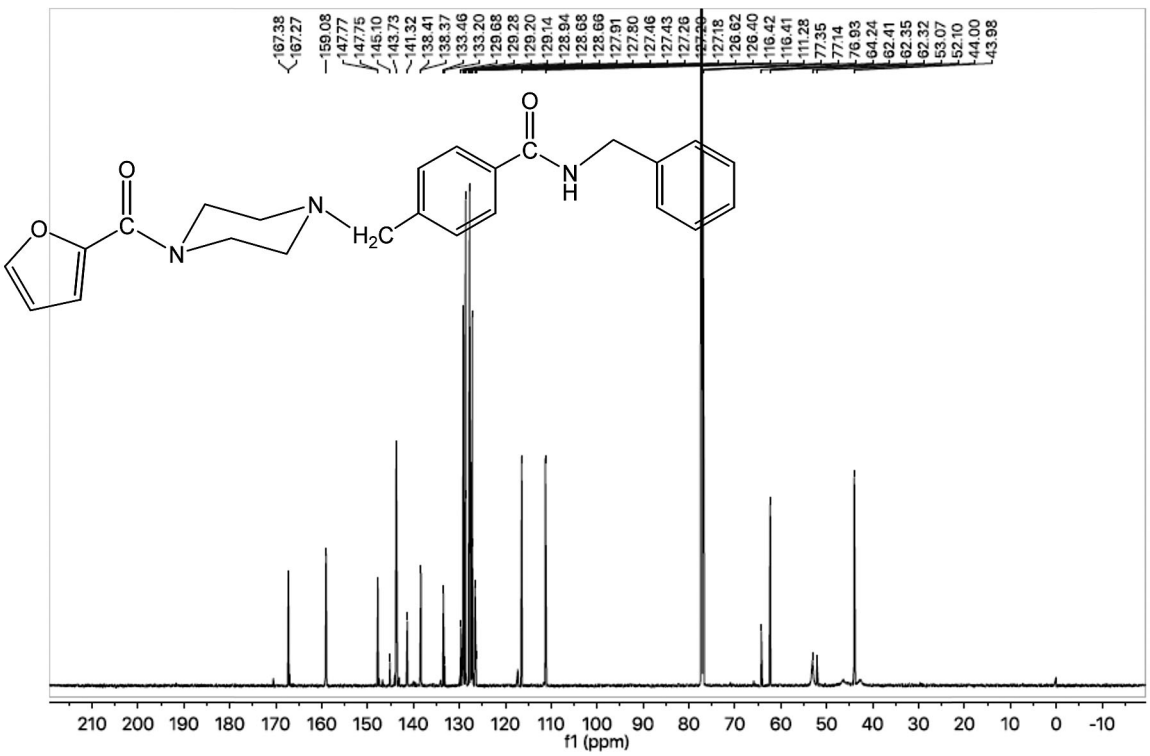


Fig. 2. ^{13}C-NMR spectrum of *N*-benzyl-4-{[4-(2-furoyl)-1-piperazinyl]methyl}benzamide (**5a**).

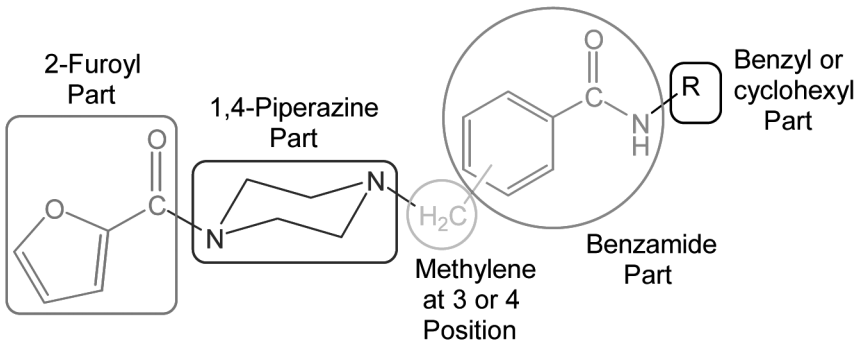


Fig. 3. General structural features of compounds **5a**, **5b**, **8a,** and **8b.**

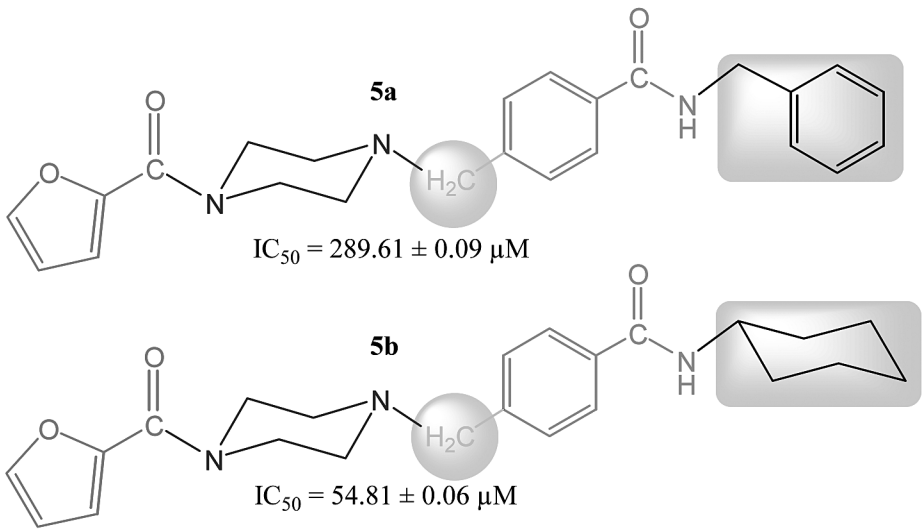


Fig. 4. Structure – activity relationship of compounds **5a** and **5b**.

benzyl group. The two doublets at δ 7.85 (d, J = 8.2 Hz, 2H, H-2 & H-6) and 7.21 (d, J = 8.4 Hz, 2H, H-3 & H-5) were allocated to four aromatic protons of 1,4-substituted benzoyl group. The three signals erecting at δ 7.45 (d, J = 1.0 Hz, 1H, H-5″), 7.03 (d, J = 2.6 Hz, 1H, H-3″) and 6.46 (dd, J = 3.4, 1.6 Hz, 1H, H-4″) were designated to three protons of furan ring. The five aromatic protons of benzyl group appeared at δ 7.38 – 7.27 (m, 5H, H-2‴ to H-6‴) as multiplet. The two signals resonating at δ 3.84 (br.s, 4H, CH_2-3′ & CH_2-5′) and 2.54 (br.s, 4H, CH_2-2′ & CH_2-6′) supported the presence of piperazine ring. The four protons attached to methylene carbons resonated as singlets at δ 3.62 (s, 2H, CH_2-8) and 2.47 (s, 2H, CH_2-7‴).

The typical ^{13}C NMR spectrum (Fig. 2) showed the most downfield signals for the quaternary carbons of two carbonyl groups at δ 167.38 (C-6″) and 167.27 (C-7). The *p*-substituted benzoyl group presented four signals for four methine carbons and two quaternary carbons at δ 159.08 (C-3 & C-5), 147.77 (C-2 & C-6), 116.41 (C-1) and 111.28 (C-4). The aromatic carbons of benzyl groups also presented four signals for five methine and one quaternary carbons at δ 141.32 (C-1‴), 129.20 (C-2‴ & C-6‴), 127.20 (C-4‴) and 126.62 (C-3‴ & C-5‴). The third moiety resonating in the aromatic region was furan ring whose three methine carbons and one quaternary carbon resonated at δ 145.10 (C-3″), 143.73 (C-2″), 138.41 (C-4″) and 133.46 (C-5″). The aliphatic region of this spectrum presented three signals nominated to methylene carbons of piperazine ring and two open chain methylene carbons. The entire spectral evidence corroborated the proposed structures of target compounds.

3.2. Enzyme Inhibition and Structure – Activity Relationship

The multi-functional molecules **5a, 5b, 8a, 8b** were synthesized and evaluated against butyryl cholinesterase (BChE). A series of such interrelated compounds have been previously reported by our research group [31]. The% inhibi-

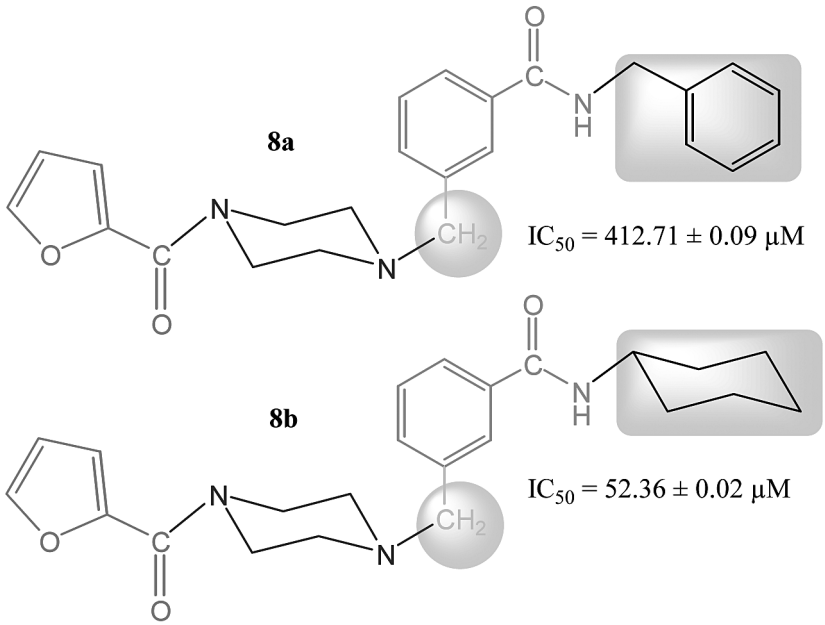


Fig. 5. Structure – activity relationship of compounds **8a** and **8b**.

tion and IC_{50} values for BChE enzyme are given in Table 1. Some of the molecules displayed promising inhibiting potential against this enzyme and well depicted from results. Though the observed activity is pertained to the cumulative effect of all functionalities embed in an entire frame work of molecule, however a brief structure activity relationship (SAR) was established by examine the effects of different entities like furoyl, piperazine and benzamide functionalities were common in these four molecules. Among these compounds, *N*-cyclohexyl-4-{[4-(2-furoyl)-1-piperazinyl]methyl}-benzamide (**5b**) and *N*-cyclohexyl-3-{[4-(2-furoyl)-1-piperazinyl]methyl}benzamide (**8b**) remained the most efficient inhibitors of BChE enzyme. The other two compounds **5a** and **8a** remained the least efficient as shown by their higher IC_{50} values. Eserine was used as reference standard having IC_{50} 0.85 ± 0.0001 μM. When we look into the structures of these molecules; we can predict that the difference lies in the *N*-substituted groups like aralkyl/aryl and position of methylene group on benzamide moiety. In compounds **5a** and **8a**, cyclohexyl group is attached to nitrogen of benzamide functionality; whereas in **5b** and **8b** benzyl group is present as *N*-substituent. In **5a** and **5b**, the methylene group is oriented para to benzamide moiety; however it is at 3rd position in case of molecules **8a** and **8b**. The general structural features of studied multifaceted compounds are presented in Fig. 3.

The presence of methylene at 3rd position of benzamide moiety lowers the IC_{50} from 252.36 ± 0.02 μmoles/L in **5b** to 54.81 ± 0.06 μmoles/L as exhibited by **8b** with respect to the reference standard, Eserine with IC_{50} value of 0.85 ± 0.0001 μmoles/L. In case of **5a** and **8a**, the occurrence of methylene group at para position to the benzamide imparted observable effect which is well illuminated from IC_{50} of compound **5a** (289.61 ± 0.09 μmoles/L), displayed better inhibition of enzyme in comparison to **8a** presenting inhibitory potential of 412.71 ± 0.09 μmoles/L (Figs. 4 and 5).

Thus, a rational from this structure – activity relationship studies was framed that molecule **8b** bearing cyclohexyl group and meta orientation of methylene moiety in the vicinity of benzamide part, was the most promising molecule in inhibiting BChE enzyme. The inhibition potential may be further enhanced by replacing the cycloalkyl group by a substituted cycloalkyl or straight chain alkyl groups. Hence, compounds **5b** and **8b** might be potent new drug candidates for BChE enzyme inhibition.

3.3. Hemolytic Activity

The hemolytic activity analysis also confirmed the low toxicity of **5b** molecule by showing red blood cells lysis of only 2.8%, which is much lower as compared to the toxicity of Triton-X-100 and close to PBS having a low toxicity of 0.09%. Compound **8b** exhibited some toxicity but that might be tolerated, which can be confirmed by further *in vivo* studies. Both these molecules should be subjected to *in vivo* analysis for further confirmation of these compounds as new drug candidates for the diseases caused by indicated enzymes.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. P. Bruder, A. Grimstvedt, T. Mejdell, et al., *Chem. Eng. Sci.,* **66**, 6193 – 6198 (2011).
2. I. Yamaguchi, A. Kado, T. Fukuda, et al., *Eur. Polym. J.*, **46**, 1119 – 1130 (2010).
3. P. Chaudhary, R. Kumar, A. K. Verma, et al., *Bioorg. Med. Chem.*, **14**, 1819 – 1826 (2006).
4. C. T. Sadashiva, J. N. N. S. Chandra, K. C. Ponnappa, et al., *Bioorg. Med. Chem. Lett.*, **16**, 3932 – 3936 (2006).
5. D. Dou, G. He, S. R. Mandadapu, et al., *Bioorg. Med. Chem. Lett.*, **22**, 377 – 379 (2012).
6. M. Han, Y. Han, C. Song, and H. Hahn, *Bull. Korean. Chem. Soc.*, **33**, 2597 – 2602 (2012).
7. E. M. Moir, K. Yoshiizumi, J. Cairns, et al., *Bioorg. Med. Chem. Lett.*, **20**, 7327 – 7330 (2010).
8. D. Nozawa, T. Okubo, T. Ishii, et al., *Bioorg. Med. Chem.*, **15**, 1989 – 2005 (2007).
9. G. Autore, A. Caruso, S. Marzocco, et al., *Molecules*, **15**, 2028 – 2038 (2010).
10. G. Ayhan-Kilcigil, S. Gurkan, T. Coban, et al., *Chem. Biol. Drug Des.*, **79**, 869 – 877 (2102).
11. H. Jawed, S. U. A. Shah, S. Jamall, and S. U. Simjee, *Int. Immunopharmacol.*, **10**, 900 – 905 (2010).
12. V. Kanagarajan, J. Thanusu, and M. Gopalakrishnan, *Eur. J. Med. Chem.*, **45**, 1583 – 1589 (2010).
13. R. Sawant and D. Kawade, *Acta Pharm.*, **61**, 353 – 361 (2011).
14. J. S. Carey, D. Laffan, C. Thomson, and M. T. Williams, *Org. Biomol. Chem.*, **4**, 2337 – 2347 (2006).
15. A. K. Ghose, V. N. Viswanadhan, and J. J. J. Wendoloski, *Comb. Chem.*, **1**, 55 – 68 (1999).
16. V. Pattabiraman and J. W. Bode, *Nature.*, **480**, 471 – 479 (2011).
17. E. Valeur, and M. Bradley, *Chem. Soc. Rev.*, **38**, 606 – 631 (2009).
18. A. A. G. N. Montalbetti, and V. Falque, *Tetrahedron*, **61**, 10827 – 10852 (2005).
19. S. Y. Han and Y. A. Kim, *Tetrahedron*, **60**, 2447 – 2467 (2004).
20. T. Kaicharla, M. Thangaraj, and A, T. Biju, *Org. Lett.*, **16**, 1728 – 1731 (2014).
21. G. Caliendo, V. Santagada, E. Perissuti, et al., *Eur. J. Med. Chem.*, **36**, 517 – 530 (2001).
22. W. N. Chan, M. S. Hadley, J. D. Harling, et al., *Bioorg. Med. Chem. Lett.*, **8**, 2903 – 2906 (1998).
23. S. J. Coats, M. J. Schulz, J. R. Carson, and E. E. Codd, *Bioorg. Med. Chem. Lett.*, **14**, 5493 – 5498 (2004).
24. J. R. Carrsoii, S. J. Coats, E. E. Codd, et al., *Bioorg. Med. Chem. Lett.*, **14**, 2109 – 2112 (2004).
25. M. Asif, *Mod. Chem. Appl.*, **4**, 1 – 10 (2016).
26. D. L. McMinn, Y. Rew, A. Sudom, et al., *Bioorg. Med. Chem. Lett.*, **19**, 1446 – 1450 (2009).
27. R. J. Knox, F. Friedlos, M. Jarman, and J. J. Roberts, *Biochem. Pharmacol.*, **37**, 4661 – 4669 (1988).
28. P. A. C. Bragay, D. A. P. Dos-Santos, M. F. D. G. F. Da-Silva, et al., *Nat. Prod. Res.*, **21**, 47 – 55 (2007).
29. A. Jason, D. Quinn, H. Garret, and K. Fred, *US Patent*., **7**, 125 – 997 (2006).
30. S. Gauthier, *Drug Aging*, **18**, 853 – 862 (2001).
31. M. A. Abbasi, A. Anwar, Aziz-ur-Rehman, et al., *Pak. J. Pharm. Sci*., **30**, 1715 – 1724 (2017).
32. G. L. Ellman, K. D. Courtney, V. Andres, and R. M. Featherstone, *Bio. Pharm*., **7**, 88 – 90 (1961).