



Synthesis, Characterization and In Vitro Acetylcholinesterase Inhibitory Evaluation of 3-Substituted 3,4-Dihydro-2H-1,2,4-benzothiadiazine 1,1-Dioxide Derivatives as Potential Anti-Alzheimer Agents

Priya M¹, Archana A², Hameedhash R³, Ajin TS⁴, Sergin P⁵, Praveen Kumar D⁶

¹⁻⁶II year M.Pharm, Department of Pharmaceutical Chemistry and Department of Pharmaceutics, College of Pharmacy, Madurai Medical College, Madurai-625020, Tamil Nadu.

Email: priyamu2002@gmail.com

ABSTRACT

Introduction: Alzheimer's disease (AD) is characterised by a cholinergic deficit, and inhibition of acetylcholinesterase (AChE) remains a mainstay of symptomatic therapy. The 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide scaffold has been explored mainly for AMPA-receptor modulation, and its activity against AChE has not been examined experimentally. The present study describes the synthesis, characterisation and *in vitro* AChE inhibitory evaluation of five 3-substituted derivatives (CPD 1–5; phenyl, 4-chlorophenyl, 2-phenylethenyl, 4-hydroxy-3-methoxyphenyl and furan-2-yl).

Materials and Methods: The derivatives were prepared by acid-catalysed condensation of 2-aminobenzenesulfonamide with the corresponding aldehydes in refluxing ethanol (78–80 °C, 2–3 h) and recrystallised from hot ethanol. They were characterised by melting point, thin-layer chromatography (TLC), FT-IR (KBr), ¹H NMR (500 MHz, CDCl₃) and mass spectrometry. AChE inhibition by CPD 3 was determined by Ellman's colorimetric method, with physostigmine as the standard.

Results and Discussion: The compounds were obtained in 75–84 % yield with melting points of 185–200 °C and a single TLC spot (*R_f* 0.45–0.60). IR spectra showed N–H stretching at 3368–3393 cm⁻¹ and SO₂ stretching at 1272–1329 cm⁻¹ (asymmetric) and 1154–1157 cm⁻¹ (symmetric); ¹H NMR spectra showed aromatic protons at δ 7.0–8.8 and ring methine protons at δ 4.2–5.7; molecular-ion peaks were observed at *m/z* 242–303. CPD 3 inhibited AChE in a concentration-dependent manner, from 10.33 % at 0.2 µg/mL to 55.37 % at 1.0 µg/mL, with an IC₅₀ of 0.93 µg/mL.

Conclusion: A one-step, acid-catalysed route gave five 3-substituted benzothiadiazine 1,1-dioxides in good yield, and the 3-(2-phenylethenyl) derivative (CPD 3) showed concentration-dependent AChE inhibition *in vitro*. The scaffold merits further evaluation, including the remaining derivatives, enzyme kinetics and selectivity studies.

Keywords: *acetylcholinesterase, Alzheimer's disease, benzothiadiazine 1,1-dioxide, synthesis, characterisation, Ellman's method.*

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia in older adults. Loss of basal forebrain cholinergic neurons and the resulting deficit in cholinergic neurotransmission underlie much of the early memory impairment, a view formalised as the cholinergic hypothesis [1–3]. Acetylcholinesterase inhibitors (AChEIs) such as donepezil, rivastigmine and galantamine raise synaptic acetylcholine and provide modest symptomatic benefit, but their efficacy is limited and dose-related adverse effects are common, which sustains interest in new chemotypes [4,5].

Human AChE possesses a narrow gorge of roughly 20 Å lined with aromatic residues. At its base lies the catalytic anionic site (CAS), containing the Ser203–His447–Glu334 triad and the choline-binding Trp86; near the entrance lies the peripheral anionic site (PAS), formed by Trp286, Tyr72, Tyr124, Asp74 and Tyr341 [6–8]. The PAS has also been implicated in AChE-promoted aggregation of amyloid-β, so ligands able to contact both sites, such as donepezil, are of particular interest [9,10].

The 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide nucleus is a cyclic sulfonamide that combines hydrogen-bond donor (N–H) and acceptor (S=O) functions with a fused aromatic ring. It has been studied principally as a template for positive allosteric modulators of AMPA/kainate receptors [11–13],

and 3-substituted members are accessible by condensation of 2-aminobenzenesulfonamide with aldehydes [14,15]. Related sulfur-containing benzo-fused heterocycles have shown AChE inhibition [16], yet the benzothiadiazine dioxide scaffold has not been systematically evaluated against this enzyme.

Here we report the synthesis of five 3-substituted benzothiadiazine 1,1-dioxide derivatives from 2-aminobenzenesulfonamide and five aromatic or heteroaromatic aldehydes, their physical, chromatographic and spectral characterisation, and the *in vitro* AChE inhibitory activity of the 3-(2-phenylethenyl) derivative (CPD 3), which was selected for biological testing on the basis of an earlier computational evaluation of the series that is reported separately.

MATERIALS AND METHODS

Materials

2-Aminobenzenesulfonamide, benzaldehyde, 4-chlorobenzaldehyde, cinnamaldehyde, vanillin, furfural, ethanol and concentrated hydrochloric acid were of analytical or laboratory reagent grade and were used as supplied. For the enzyme assay, acetylthiocholine iodide (ATCI), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), potassium phosphate buffer, AChE solution and physostigmine (standard) were used.

General procedure for synthesis (CPD 1–5)

2-Aminobenzenesulfonamide (2.5 g) and the appropriate aldehyde (Table 1) were dissolved in ethanol, a few drops of concentrated HCl were added, and the mixture was refluxed with continuous stirring at 78–80 °C for 2–3 h (Fig 2) [14,15]. After completion of the reaction, the mixture was cooled **and the precipitated product was collected by filtration and washed with ice-cold water. The crude product was purified by recrystallisation from hot** ethanol.

Physical and chromatographic characterisation

Colour, state, texture and solubility (water, ethanol, methanol, chloroform and dimethyl sulfoxide, qualitatively at room temperature) were recorded. Melting points were determined by the open-capillary method on a digital melting-point apparatus. Reaction progress and purity were monitored by TLC on pre-coated silica gel GF₂₅₄ plates, with ethyl acetate–hexane (3:7, 5:5 and 4:6 were tried) as the mobile phase and visualisation in a UV chamber; retardation factors (*R_f*) of reactants and products were compared [19].

Spectral characterisation

IR spectra were recorded as KBr pellets (pellet press model M15) on an ABB IR spectrophotometer (model 3000). ¹H NMR spectra were recorded on a Bruker TopSpin Advance 500 MHz spectrometer in CDCl₃ with tetramethylsilane (TMS) as internal standard; chemical shifts are given in δ (ppm). Mass spectra were recorded and the molecular-ion (*M*⁺) and fragment peaks were noted from the plot of relative abundance against *m/z* [20].

In vitro AChE inhibition assay

AChE activity was measured by a spectrophotometric adaptation of Ellman's method [17,18]. The reaction mixture contained 500 µL of 3 mM DTNB in 0.1 M potassium phosphate buffer (pH 8), 100 µL of 15 mM ATCI in water, 275 µL of 0.1 M phosphate buffer (pH 8) and 100 µL of CPD 3 solution at 0.2, 0.4, 0.6, 0.8 and 1.0 µg/mL. A blank without enzyme corrected for non-enzymatic hydrolysis; in the reaction cuvette, 25 µL of buffer was replaced by AChE solution (0.16 U/mL). Thiocholine released by enzymatic hydrolysis reacts with DTNB to give a yellow product, and absorbance was recorded every minute for 5 min at 405 nm. Percentage inhibition was calculated as $(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100$, where the control contained buffer instead of inhibitor, and the IC₅₀ was obtained from the concentration–response relationship. Physostigmine served as the standard.

RESULTS AND DISCUSSION

Synthesis

Acid-catalysed condensation of 2-aminobenzenesulfonamide with the five aldehydes in refluxing ethanol gave the corresponding 3-substituted 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxides CPD 1–5 (Fig 1, Fig 2, Table 1). The products precipitated on cooling and were purified by a single recrystallisation from ethanol, and isolated yields ranged from 75 to 84 % (Table 2). The route is a single step from inexpensive starting materials under mild conditions and is consistent with the condensation approach reported for this ring system [14,15].

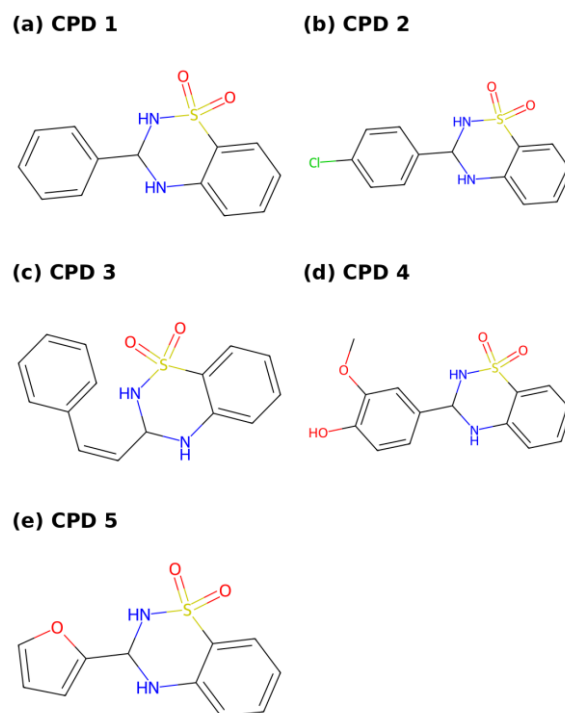


Fig 1: Structures of the synthesised derivatives CPD 1–5.

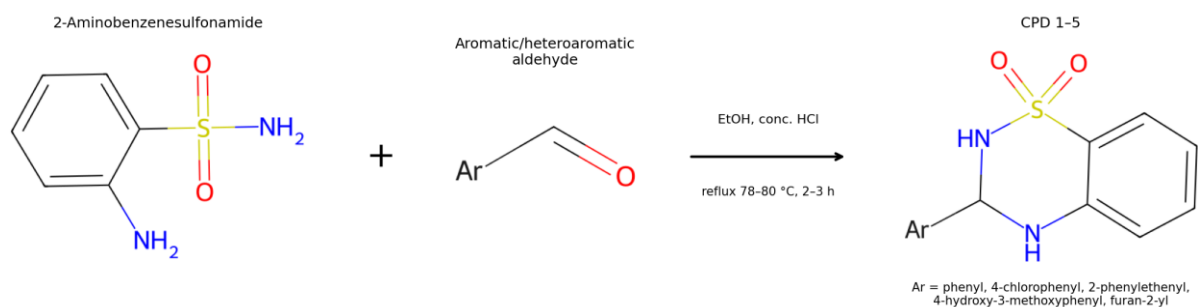


Fig 2: General synthetic scheme for CPD 1–5 (Ar = C-3 substituent given in Table 1).

Table 1: Reactants, solvent volumes and molecular formulae of the synthesised 3-substituted 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxides (2-aminobenzenesulfonamide, 2.5 g, in each case)

ID	C-3 substituent	Aldehyde used (amount)	Ethanol (mL)	Formula	MW (g/mol)
CPD 1	Phenyl	Benzaldehyde (2.23 mL)	36	C ₁₃ H ₁₂ N ₂ O ₂ S	260.31
CPD 2	4-Chlorophenyl	4-Chlorobenzaldehyde (2.25 mL)	36	C ₁₃ H ₁₁ ClN ₂ O ₂ S	294.76
CPD 3	2-Phenylethenyl	Cinnamaldehyde (1.23 mL)	41	C ₁₅ H ₁₄ N ₂ O ₂ S	286.35
CPD 4	4-Hydroxy-3-methoxyphenyl	Vanillin (1.88 g)	50	C ₁₄ H ₁₄ N ₂ O ₄ S	306.34
CPD 5	Furan-2-yl	Furfural (1.25 mL)	45	C ₁₁ H ₁₀ N ₂ O ₃ S	250.27

Physical and chromatographic characteristics

The compounds were off-white, sky-blue, yellow-orange, white and brown solids obtained as powders or fine crystals (Table 2). All were soluble in ethanol and showed poor solubility in water. Melting points fell between 185 and 200 °C; individual melting ranges were narrow (2–5 °C) for CPD 1, 2 and 4 and wider (8–9 °C) for CPD 3 and 5. Each recrystallised product gave a single spot on TLC (R_f 0.45–0.60; ethyl acetate–hexane, 3:7) that differed from the R_f values of the starting materials, indicating consumption of the reactants and chromatographic homogeneity of the products.

Table 2: Physical and chromatographic characteristics of CPD 1–5 (ranges across the series)

Parameter	Observation / range (CPD 1–5)
Colour	Off-white, sky-blue, yellow-orange, white, brown
State / texture	Solid; powder to fine crystalline
Solubility	Soluble in ethanol; poorly soluble in water
Yield (%)	75–84
Melting point (°C)	185–200
R_f (ethyl acetate–hexane, 3:7)	0.45–0.60 (single spot)

Spectral characterisation

Spectral data are summarised as ranges across the series in Table 3. All five IR spectra showed N–H stretching at 3368–3393 cm^{-1} (overlapping with the phenolic O–H of CPD 4) and the strong sulfonyl (SO_2) bands characteristic of the cyclic sulfonamide, namely asymmetric stretching at 1272–1329 cm^{-1} and, in CPD 1, 2, 3 and 5, symmetric stretching at 1154–1157 cm^{-1} [20]. Aromatic C=C stretching (1599–1682 cm^{-1}), ring skeletal vibrations (1484–1498 cm^{-1}) and out-of-plane aromatic C–H bending (700–758 cm^{-1}) were also present; CPD 3 showed a conjugated C=C band at 1599 cm^{-1} , and CPD 4 showed methoxy C–H and C–O absorptions at 2917 and 1037 cm^{-1} .

In the ^1H NMR spectra, aromatic protons appeared as multiplets (doublets at δ 7.62 and 7.78 for CPD 2) within δ 7.0–8.8. The ring N–CH–N methine proton resonated at δ 4.2–5.7, exchangeable N–H and O–H protons appeared as broad singlets, and the diagnostic signals of the C-3 substituents were observed for CPD 3 (–CH=CH–, δ 6.4–6.8) and CPD 4 (OCH_3 singlet at δ 3.3). Mass spectra showed molecular-ion peaks at m/z 242–303 with fragment ions, and ions at m/z 121–124, 135 and 146–148 recurred in CPD 2–5. Together, the IR and NMR data are consistent with the benzothiadiazine 1,1-dioxide framework carrying the respective C-3 substituent.

Table 3: Spectral characteristics of CPD 1–5 (ranges across the series)

Technique	Assignment	Range observed (CPD 1–5)
FT-IR (KBr), cm^{-1}	N–H stretching (O–H in CPD 4)	3368–3393
	Aliphatic / methoxy C–H stretching (CPD 4, 5)	2917–2927
	Aromatic / conjugated C=C stretching	1599–1682
	Aromatic ring skeletal vibration	1484–1498
	SO_2 asymmetric stretching	1272–1329
	SO_2 symmetric stretching	1154–1157
	C–N / C–O stretching	1037–1087
	Aromatic C–H out-of-plane bending	700–758
^1H NMR (500 MHz, CDCl_3), δ ppm	Aromatic H (m / d)	7.0–8.8

Technique	Assignment	Range observed (CPD 1–5)
	Vinylic –CH=CH– (CPD 3)	6.4–6.8
	Ring N–CH–N methine	4.2–5.7
	Exchangeable N–H / O–H (br s)	4.5–10.2
	OCH ₃ (CPD 4, s)	3.3
Mass spectrometry, <i>m/z</i>	Molecular ion (M ⁺)	242–303
	Recurring fragment ions (CPD 2–5)	121–124, 135, 146–148

In vitro AChE inhibitory activity

CPD 3 inhibited AChE in a concentration-dependent manner (Table 4, Fig 3). Inhibition rose from 10.33 % at 0.2 µg/mL to 55.37 % at 1.0 µg/mL, and the IC₅₀ was 0.93 µg/mL; linear regression of inhibition against concentration over the five points gave $r^2 = 0.995$. Under the same assay conditions the standard, physostigmine, produced 100 % inhibition, compared with 55.37 % for CPD 3 at the highest concentration tested.

Table 4: Inhibition of AChE by CPD 3 (Ellman's method)

S. No.	CPD 3 concentration (µg/mL)	Absorbance (405 nm)	Inhibition (%)
1	Control	1.210	0.00
2	0.2	1.085	10.33
3	0.4	0.964	20.33
4	0.6	0.845	30.17
5	0.8	0.706	41.65
6	1.0	0.540	55.37

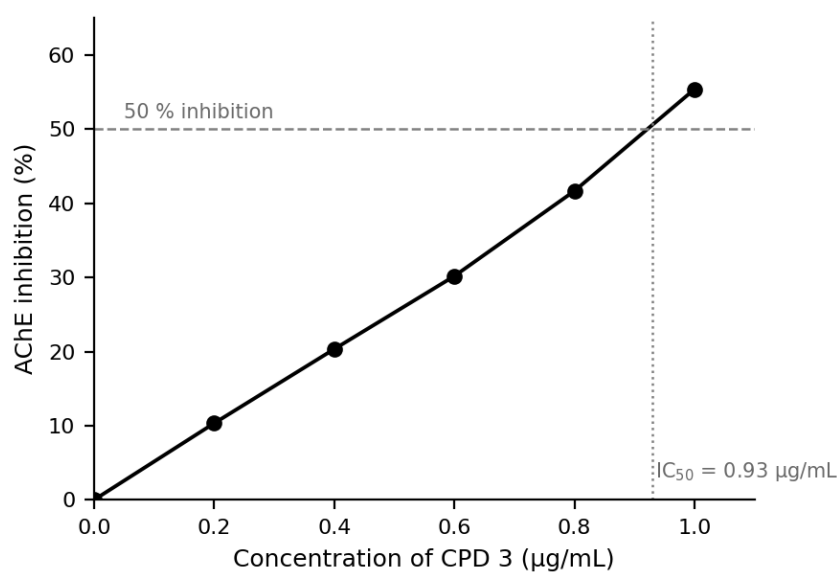


Fig 3: Concentration-dependent inhibition of AChE by CPD 3 (dashed line: 50 % inhibition).

Discussion

The five derivatives were obtained in a single step in good yield and showed the spectral features expected for the 3-substituted benzothiadiazine 1,1-dioxide core: the N–H and SO₂ absorptions common to all compounds, the aromatic and methine proton signals, and the substituent-specific bands of the vinyl (CPD 3) and vanillyl (CPD 4) groups. The molecular-ion peaks recorded for the series (*m/z* 242–303) will be complemented by ¹³C NMR and high-resolution mass spectrometry to complete the structural assignment.

CPD 3 was prioritised for biological testing because of its favourable profile in the earlier computational evaluation of the series. In the Ellman assay it showed clear concentration dependence, with an IC₅₀ of 0.93 µg/mL, but it was less effective than physostigmine at the highest concentration tested (55.37 % against 100 %). The extended, conjugated 2-phenylethenyl substituent may favour contacts with the aromatic residues lining the AChE gorge, but this remains a hypothesis that requires kinetic and structural confirmation. Because CPD 1, 2, 4 and 5 were not assayed, no structure–activity relationship can yet be drawn from the present data.

Several limitations apply. Only one derivative was assayed against a single standard; the mode of inhibition, selectivity over butyrylcholinesterase, cell-based neuroprotection and cytotoxicity were not examined, and the IC₅₀ should be compared with that of physostigmine determined under identical conditions. Evaluation of the complete series, enzyme kinetics and selectivity studies are therefore the next steps.

CONCLUSION

Five 3-substituted 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide derivatives were synthesised by a simple acid-catalysed condensation in 75–84 % yield and characterised by melting point, TLC, IR, ¹H NMR and mass spectrometry. The 3-(2-phenylethenyl) derivative (CPD 3) inhibited AChE *in vitro* in a concentration-dependent manner (IC₅₀ 0.93 µg/mL), identifying the benzothiadiazine 1,1-dioxide scaffold as a starting point for further development of AChE inhibitors for Alzheimer's disease. Testing of the remaining derivatives, kinetic and selectivity studies, and *in vivo* evaluation are required to establish its therapeutic potential.

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Conflicts of Interest: The authors declare no conflict of interest.

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