2-Phenitidine derivatives as suitable inhibitors of butyrylcholinesterase

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This manuscript reports the synthesis of a series of *N*-substituted derivatives of 2-phenitidine. First, the reaction of 2-phenitidine (**1**) with benzene sulfonyl chloride (**2**) yielded *N*-(2-ethoxyphenyl) benzenesulfonamide (**3**), which further on treatment with sodium hydride and alkyl halides (**4a-g**) furnished into new sulfonamides (**5a-g**). Second, the phenitidine reacted with benzoyl chloride (**6**) and acetyl chloride (**8**) to yield the reported *N*-benzoyl phenitidine (**7**) and *N*-acetyl phenitidine (**9**), respectively. These derivatives were characterized by infrared spectroscopy, ¹H-NMR, and EI-MS, and then screened against acetylcholinesterase, butylcholinesterase, and lipoxygenase enzyme, and were found to be potent inhibitors of butyrylcholinesterase alone.

Uniterms: 2-Phenitidine/inhibitor activity. Sulfonamides. Acetamide. Benzamide. Butyrylcholinesterase.

Este trabalho apresenta a síntese de uma série de derivados da 2-fenetidina *N*-substituídos. Primeiro, a reação da 2-fenetidina (**1**) com cloreto de benzenossulfonila (**2**) conduziu à *N*-(2-etoxifenil) benzenossulfonamida (**3**) que, após tratamento com hidreto de sódio e haletos de alquila (**4a-g**), originou novas sulfonamidas (**5a-g**). Em segundo lugar, a reação da fenetidina com cloreto de benzoíla (**6**) e cloreto de acetila (**8**) conduziu, respectivamente, à *N*-benzoilfenetidina (**7**) e *N*-acetilfenetidina (**9**). A caracterização destes derivados fez-se por IV, ¹H-RMN e EM-IE. Procedeu-se à avaliação da atividade inibidora destes compostos em relação às enzimas acetilcolinesterase, butirilcolinesterase e lipoxigenase. No entanto, apenas revelaram atividade inibidora da butirilcolinesterase.

Unitermos: 2-Fenetidina/atividade inibidora. Sulfonamidas. Acetamida. Benzamida. Butirilcolinesterase.

INTRODUCTION

Compounds bearing the -SO₂-NH- group (the sulfonamide group) have long been known to be potent inhibitors of carbonic anhydrase (CA) (Remko *et al.,* 2010; Supuran *et al.*, 2003), and are widely used as antibacterial agents. Synthesis of sulfonamides involves the nucleophilic attack of ammonia or amine on a sulfonyl halide (Chan *et al.,* 2002; Shaabani *et al.,* 2007). On the other hand, arylsulfonyl azides can be reduced to convert into arylsulfonamides (Boruah *et al.,* 1997). It is also known that aryl sulfonamides act as antitumor agents through the perturbation of the cell cycle in the G1 phase, during angiogenesis inhibition or distribution of the microtubule assembly (Saczewski *et al.*, 2008; Garaj *et al.*, 2005; Saczewski *et al.*, 2006; Supuran *et al.*, 2003). Various sulfonamides have also been reported to act as antitumor agents through carbonic anhydrase (CA) inhibition (Supuran *et al.*, 2003; Supuran *et al.*, 2004; Kivela *et al.*, 2005; Supuran *et al.*, 2007).

Acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8) consist of a family of enzymes, which include serine hydrolases. The different specificities for the substrates and the inhibitors for these enzymes are due to the differences in the amino acid residues of the active sites of AChE and BChE. The enzyme

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system is responsible for the termination of acetylcholine at the cholinergic synapses. These are key components of cholinergic brain synapses and neuromuscular junctions. The major functions of AChE and BChE are to catalyze the hydrolysis of the neurotransmitter acetylcholine, and to terminate the nerve impulse at the cholinergic synapses (Cygler *et al.*, 1993; Tougu *et al.*, 2001). It has been found that BChE (E.C 3.1.1.8) inhibition is an effective tool for the treatment of Alzheimer's disease and related dementias. BChE is found in significantly higher quantities in Alzheimer's plaques than in plaques found among normal age-related brains without dementia. BChE is produced in the liver and enriches blood circulation. In addition, it is also present in adipose tissue, and can also be found in the intestine, smooth muscle cells, white matter of the brain, and in many other tissues (Gauthier *et al.*, 2001). Therefore, the search for new cholinesterase inhibitors is considered an important and ongoing strategy in the introduction of new drug candidates for the treatment of Alzheimer's disease and other related diseases (Bertaccini *et al.*, 1982).

In this work, we report on the various *N*-substituted derivatives of 2-phenitidine. First, a new parent sulfonamide [*N*-(2-ethoxyphenyl)benzenesulfonamide] was prepared by reacting benzenesulfonyl chloride with 2-phenitidine at room temperature in excellent yield. Simple stirring in basic media gave the desired compound that was further processed to obtain different new *N*-alkyl substituted sulfonamides, and then 2-phenitidine was also acylated with benzoyl chloride and acetyl chloride, which have already been reported (Downer, Jackson, 2007; Smith *et al.*, 2003).

A survey of the literature revealed that slight modifications in the structure can result in qualitative as well as quantitative changes in activity, which prompted us to undertake the synthesis of various 2-phenitidine derivatives and to study their structure-activity relationship by screening them against DPPH, AChE, BChE, and lipoxygenase (LOX), and these were found to be active against BChE, and were also possible entrants for the treatment of Alzheimer's disease.

MATERIALS AND METHODS

General

Thin-layer chromatography (TLC) was performed on pre-coated silica gel G-25-UV$_{254}$ plates. Detection was carried out at 254 nm with a ceric sulphate reagent. Purity was checked on TLC with different solvent systems using ethyl acetate and *n*-hexane, giving a single spot. The infra-red (IR) spectra were recorded in KBr on a Jasco-320-A spectrophotometer (wave number in cm^{-1}). ^{1}H-NMR spectra were recorded in CDCl$_3$ on a Bruker spectrometer operating at 400 MHz. Chemical shifts are given in ppm. Mass spectra (EI-MS) were recorded on a JMS-HX-110 spectrometer with a data system. The melting points were recorded on a Griffin and George melting point apparatus by open capillary tube, and these were uncorrected.

Procedure for the synthesis of sulfonamide in aqueous media

The nucleophilic substitution reaction of amine with benzene sulfonyl chloride was carried out as follows: a mixture of benzenesulfonyl chloride (10.0 mmol; 1.27 mL) and 2-phenitidine (10.0 mmol; 1.43 mL) was suspended in 25 mL of water. The pH of the suspension was adjusted and was maintained at 9.0 by adding a basic aqueous solution at room temperature. The reaction solution was stirred and monitored with TLC, and it took 2 hours for the completion of the reaction. Then, concentrated HCl was gradually added to adjust the pH to 2.0. The precipitates were collected by filtration, washed with distilled water, and dried to afford the title compound **3**. The product was dissolved in methanol and recrystallized by slow evaporation of the solvent in order to generate colorless, bead-like crystals of *N*-(2-ethoxyphenyl) benzenesulfonamide (yield 89%; m.p. 88 °C).

General procedure for the synthesis of *N*-alkyl substituted sulfonamides in DMF

The calculated amount of 3 (0.1 mmol) was taken in a round-bottomed flask (50 mL), and dimethyl formamide (DMF; 10 mL) was added to dissolve it; sodium hydride (0.1 mmol) was then added to the mixture. The mixture was stirred for 30 minutes at room temperature, then the alkyl halide was slowly added to the mixture, and the solution was stirred for a further 3 hours. The progress of the reaction was monitored via TLC till single spot. The product was precipitated by adding water. It was filtered, washed with water, and crystallized from aqueous methanol.

General procedure for the synthesis of *N*-acylated derivatives

The calculated amount of 1 was taken in the round-bottomed flask (50 mL), then a small amount of sodium carbonate (Na$_2$CO$_3$) was added to make the medium basic. Acyl chloride (8 and 10 separately) was then added, and

the solution was shaken vigorously. The evolved CO_2 was liberated at intervals. The reaction progress was monitored with TLC till single spot. After completion of the reaction, water was added, and the precipitates that were formed were filtered and dried.

Acetylcholinesterase assay

AChE inhibition activity was performed according to the method of Ellman *et al.* (1961) with slight modifications. The total volume of the reaction mixture was 100 µL. It contained 60 µL of Na_2HPO_4 buffer with a concentration of 50 mM and a pH of 7.7. Following this, 10 µL of the test compound (0.5 mM well^{-1}) was added, followed by the addition of 10 µL (0.005 unit well^{-1}) of enzyme. The contents were mixed and preread at 405 nm, then contents were preincubated for 10 minutes at 37 ºC. The reaction was initiated by the addition of 10 µL of 0.5 mM well^{-1} substrate (acetylthiocholine iodide), followed by the addition of 10 µL of DTNB (0.5 mM well^{-1}). After 15 minutes of incubation at 37ºC, absorbance was measured at 405 nm. A Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM well^{-1}) was used as a positive control. The percent of inhibition was calculated using the following equation:

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

Butyrylcholinesterase assay

BChE inhibition activity was performed according to the method of Ellman *et al.* (1961) with slight modifications. The total volume of the reaction mixture was 100 µL containing 60 µL of the Na_2HPO_4 buffer (50 mM and a pH of 7.7). A total of 10 µL of the test compounds of 0.5 mM well^{-1} were added, followed by the addition of 10 µL (0.5 unit well^{-1}) of BChE. The contents were mixed and preread at 405 nm and then preincubated for 10 minutes at 37 ºC. The reaction was initiated by the addition of 10 µL of the 0.5 mM well^{-1} substrate (butyrylthiocholine bromide) followed by the addition of 10 µL of DTNB, 0.5 mM well^{-1}. After 15 minutes of incubation at 37 ºC, absorbance was measured at 405 nm. A Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM well^{-1}) was used as a positive control. The percent of inhibition was calculated by the help of following equation:

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

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

Lipoxygenase assay

LOX activity was assayed according to the reported method (Clapp *et al.*, 1985; Kemal *et al.*, 1987), but with slight modifications. A total volume of 200 µL of the assay mixture contained 150 µL sodium phosphate buffer (100 mM, pH 8.0), 10 µL of the test compound, and 15 µL of the purified LOX enzyme (Sigma, USA). The contents were mixed and preread at 234 nm, and then preincubated for 10 minutes at 25 °C. The reaction was initiated by the addition of 25 µL of substrate solution. The change in absorbance was observed after 6 minutes at 234 nm. A Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All reactions were performed in triplicate. The positive and negative controls were included in the assay. Quercetin (0.5 mM well^{-1}) was used as a positive control. The percentage of inhibition was calculated by the formula given below:

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

where Control = Total enzyme activity without inhibitor; Test = Activity in the presence of the test compound.

DPPH assay

The stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for the determination of antioxidant activity. Different concentrations of the compounds in their respective solvents were added at an equal volume (10 µL) to 90 µL of 100 µM of methanolic DPPH in a total volume of 100 µL in 96-well plates. The contents were mixed and incubated at 37 ºC for 30 minutes. The absorbance was measured at 517 nm using a Synergy HT BioTek® USA microplate reader. Quercetin and L-ascorbic acid were used as standard antioxidants. The experiments were carried out in triplicate. IC_{50} values were calculated using the EZ-Fit5 (Perrella Scientific Inc., Amherst, USA) software. The decrease in absorbance indicates increased radical scavenging activity, which was determined by the following formula (Huang *et al.,* 2005):

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

Structural characterization

N-(2-Ethoxyphenyl)benzenesulfonamide (3)

Dark brown beads, yield 89%, m.p. 88 °C; IR (KBr): ν_{max}: 3430 (N-H), 3056 (Ar-H), 1341 (-SO$_2$NH-), 1258 (Ar-O-R); EIMS: m/z 277 [M]$^+$, 232 [M-OC$_2$H$_5$]$^+$, 213 [M-SO$_2$]$^+$, 141[C$_6$H$_5$SO$_2$]$^+$.

N-Methyl-N-(2-ethoxyphenyl)benzenesulfonamide (5a)

Dark brown powder, yield 83%, m.p. 70 °C; IR (KBr): ν_{max}: 3466 (N-R), 3040 (Ar-H), 1344 (-SO$_2$NR-), 1279 (Ar-O-R); EIMS: m/z 291 [M]$^+$, 246 [M-OC$_2$H$_5$]$^+$, 227 [M-SO$_2$]$^+$, 141 [C$_6$H$_5$SO$_2$]$^+$.

N-Ethyl-N-(2-ethoxyphenyl)benzenesulfonamide (5b)

Dark brown powder, yield 86%, m.p. 54 °C IR (KBr): ν_{max}: 3401 (N-R), 3031 (Ar-H), 1343 (-SO$_2$NR-), 1260 (Ar-O-R); EIMS: m/z 305 [M]$^+$, 260 [M-OC$_2$H$_5$]$^+$, 241 [M-SO$_2$]$^+$, 141 [C$_6$H$_5$SO$_2$]$^+$.

N-Isopropyl-N-(2-ethoxyphenyl)benzenesulfonamide (5c)

Dark brown powder, yield 81%, m.p. 60 °C. IR (KBr): ν_{max}: 3432 (N-R), 3075 (Ar-H), 1323 (-SO$_2$NR-), 1268 (Ar-O-R); EIMS: m/z 319 [M]$^+$, 274 [M-OC$_2$H$_5$]$^+$, 255 [M-SO$_2$]$^+$, 141 [C$_6$H$_5$SO$_2$]$^+$.

N-Propyl-N-(2-ethoxyphenyl)benzenesulfonamide (5d)

Dark brown powder, yield 89%, m.p. 56 °C IR (KBr): ν_{max}: 3467 (N-R), 3065 (Ar-H), 1343 (-SO$_2$NR-) 1255 (Ar-O-R); EIMS: m/z 319 [M]$^+$, 264 [M-OC$_2$H$_5$]$^+$, 255 [M-SO$_2$]$^+$, 141 [C$_6$H$_5$SO$_2$]$^+$.

N-Butyl-N-(2-ethoxyphenyl) benzenesulfonamide (5e)

Dark brown powder, yield 82%, m.p. 59 °C. IR (KBr): ν_{max}: 3484 (N-R), 3049 (Ar-H), 1315 (-SO$_2$NR-), 1286 (Ar-O-R); EIMS: m/z 333 [M]$^+$, 288 [M-OC$_2$H$_5$]$^+$, 269 [M-SO$_2$]$^+$, 141 [C$_6$H$_5$SO$_2$]$^+$.

N-Pentyl-N-(2-ethoxyphenyl)benzenesulfonamide (5f)

Dark brown powder, yield 84%, m.p. 58 °C. IR (KBr): ν_{max}: 3452 (N-R), 3063 (Ar-H), 1311 (-SO$_2$NR-), 1263 (Ar-O-R); EIMS: m/z 347 [M]$^+$, 302 [M-OC$_2$H$_5$]$^+$, 283 [M-SO$_2$]$^+$, 141 [C$_6$H$_5$SO$_2$]$^+$.

N-Allyl-N-(2-ethoxyphenyl)benzenesulfonamide (5g)

Gummy solid, yield 80%. IR (KBr): ν_{max}: 3488 (N-R), 3286 (Ar-H), 1313 (-SO$_2$NR-), 1255 (Ar-O-R); EIMS: m/z 317 [M]$^+$, 272 [M-OC$_2$H$_5$]$^+$, 253 [M-SO$_2$]$^+$, 141 [C$_6$H$_5$SO$_2$]$^+$.

N-Benzoyl-2-ethoxyaniline (7)

Gummy solid, yield 83%. IR (KBr): ν_{max}: 3429 (N-H), 3067 (Ar-H), 1253 (Ar-O-R); EIMS: m/z 241 [M]$^+$, 196 [M-OC$_2$H$_5$]$^+$, 213 [M-CO]$^+$, 105 [C$_6$H$_5$CO]$^+$.

N-Acetyl-2-ethoxyaniline (9)

Gummy solid, yield 87%. IR (KBr): ν_{max}: 3424 (N-H), 3034 (Ar-H), 1244 (Ar-O-R); EIMS: m/z 179 [M]$^+$, 134 [M-OC$_2$H$_5$]$^+$, 151 [M-CO]$^+$, 43 [CH$_3$CO]$^+$.

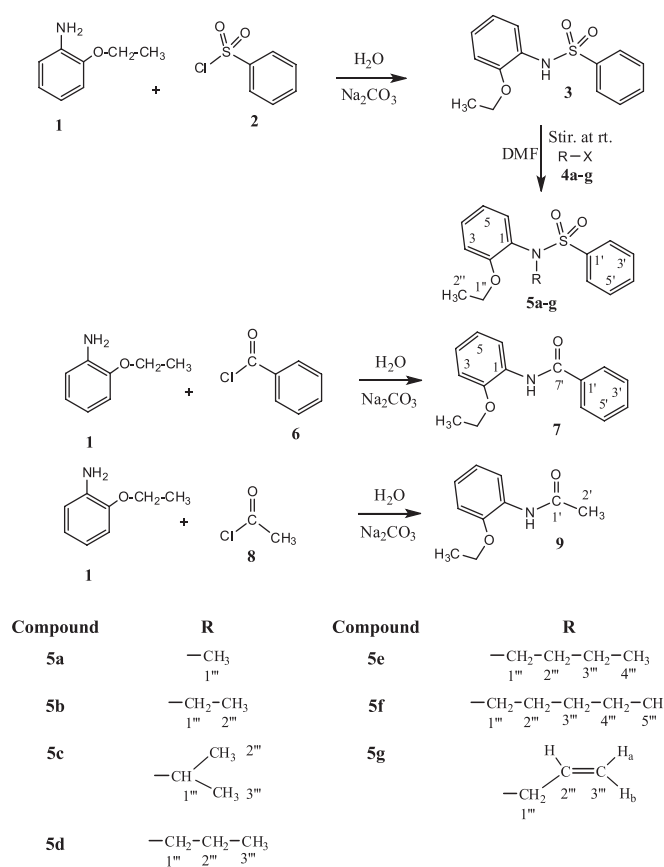| Compound	R	Compound	R
5a	—CH$_3$ 1'''	5e	—CH$_2$—CH$_2$—CH$_2$—CH$_3$ 1''' 2''' 3''' 4'''
5b	—CH$_2$—CH$_3$ 1''' 2'''	5f	—CH$_2$—CH$_2$—CH$_2$—CH$_2$—CH$_3$ 1''' 2''' 3''' 4''' 5'''
5c	—CH(CH$_3$ 2''')(CH$_3$ 3''') 1'''	5g	—CH$_2$—CH═CH$_2$
5d	—CH$_2$—CH$_2$—CH$_3$ 1''' 2''' 3'''		

FIGURE 1 - Synthesis of 2-phenitidine derivatives 5a-g, 7, and 9.

RESULTS AND DISCUSSION

The designed *N*-substituted new derivatives of 2-phenitidine were synthesized according to Figure 1. The parent compound **3** was synthesized as dark brown beads. The molecular formula, C$_{14}$H$_{15}$NO$_3$S, was established with the help of EI-MS showing a molecular ion peak at m/z 277, and by counting the number of protons in the ^{1}H-NMR spectrum (Table-I). Its IR spectrum revealed the presence of an ethoxy group (1258 cm^{-1}), a sulfonyl group

TABLE I - ¹H-NMR^a data (CDCl$_3$) of compounds **3, 5a-g, 7, 9**. δ in ppm, J in Hz

C.No.	3	5a	5b	5c	5d	5e	5f	5g	7	9
	δ (H)	δ (H)	δ (H)	δ (H)	δ (H)	δ (H)	δ (H)	δ (H)	δ (H)	δ (H)
1	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
3	6.67 (dd, J = 1.0, 7.6)	6.74 (dd, J = 1.0, 8.0)	6.74 (dd, J = 1.2, 8.4)	6.68 (dd, J = 1.0, 8.0)	6.73 (dd, J = 1.0, 8.4)	6.72 (dd, J = 1.0, 8.4)	6.74 (dd, J = 1.0, 8.4)	6.8 (dd, J = 1.0, 8.0)	6.88 (dd, J = 1.2, 7.6)	6.84 (dd, J = 1.0, 7.6)
4	7.00 (ddd, J = 1.2, 7.6, 8.0)	7.24 (ddd, J = 1.6, 7.6, 8.0)	7.24 (ddd, J = 1.6, 7.6, 8.0)	7.28 (ddd, J = 1.6, 8.4, 8.0)	7.24 (ddd, J = 1.6, 7.6, 8.0)	7.20 (ddd, J = 1.6, 8.0, 8.0)	7.24 (ddd, J = 1.6, 8.0, 8.4)	7.21 (ddd, J = 1.6, 8.4, 8.4)	7.04 (ddd, J = 2.0, 7.6, 8.0)	6.98 (ddd, J = 1.2, 7.6, 8.0)
5	6.87 (ddd, J = 1.2, 7.6, 8.0)	6.91 (ddd, J = 1.6, 8.0, 8.0)	6.91 (ddd, J = 1.2, 7.6, 7.6)	6.89 (ddd, J = 1.2, 7.6, 7.6)	6.91 (ddd, J = 1.2, 7.6, 7.6)	6.91 (ddd, J = 1.0 7.2, 7.6)	6.91 (ddd, J = 1.2, 7.6, 7.6)	6.87 (ddd, J = 1.2 ,7.6, 8.4)	6.99 (ddd, J = 1.6, 7.6, 7.6)	6.91 (ddd, J = 1.2, 7.6, 8.0)
6	7.52 (dd, J = 1.2, 7.6)	7.36 (dd, J = 2.0, 8.0)	7.31 (dd, J = 1.6, 7.6)	7.13 (dd, J = 1.6, 7.6)	7.30 (dd, J = 2.0, 8.0)	7.31 (dd, J = 2, 8.0)	7.32 (dd, J = 1.6, 7.6)	7.27 (dd, J = 1.6, 7.6)	8.52 (dd, J = 1.6, 7.6)	8.33 (br d, J = 7.6)
1'	-	-	-	-	-	-	-	-	-	2.18 (s)
2'	7.71 (dd, J = 1.6, 8.8)	7.66 (dd, J = 1.6, 8.8)	7.66 (dd, J = 1.2, 8.4)	7.82 (dd, J = 1.2, 8.4)	7.63 (dd, J = 1.2, 7.2)	7.63 (dd, J = 1.2, 7.2)	7.63 (dd, J = 1.2, 8.8)	7.63 (dd, J = 1.2, 8.4)	7.88 (dd, J = 1.6, 8.4)	-
3'	7.36 (br t, J = 7.6)	7.41 (br t, J = 7.6)	7.38 (br t, J = 7.2)	7.41 (br t, J = 7.2)	7.38 (br t, J = 8.0)	7.39 (br t, J = 7.6)	7.38 (br.t, J = 7.6)	7.38 (br t, J = 7.2)	7.45 (br t, J = 8.6)	-
4'	7.47 (br t, J = 7.6)	7.51 (br t, J = 7.6)	7.48 (br t, J = 7.6)	7.49 (br t, J = 7.6)	7.48 (br t, J = 7.6)	7.49 (br t, J = 7.6)	7.49 (br.t, J = 7.6)	7.48 (br t, J = 7.6)	7.54 (br t, J = 8.2)	-
5'	7.36 (br t, J = 7.6)	7.41 (br t, J = 7.6)	7.38 (br t, J = 7.2)	7.41 (br t, J = 7.2)	7.38 (br t, J = 8.0)	7.39 (br t, J = 7.6)	7.38 (br.t, J = 7.6)	7.38 (br t, J = 7.2)	7.45 (br t, J = 8.6)	-
6'	7.71 (dd, J = 1.6, 8.8)	7.66 (dd, J = 1.6, 8.8)	7.66 (dd, J = 1.2, 8.4)	7.82 (dd, J = 1.2, 8.4)	7.63 (dd, J = 1.2, 7.2)	7.63 (dd, J = 1.2, 7.2)	7.63 (dd, J = 1.2, 8.8)	7.63 (dd, J = 1.2, 8.4)	7.88 (dd, J = 1.6, 8.4)	-
1"	3.78 (q, J = 7.2)	3.64 (q, J = 6.8)	3.64 (m, 2H)	3.83 (q, J = 6.8)	3.59 (m, 4H)	3.59 (m, 2H, CH2-1″),	3.59 (m)	4.20 (m)	4.14 (q, J = 6.8)	-
2"	1.22 (t, J = 7.2)	0.93 (t, J = 6.8)	0.94 (t, J = 7.2)	1.20 (t, J = 6.8)	0.93 (t, J = 6.8)	0.93 (t, J = 6.8)	0.93 (t, J = 6.8)	0.92 (t, J = 6.8)	1.47 (t, J = 6.8)	-
1‴	-	3.21(s)	3.64 (m, CH$_2$-1″)	4.35 (septet, J = 6.4)	3.59 (m, 4H)	3.59 (m)	3.59 (m)	4.20 (m)		4.09 (q, J = 6.8)
2‴	-	-	1.05 (t, J = 7.2)	1.08 (d, J = 6.4)	1.30 (m)	1.38 (m)	1.40 (m)	5.76 (m)		1.43 (t, J = 6.8)
3‴	-	-	-	1.08 (d, J = 6.4)	0.83 (t, J = 7.2)	1.32 (m)	1.26 (m)	5.03 (dd, J =1.6, 17.3 H$_b$) & , 4.96 (dd , J =1.2, 10 Hz, H$_a$)	-	-
4‴	-	-	-	-	-	0.83 (t, J =7.2)	1.24 (m)	-	-	-
5‴	-	-	-	-	-	-	0.82 (t, J = 7.2)	-	-	-

^{a} All spectra were recorded at 400 MHz.

(1341 cm^{-1}), and a –NH group (3430 cm^{-1}) in the molecule. The EI-MS also gave a distinct peak at *m/z* 213 after the removal of the –SO$_2$ group, and a further two fragments of ion peaks were observed at *m/z* 232 [M-OC$_2$H$_5$]$^+$ and 141 [C$_6$H$_5$SO$_2$]$^+$, which showed the presence of ethoxy and benzene sulfonyl groups, respectively, in the molecule. In its ¹H-NMR spectrum, the signals in the aromatic region appeared at δ 7.52 (dd, J = 1.2, 7.6 Hz, H-6), 7.00 (ddd, J = 1.2, 7.6, 8.0 Hz, H-4), 6.87 (ddd, J = 1.2, 7.6, 8.0 Hz, H-5), and 6.67 (dd, J = 1.0, 7.6 Hz, H-3), which were assigned to the protons of the disubstituted ring of phenitidine, and the signals appearing at δ 7.71 (dd, J = 1.6, 8.8 Hz, 2H, H-2' & H-6'), 7.47 (brt, J = 7.6 Hz, H-4'), and 7.36 (br t, J = 7.6 Hz, H-3' & H-5') were typical for the protons of the mono-substituted ring derived from benzenesulphonyl chloride. Moreover, a characteristics quartet at δ 3.78 (q, J = 7.2 Hz,

TABLE II - Enzyme inhibition studies of 2-phenitidine derivatives (n = 3, mean ± SEM)

Compound No.	DPPH		AChE		BChE		LOX	
	(%) at 0.5 mM	(IC_{50}) µmoles	(%) at 0.5 mM	(IC_{50}) µmoles	(%) at 0.5 mM	(IC_{50}) µmoles	(%) at 0.5 mM	(IC_{50}) µmoles
3	9.10±0.59	Nil	38.44±0.47	Nil	72.05±0.98	68±0.99	13.48±0.66	Nil
5a	14.66±0.78	Nil	40.39±0.95	Nil	81.84±0.87	132±1.01	4.24±0.45	Nil
5b	14.59±0.75	Nil	50.65±0.83	> 500	82.18±0.45	10±0.85	4.02±1.06	Nil
5c	17.86±0.91	Nil	37.79±1.01	Nil	90.51±1.02	07±0.81	3.70±0.92	Nil
5d	25.30±0.84	Nil	36.49±0.93	Nil	78.14±1.11	72±1.00	15.87±0.72	Nil
5e	27.87±0.73	Nil	34.68±0.89	Nil	69.67±0.98	131±0.69	18.48±0.76	Nil
5f	46.56±1.02	Nil	36.10±0.77	Nil	66.67±0.65	169±0.77	10.54±0.84	Nil
5g	16.89±0.59	Nil	47.01±0.84	Nil	83.35±0.59	78±0.78	13.59±0.69	Nil
7	22.86±0.68	Nil	30.65±0.91	Nil	77.60±0.48	141±1.03	59.78±0.93	257±0.88
9	10.42±1.01	Nil	31.95±1.00	Nil	60.81±0.98	298±1.10	13.26±0.85	Nil
Control	Quercetin	16.96±0.14	Eserine	0.04±0.001	Eserine	0.85±0.001	Quercetin	37.12±0.07

Note: DPPH = 1,1-diphenyl-2-picrylhydrazyl radical

2H, H_2-1") and a triplet at δ 1.22 (t, $J = 7.2$ Hz, 3H, H_3-2") revealed the presence of an ethoxy group in the molecule. On the basis of the cumulative evidence reported above, the structure of **3** was assigned as N-(2-ethoxyphenyl) benzenesulfonamide, which is a new sulfonamide. Similarly, on the basis of spectral evidences from IR, EI-MS (experimental section) and, ^{1}H-NMR (Table I), the structures of other derivatives of 2-phenitidine were elucidated as described in Figure 1.

The screening of these derivatives showed that these were inactive against LOX and AChE, but they exhibited moderate inhibitory potential against BChE, as evident from their IC_{50} values (Table II). Among these, N-isopropyl-N-(2-ethoxyphenyl) benzenesulfonamide (**5c**) was found to be the most suitable inhibitor, having an IC_{50} value of 07 ± 0.81 µmoles/L relative to eserine, a reference standard, with an IC_{50} value of 0.85 ± 0.001 µmoles/L, probably due to the substitution of the isopropyl group (a branched chain) in this molecule. Antioxidant activity using the DPPH method showed little antioxidant activity of these compounds; the highest activity was shown by 5f (46.56% ± 1.02% at 0.5 mM) and the lowest activity by parent compound 3 (9.10% ± 0.59% at 0.5 mM). All other compounds showed antioxidant activity between these two extremes. The present investigation generally concluded that the synthesized 2-phenitidine derivatives, 5a-5g, 7, and 9 – due to their suitable structural features – could be used as valuable inhibitors of BChEs, and that these are also ideally suited for further structural modification to obtain more potent and less cytotoxic therapeutic agents for the treatment of Alzheimer's disease.

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