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Original Research

Acetylcholinesterase inhibitor and cytotoxic activity of some novel acetazolamide cyclocondensed azetidinones

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Abstract

Objective: The purpose of the study was to synthesize potent acetylcholinesterase (AChE) inhibitor and cytotoxic compounds by condensing acetazolamide with azetidinones.
Methods: A new series of novel acetazolamide condensed azetidinones was prepared by Schiff's bases of acetazolamide with chloroacetylchloride in the presence of a catalytic amount of 1,4-dioxane and triethyl amine. Confirmation of the chemical structure of the synthesized compounds (5a–k) was substantiated by melting point, thin layer chromatography, different spectral data infrared, ^{1}H-nuclear magnetic resonance, and mass spectra. The synthesized compounds were evaluated for AChE inhibitory activity and *in vitro* cytotoxicity against Vero cells.
Results: The titled compounds exhibited weak, moderate, or high AChE inhibitory activity and *in vitro* cytotoxicity. Compound 5c exhibited potential AChE inhibitory activity, with a concentration of 0.10μM giving 50% inhibition, when compared with the current therapeutic agent donepezil HCl and *in vitro* cytotoxicity with a lower concentration required to kill 50% of the cells.
Conclusion: Acetazolamide-cyclocondensed azetidinones might prove interesting as potential AChE inhibitors and cytotoxic compounds, and should be investigated further.

Keywords: Acetazolamide cyclocondensed; Acetylcholinesterase inhibitor; Azetidinones; *In vitro* cytotoxicity; Vero cells

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1. Introduction

The activity of famous antibiotic classes such as the penicillins, cephalosporins, carumonam, aztreonam, thienamicine, nocardicins, and carbapenems is attributed to the presence of a 2-azetidinone ring.[1] Unfortunately, the most widely used of them exert selective pressure on bacteria and permit the proliferation of resistant organisms. Several synthetic and semisynthetic β-lactam antibiotics have been developed due to the growing resistance of bacteria towards the classics β-lactam antibiotics and the need for drugs with a more specific antibacterial activity.[2] The name lactam is given to cyclic amides. In older nomenclature the second carbon in an aliphatic carboxylic acid was designated as α, the third as β and so on. Thus a β-lactam is a cyclic amide with four atoms in its ring. The contemporary name for this ring system is azetidinone although β-lactam has come to be a generic descriptor for the penicillin family. The ring ultimately proved to be the main component of the pharmacophore.[3,4] Therefore, the term possesses medicinal as well as chemical significance. Ketene–imine cycloaddition was reported by Staudinger[5] to

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be a smooth, well-documented route to the synthesis of substituted β-lactam derivatives. In an effort to investigate a suitably substituted monocyclic β-lactam as a minimum requirement for biological activity, many scientists reported the trans-stereoselective synthesis of butadienyl azetidinones and their Diels–Alder cycloaddition.[5,6] This included the preparation of a series of Schiff's bases and their reaction with dienylketene to produce a transazetidinone. This involved the *in situ* formation of the ketene and its subsequent addition to the imine.[7,8]

The 2-azetidinone skeleton, otherwise known as the β-lactam ring, has been recognized as a useful building block in the synthesis of biologically important compounds. Azetindin-2-one derivatives display interesting biological activities such as antimicrobial,[9–11] antitubercular,[12,13] analgesic, anti-inflammatory,[14] chymase inhibitory,[15] antitumor,[16,17] cholesterol absorption inhibitory properties,[18] antinociceptive,[19] and anticonvulsant.[20] The chemical structure of acetazolamide provides a most valuable molecular template for the development of agents able to interact with a wide variety of biological activities. The synthesis of acetazolamide condensed with azetidinones has not been reported previously. Hence, it was thought worthwhile to synthesize new congeners by incorporating acetazolamide and azetidinone moieties in a single molecular framework and to evaluate their acetylcholinesterase (AChE) inhibitor activity and *in vitro* cytotoxicity.

2. Materials and methods

All chemicals were supplied by E. Merck (Merck Feldbergstr. 78, 64293 Darmstadt, Germany) and S.D. Fine Chemicals (315–317, T V Industrial Estate, 248, Worli Road, BDD Chawls Worli, Worli, Mumbai, Maharashtra 400018, India). The melting points (MP) of the synthesized compounds were determined in open capillaries using a Veego VMP-1 apparatus and are expressed in °C and are uncorrected. The IR spectra of compounds were recorded on Shimadzu FT-IR spectrometer using the KBr pellet technique and are expressed in cm^{-1}. ^{1}H NMR spectra was recorded on Bruker DRX-300 (300 MHz FT-NMR) using dimethyl sulfoxide (DMSO)-d_6 as solvent and tetramethylsilane as internal standard. The chemical shifts were reported in ppm. Mass spectra MS were obtained using Shimadzu LCMS 2010A under ESI ionization technique.

2.1. Synthesis of Schiff's bases (1a–j)

A mixture of equimolar quantities of compound acetazolamide (0.01M) and appropriate aryl and heteroaryl aldehyde (0.01M) was dissolved in ethanol (95%). The contents were relaxed for 3.5 hours on a steam bath. The solid obtained was separated out and crystallized from ethanol. The synthesized compounds yield ranges were found to be 62–78% and melting point range was 152–208°C. The infrared (IR) spectra of compounds 1a–j showed strong absorption bands for carbonyl group (1720 cm^{-1}), aromatic C–H Stretching (3186 cm^{-1}), and aromatic C=C Stretching (1630 and 1520 cm^{-1}). ^{1}H-nuclear magnetic resonance (NMR) spectra of compounds 1a–j showed a quartet for methine protons at 7.8 (^{1}H, N=CH–R), multiplets at 6.2–7.8 (Ar–H). Compounds 1d and 1g showed a singlet at 2.24 due to the signals of the third CH group.

2.2. Synthesis of azetidinones (5a–k)

Triethylamine (0.01M) in 1,4-dioxane, chloroacetyl chloride (0.01M) was added drop wise to a solution of the compounds 1a-k (0.005M) at room temperature. The reaction mixture was stirred for 25 minutes and then refluxed for 4.5 hours on a water bath. The solid obtained after removal of 1,4-dioxane was crystallized from ethanol. The yield, melting point, and spectral characterization of the compounds were reported in analytical data.

2.3. Analytical data

2.3.1. N-{5-[(3-chloro-2-oxo-4-phenylazetidin–1-yl) sulfonyl]–1,3,4-thiadiazol-2-yl}acetamide (5a)

Colorless amorphous solid; MP: 218–220°C; yield: 72%; IR (KBr, cm^{-1}): 3310 (N–H), 3048 (ArC–H), 1678 (C=O, amide), 1520 (ArC=C), 1380 (C–N), and 710 (C–Cl); ^{1}H-NMR (DMSO-d6) δ: 1.78 (s, 1H, N–CH–C), 2.62 (s, 1H, C–CH–Cl), 7.08–7.36 (m, 4H, ArH), 7.44–7.78 (m,4H, ArH), and 10.21 (s, 1H, CONH); MS (m/z): M^{+} calculated 385.99; found: 386.04.

2.3.2. N-{5-[(3-chloro-2-oxo-4-pyridin-4-ylazetidin–1-yl) sulfonyl]-1,3,4-thiadiazol-2-yl}acetamide (5b)

Colorless amorphous solid; MP: 236–239°C; yield: 76%; IR (KBr, cm^{-1}): 3292 (N–H), 3110 (ArC–H), 1672 (C=O, amide), 1584 (ArC=C), 1368 (C–N), and 692 (C–Cl); ^{1}H-NMR (DMSO-d6) δ: 1.92 (s, 1H, N–CH–C), 2.42 (s, 1H, C–CH–Cl), 7.21–7.38 (m, 4H, ArH), 7.42-7.68 (m,4H, ArH), and 10.34 (s, 1H, CONH); MS (m/z): M^{+} calculated 386.98; found: 387.06.

2.3.3. N-(5-{[3-chloro-2-(4-fluorophenyl)-4-oxoazetidin-1-yl]sulfonyl}-1,3,4-thiadiazol-2-yl)acetamide (5c)

Colorless amorphous solid; MP: 266–268°C; yield: 81%; IR (KBr, cm^{-1}): 3286 (N–H), 3162 (ArC–H), 1720 (C=O, amide), 1565 (ArC=C), 1472 (C–N), and 708 (C–Cl); ^{1}H-NMR (DMSO-d6) δ: 1.87 (d, 1H, N-CH-C), 2.56 (d, 1H, C-CH-Cl), 7.10 (m, 4H, ArH), 7.45 (m, 4H, ArH), and 10.12 (s, 1H, CONH); MS (m/z): M^{+} calculated 403.98.05; found: 404.04.

2.3.4. N-(5-{[3-chloro-2-(3-fluorophenyl)-4-oxoazetidin-1-yl]sulfonyl}-1,3,4-thiadiazol-2-yl)acetamide (5d)

Colorless amorphous solid; MP: 254–257°C; yield: 79%; IR (KBr, cm^{-1}): 3314 (N–H), 3084 (ArC–H), 1672 (C=O, amide), 1448 (ArC=C), 1392 (C–N), and 694 (C–Cl); ^{1}H-NMR (DMSO-d6) δ: 1.95 (s, 1H, N-CH-C), 2.38 (s, 1H, C-CH-Cl), 7.08–7.36 (m, 4H, ArH), 7.23–7.58 (m,4H, ArH), and 10.14 (s, 1H, CONH); MS (m/z): M^{+} calculated 403.98; found: 404.04.