Biotechnology Reports 5 (2015) 1–6

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Acetylcholinesterase enzyme inhibitor activity of some novel pyrazinamide condensed 1,2,3,4-tetrahydropyrimidines

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ARTICLE INFO

Article history:
Received 31 July 2014
Received in revised form 19 October 2014
Accepted 20 October 2014
Available online 29 October 2014

Keywords:
Pyrazinamide
Tetrahydropyrimidines
Biginelli reaction
Acetyl cholinesterase inhibitor

ABSTRACT

A new series of some novel pyrazinamide condensed 1,2,3,4-tetrahydropyrimidines was prepared by reacting of *N*-(3-oxobutanoyl)pyrazine-2-carboxamide with urea/thiourea and appropriate aldehyde in the presence of catalytic amount of laboratory made *p*-toluenesulfonic acid as an efficient catalyst. Confirmation of the chemical structure of the synthesized compounds (4a–l) was substantiated by TLC, different spectral data IR, ^{1}H NMR, mass spectra and elemental analysis. The synthesized compounds were evaluated for acetyl and butyl cholinesterase (AChE and BuChE) inhibitor activity. The titled compounds exhibited weak, moderate or high AChE and BuChE inhibitor activity. Especially, compound (4l) showed the best AChE and BuChE inhibitory activity of all the 1,2,3,4-tetrahydropyrimidine derivatives, with an IC_{50} value of 0.11 μM and 3.4 μM.

1. Introduction

Acetylcholine (ACH) acts as an excitatory neurotransmitter for voluntary muscles in the somatic nervous system and as a preganglionic and a postganglionic transmitter in the parasympathetic nervous system of vertebrates and invertebrates [1,2]. Acetyl cholinesterase (AChE) is a terminator enzyme of nerve impulse transmission at the cholinergic synapses by quick hydrolysis of ACH to choline and acetate. Inhibition of AChE evolves a strategy for the treatment of several diseases as Alzheimer's disease (AD), senile dementia, ataxia, myasthenia gravis and Parkinson's disease [3]. AD is one form of senile dementia, which occurs due to various neuropathological conditions such as senile plaques and neurofibrillary tangles. It is the most common dementias that affect half of the population aged 85 years [4,5] and seventh main cause of life lost affecting 5.3 million people over the world. In AD, growing numbers of nerve cells degenerate and die along with loss in synapse through which information flows from and to the brain. As a result, cognitive impairment and dementia occur [6]. The neuropathology of AD is generally characterized by the presence of numerous amyloidal β-peptide (Aβ) plaques, neurofibrillary tangles (NFT), and degeneration or atrophy of the basal forebrain cholinergic neurons. The loss of basal forebrain cholinergic cells results in an important reduction in ACh level, which plays an important role in the cognitive impairment associated with AD [7].

Both cholinesterase enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are involved in the hydrolysis of acetylcholine; however, studies showed that as the disease progresses, the activity of AChE decreases while the activity of BChE remains unaffected or even increases [8]. In the brain of advanced staged AD patients, BChE can compensate for AChE when the activity of AChE is inhibited by AChE inhibitors. Thus, BChE hydrolyses the already depleted levels of ACh in these patients. Furthermore, restoration of ACh levels by BChE inhibition seems to occur without apparent adverse effects [9,10]. It has been also proposed that individuals with low-activity of BChE can sustain cognitive functions better comparing two individuals with normal BChE activity [11].

Pyrimidine derivatives comprise a diverse and interesting group of drugs is extremely important for their biological activities. Dihydropyrimidine and their derivatives have attracted increasing interest owing to their therapeutic and pharmaceutical properties, such as antiviral, antitubercular [12,13], antimicrobial agent [14–18] antagonists of the human adenosine A2A receptor [19], cyclooxygenase-2 inhibitory activity [20,21], tyrosine kinase inhibitors, antiamoebic activity [22,23], cytotoxicity [24,25] and acetyl cholinesterase inhibitor activity [26]. The discovery during

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http://dx.doi.org/10.1016/j.btre.2014.10.007

the 1930s that a dihydropyridine (dihydronicotinamide derivative, NADH), "hydrogen-transferring coenzyme" consequently became important in biological system, has generated numerous studies on the biochemical properties of dihydropyridines and their bio-isosteres dihydropyrimidines. The search for more suitable preparation of tetrahydropyrimidinones continues today.

The chemical structure of pyrazinamide provides a most valuable molecular template for the development of agents able to interact with a wide variety of biological activities [27]. Tetrahydropyrimidines are structurally similar to dihydropyrimidines. Hence, it was thought worthwhile to synthesize new congeners by incorporating pyrazinamide with 1,2,3,4-tetrahydropyrimidinones moieties in a single molecular framework and to evaluate their acetyl and butyl cholinesterase inhibitor activity.

2. Experimental

2.1. Materials and methods

All chemicals were supplied by E. Merck (Germany) and SD fine chemicals (India). Melting points were determined by the open tube capillary method and are uncorrected. The purity of the compounds was checked on thin layer chromatography (TLC) plates (silica–gel G) in the solvent system, ethanol, chloroform, ethyl acetate (6:2:2); the spots were located under iodine vapors or UV light. IR spectrum was obtained on a PerkinElmer 1720 FT-IR spectrometer (KBr Pellet). ^{1}H NMR spectra were recorded or a Bruker DRX-300 (300 MHz FT-NMR) spectrometer using DMSO-d_6 as solvent and TMS as internal standard. Mass spectra were obtained using Shimadzu LCMS 2010A under ESI ionization technique. Elemental analyses (C, H, and N) were performed on PerkinElmer model 240C analyzer.

2.2. Preparation of N-(3-oxobutanoyl)pyrazine-2-carboxamide (3)

Pyrazinamide **1** (0.01 M) and ethyl acetoacetate **2** (0.01 M) were mixed in presence 10 ml of glacial acetic acid and refluxed for approximately 3.0 h. The colorless liquid formed was then heated on a water bath to remove the alcohol formed during the reaction. After allowing the reaction mixture to cool, crude crystals were obtained. Purification was performed by stirring crude crystals with cold diethyl ether for approximately 20 min using a mechanical stirrer. Allowing it to stand for 15 min, followed by filtration, resulted in the third compound in a pure form of *N*-(3-oxobutanoyl)pyrazine-2-carboxamide **3**.

2.2.1. General procedure

2.2.1.1. Preparation of 1,2,3,4-tetrahydropyrimidines by microwave irradiation method (4a–l). The mixture of *N*-(3-oxobutanoyl) pyrazine-2-carboxamide (0.005 M), urea/thiourea (0.0075 M), and appropriate aldehyde (0.005 M) with a catalytic amount of laboratory made *p*-toluenesulfonic acid in 10 ml of ethanol was subjected to microwave irradiation (300 W) for 12 min at the interval of 10 s. The reactions were monitored through TLC using the appropriate solvent system. After the reaction was complete, the reaction mixture was cooled in a refrigerator and filtered. The precipitate obtained was washed thoroughly with water to remove unreacted urea/thiourea and dried. The crude solid product was recrystallized with ethanol to give the pure compounds (4a–l).

2.3. Analytical data

2.3.1. N-(3-Oxobutanoyl)pyrazine-2-carboxamide (3)

Light-red-colored solid, M.P.: 162–164 °C; yield: 69%; IR (KBr, cm^{-1}): 3324 (N—H), 2952 (AliC—H), 1728 (C=O, ketone), 1688 (C=O, amide), 1592 (C=C), 1343 (C—N); ^{1}H NMR (DMSO-d_6) δ: 2.05 (s, ^{3}H, CH_3), 2.87 (s, ^{2}H, CH_2), 8.78 (s, ^{1}H, Ar—H), 8.93 (s, ^{1}H, Ar—H), 9.08 (s, ^{1}H, Ar—H), 9.43 (s, ^{1}H, NH); calculated for $C_9H_9N_3O_3$: C, 52.17; H, 4.38; N, 20.28; found C, 52.12; H, 4.52; N, 20.33.

2.3.2. 6-Methyl-2-oxo-4-phenyl-N-(pyrazin-2-ylcarbonyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide (4a)

Dark-brownish solid, M.P.: 284–286 °C; yield: 70%; IR (KBr, cm^{-1}): 3246 (N—H), 3152 (Ar—C—H), 2968 (Ali—C—H), 1674 (C=O, amide), 1583 (C=C), 1248 (O—C); ^{1}H NMR (DMSO-d_6) δ: 2.09 (s, ^{3}H, CH_3), 5.45 (s, ^{1}H, CH), 7.12–7.23 (m, ^{5}H, Ar—H), 8.78 (s, ^{1}H, Ar—H), 8.93 (s, ^{1}H, Ar—H), 9.08 (s, ^{1}H, Ar—H), 9.41 (s, ^{1}H, NH), 9.76 (s, ^{1}H, NH), 10.11 (s, ^{1}H, NH); MS (*m*/*z*): (M + 1) calculated 338.12; found 338.07; calculated for $C_{17}H_{15}N_5O_3$: C, 60.53; H, 4.48; N, 20.76; found C, 60.48; H, 4.53; N, 20.82.

2.3.3. 6-Methyl-4-phenyl-N-(pyrazin-2-ylcarbonyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (4b)

Ash-colored solid, M.P.: 296–298 °C; yield: 77%; IR (KBr, cm^{-1}): 3253 (N—H), 3166 (Ar—C—H), 2948 (Ali—C—H), 1677 (C=O, amide), 1584 (C=C), 1888 (C=S), 1192 (O—C); ^{1}H NMR (DMSO-d_6) δ: 2.06 (s, ^{3}H, CH_3), 5.38 (s, ^{1}H, CH), 7.09–7.25 (m, ^{5}H, Ar—H), 8.78 (s, ^{1}H, Ar—H), 8.93 (s, ^{1}H, Ar—H), 9.08 (s, ^{1}H, Ar—H), 9.39 (s, ^{1}H, NH), 9.82 (s, ^{1}H, NH), 10.08 (s, ^{1}H, NH); MS (*m*/*z*): (M + 1) calculated 354.10; found 354.04. Calculated for $C_{17}H_{15}N_5O_2S$: C, 57.78; H, 4.28; N, 19.82; found C, 57.83; H, 4.22; N, 19.87.

2.3.4. 6-Methyl-4-(3-nitrophenyl)-2-oxo-N-(pyrazin-2-ylcarbonyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide (4c)

Light-yellowish solid, M.P.: 313–315 °C; yield: 76%; IR (KBr, cm^{-1}): 3276 (N—H), 3168 (Ar—C—H), 2984 (Ali—C—H), 1678 (C=O, amide), 1558 (C=C), 1162 (O—C); ^{1}H NMR (DMSO-d_6) δ: 2.07 (s, ^{3}H, CH_3), 5.49 (s, ^{1}H, CH), 7.39–7.43 (d, 2H, Ar—H), 7.97–8.02 (d, ^{2}H, Ar—H), 8.78 (s, ^{1}H, Ar—H), 8.93 (s, ^{1}H, Ar—H), 9.08 (s, ^{1}H, Ar—H), 9.24 (s, ^{1}H, NH), 9.68 (s, ^{1}H, NH), 10.06 (s, ^{1}H, NH); MS (*m*/*z*): (M + 1) calculated 383.10; found 383.15; calculated for $C_{17}H_{14}N_6O_5$: C, 53.40; H, 3.69; N, 21.98; found C, 53.44; H, 3.75; N, 21.94.

2.3.5. 6-Methyl-4-(3-nitrophenyl)-N-(pyrazin-2-ylcarbonyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (4d)

Light-bluish solid, M.P.: 357–359 °C; yield: 71%; IR (KBr, cm^{-1}): 3257 (N—H), 3164 (Ar—C—H), 2971 (Ali—C—H), 1678 (C=O, amide), 1562 (C=C), 1865 (C=S), 1174 (O—C); ^{1}H NMR (DMSO-d_6) δ: 2.03 (s, ^{3}H, CH_3), 5.39 (s, ^{1}H, CH), 7.42–7.47 (d, ^{2}H, Ar—H), 7.98–8.04 (d, ^{2}H, Ar—H), 8.78 (s, ^{1}H, Ar—H), 8.93 (s, ^{1}H, Ar—H), 9.08 (s, ^{1}H, Ar—H), 9.17 (s, ^{1}H, NH), 9.61 (s, ^{1}H, NH), 10.04 (s, ^{1}H, NH); MS (*m*/*z*): (M + 1) calculated 399.08; found 400.03; calculated for $C_{17}H_{14}N_6O_4S$: C, 51.25; H, 3.54; N, 21.09; found C, 51.30; H, 3.59; N, 21.15.

2.3.6. 4-(3-Chlorophenyl)-6-methyl-2-oxo-N-(pyrazin-2-ylcarbonyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide (4e)

Light-greenish solid, M.P.: 357–359 °C; yield: 79%; IR (KBr, cm^{-1}): 3276 (N—H), 3134 (Ar—C—H), 2948 (Ali—C—H), 1672 (C=O, amide), 1569 (C=C), 1189 (O—C); ^{1}H NMR (DMSO-d_6) δ: 2.09 (s, ^{3}H, CH_3), 5.51 (s, ^{1}H, CH), 6.98–7.13 (m, ^{4}H, Ar—H), 8.78 (s, ^{1}H, Ar—H), 8.93 (s, ^{1}H, Ar—H), 9.08 (s, ^{1}H, Ar—H), 9.14 (s, ^{1}H, NH), 9.49 (s, ^{1}H, NH), 10.05 (s, ^{1}H, NH); MS (*m*/*z*): (M + 1) calculated 372.08; found 372.02; calculated for $C_{17}H_{14}ClN_5O_3$: C, 54.92; H, 3.80; N, 18.84; found C, 54.97; H, 3.74; N, 18.90.

2.3.7. 4-(3-Chlorophenyl)-6-methyl-N-(pyrazin-2-ylcarbonyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (4f)

Ash-colored solid, M.P.: 324–326 °C; yield: 80%; IR (KBr, cm^{-1}): 3254 (N—H), 3163 (Ar—C—H), 2978 (Ali—C—H), 1681 (C=O, amide), 1548 (C=C), 1879 (C=S), 1146 (O—C); ^{1}H NMR (DMSO-d_6) δ: 2.07 (s, ^{3}H, CH_3), 5.44 (s, ^{1}H, CH), 7.06–7.24 (m, ^{4}H, Ar—H), 8.78 (s, ^{1}H,

Ar—H), 8.93 (s, ^{1}H, Ar—H), 9.08 (s, ^{1}H, Ar—H), 9.25 (s, ^{1}H, NH), 9.48 (s, ^{1}H, NH), 10.12 (s, ^{1}H, NH); MS (*m*/*z*): (M + 1) calculated 388.06; found 388.11; calculated for $C_{17}H_{14}ClN_5O_2S$: C, 52.65; H, 3.64; N, 18.06; found C, 52.71; H, 3.69; N, 18.12.

*2.3.8. 4-(4-Fluorophenyl)-6-methyl-2-oxo-*N*-(pyrazin-2-ylcarbonyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide (4g)*

Light-bluish solid, M.P.: 356–358 °C; yield: 81%; IR (KBr, cm^{-1}): 3274 (N—H), 3186 (Ar—C—H), 2951 (Ali—C—H), 1678 (C=O, amide), 1547 (C=C), 1175 (O-C); ^{1}H NMR (DMSO-d_6) δ: 2.05 (s, ^{3}H, CH_3), 5.52 (s, ^{1}H, CH), 6.95 (d, 2H, Ar—H), 7.15 (d, ^{2}H, Ar—H), 8.78 (s, ^{1}H, Ar—H), 8.93 (s, ^{1}H, Ar—H), 9.08 (s, ^{1}H, Ar—H), 9.17 (s, ^{1}H, NH), 9.51 (s, ^{1}H, NH), 10.02 (s, ^{1}H, NH); MS (*m*/*z*): (M + 1) calculated 356.11; found 356.17; calculated for $C_{17}H_{14}FN_5O_3$: C, 57.46; H, 3.97; N, 19.71; found C, 57.51; H, 4.03; N, 19.76.

*2.3.9. 4-(4-Fluorophenyl)-6-methyl-*N*-(pyrazin-2-ylcarbonyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (4h)*

Light-yellowish solid, M.P.: 367–369 °C; yield 83%; IR (KBr, cm^{-1}): 3242 (N—H), 3181(Ar—C—H), 2948 (Ali—C—H), 1678 (C=O, amide), 1564 (C=C), 1858 (C=S), 1148 (O—C); ^{1}H NMR (DMSO-d_6) δ: 2.03 (s, ^{3}H, CH_3), 5.48 (s, ^{1}H, CH), 6.98 (d, ^{2}H, Ar—H), 7.21 (d, ^{2}H, Ar—H), 8.78 (s, ^{1}H, Ar—H), 8.93 (s, ^{1}H, Ar—H), 9.08 (s, ^{1}H, Ar—H), 9.28 (s, ^{1}H, NH), 9.59 (s, ^{1}H, NH), 10.04 (s, ^{1}H, NH); MS (*m*/*z*): (M + 1) calculated 372.09; found 372.15; calculated for $C_{17}H_{14}FN_5O_2S$: C, 54.98; H, 3.80; N, 18.86; found C, 55.03; H, 3.86; N, 18.92.

*2.3.10. 4-(4-Chlorophenyl)-6-methyl-2-oxo-*N*-(pyrazin-2-ylcarbonyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide (4i)*

Ash-colored solid, M.P.: 341–343 °C; yield 79%; IR (KBr, cm^{-1}): 3256 (N—H), 3162 (Ar—C—H), 2974 (Ali—C—H), 1681 (C=O, amide), 1548 (C=C), 1883 (C=S), 1168 (O—C); ^{1}H NMR (DMSO-d_6) δ: 2.07 (s, ^{3}H, CH_3), 5.45 (s, ^{1}H, CH), 7.05 (d, ^{2}H, Ar—H), 7.23 (d, ^{2}H, Ar—H), 8.78 (s, ^{1}H, Ar—H), 8.93 (s, ^{1}H, Ar—H), 9.08 (s, ^{1}H, Ar—H), 9.09 (s, ^{1}H, NH), 9.54 (s, ^{1}H, NH), 10.12 (s, ^{1}H, NH); MS (*m*/*z*): (M + 1) calculated 372.08; found 372.13; calculated for $C_{17}H_{14}ClN_5O_3$: C, 54.92; H, 3.80; N, 18.84; found C, 54.97; H, 3.84; N, 18.90.

*2.3.11. 4-(4-Chlorophenyl)-6-methyl-*N*-(pyrazin-2-ylcarbonyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (4j)*

Light-bluish solid, M.P.: 331–333 °C; yield 74%; IR (KBr, cm^{-1}): 3238 (N—H), 3164 (Ar—C—H), 2937 (Ali—C—H), 1676 (C=O, amide), 1574 (C=C), 1889 (C=S), 1194 (O—C); ^{1}H NMR (DMSO-d_6) δ: 2.08 (s, ^{3}H, CH_3), 5.44 (s, ^{1}H, CH), 7.08 (d, ^{2}H, Ar—H), 7.23 (d, ^{2}H, Ar—H), 8.78 (s, ^{1}H, Ar—H), 8.93 (s, ^{1}H, Ar—H), 9.08 (s, ^{1}H, Ar—H), 9.21 (s, ^{1}H, NH), 9.59 (s, ^{1}H, NH), 10.11 (s, ^{1}H, NH); MS (*m*/*z*): (M + 1) calculated 388.06; found 388.00; calculated for $C_{17}H_{14}ClN_5O_2S$: C, 52.65; H, 3.64; N, 18.06; found C, 52.59; H, 3.69; N, 18.12.

*2.3.12. 6-Methyl-2-oxo-*N*-(pyrazin-2-ylcarbonyl)-4-(pyridin-4-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide (4k)*

Light-yellowish solid, M.P.: 295–297 °C; yield 77%; IR (KBr, cm^{-1}): 3228 (N—H), 3172 (Ar—C—H), 2957 (Ali—C—H), 1684 (C=O, amide), 1581 (C=C), 1848 (C=S), 1175 (O—C); ^{1}H NMR (DMSO-d_6) δ: 2.01 (s, ^{3}H, CH_3), 5.43 (s, ^{1}H, CH), 7.45 (d, ^{2}H, Ar—H), 8.34 (d, ^{2}H, Ar—H), 8.78 (s, ^{1}H, Ar—H), 8.93 (s, ^{1}H, Ar—H), 9.08 (s, ^{1}H, Ar—H), 9.16 (s, ^{1}H, NH), 9.51 (s, ^{1}H, NH), 10.01 (s, ^{1}H, NH); MS (*m*/*z*): (M + 1) calculated 339.12; found 339.18; calculated for $C_{16}H_{14}N_6O_3$: C, 56.80; H, 4.17; N, 24.84; found C, 56.85; H, 4.12; N, 24.90.

*2.3.13. 6-Methyl-*N*-(pyrazin-2-ylcarbonyl) -4-(pyridin-4-yl) -2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (4l)*

Ash-colored solid, M.P.: 317–319 °C; yield 73%; IR (KBr, cm^{-1}): 3258 (N—H), 3192 (Ar—C—H), 2936 (Ali—C—H), 1677 (C=O, amide), 1583 (C=C), 1891 (C=S), 1138 (O—C); ^{1}H NMR (DMSO-d_6) δ: 2.05 (s, ^{3}H, CH_3), 5.49 (s, ^{1}H, CH), 7.36 (d, ^{2}H, Ar—H), 8.54 (d, ^{2}H, Ar—H), 8.78 (s, ^{1}H, Ar—H), 8.93 (s, ^{1}H, Ar—H), 9.08 (s, ^{1}H, Ar—H), 9.32 (s, ^{1}H, NH), 9.76 (s, ^{1}H, NH), 10.18 (s, ^{1}H, NH); MS (*m*/*z*): (M + 1) calculated 355.09; found 355.14; calculated for $C_{16}H_{14}N_6O_2S$: C, 54.23; H, 3.98; N, 23.71; found C, 54.29; H, 3.95; N, 23.77.

2.4. Acetylcholinesterase inhibitory activity

2.4.1. In vitro *inhibition studies on AChE and BuChE*

Acetylcholinesterase (AChE, from electric eel), butyl cholinesterase (BuChE, from equine serum), 5,5′-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, DTNB), acetylthiocholine chloride (ATC), butylthiocholine chloride (BTC), and hydrochloride were purchased from Sigma–Aldrich. The 1,2,3,4-tetrahydropyrimidines derivatives were dissolved in DMSO and diluted in 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 8.0) to provide a final concentration range. DMSO was diluted to a concentration in excess of 1 in 10,000, and no inhibitory action on either AChE or BuChE was detected in separate prior experiments.

2.4.2. In vitro *AChE assay*

All the assays were carried out under 0.1 M KH_2PO_4/K_2HPO_4 buffers, pH 8.0, using a Shimadzu UV-2450 spectrophotometer. Enzyme solutions were prepared to give 2.0 units/ml in 2 ml aliquots. The assay medium (1 ml) consisted of phosphate buffer (pH 8.0), 50 μl of 0.01 M DTNB, 10 μl of enzyme, and 50 μl of 0.01 M substrate (ACh chloride solution). Test compounds were added to the assay solution and preincubated at 37 °C with the enzyme for 15 min followed by the addition of substrate. The activity was determined by measuring the increase in absorbance at 412 nm at 1 min intervals at 37 °C. Calculations were performed according to the method of the equation in Ellman's method [28]. Each concentration was assayed in triplicate. *In vitro* BuChE assay was similar to the method used for AChE.

3. Results and discussion

A series of 12 novel pyrazinamide condensed 1,2,3,4-tetrahydropyrimidines of biological interest were synthesized and evaluated for acetyl and butyl cholinesterase inhibitor activity, all the compounds were characterized by IR, ^{1}H NMR, MS and elemental analysis of their structures.

3.1. Chemistry

Synthesis of 1,4-dihydropyrimidines by adopting the Biginelli synthetic protocol [29] involving one pot multicomponent reaction was performed by following steps as outlined in Fig. 1. In the first step, ethyl acetoacetate **2** and pyrazinamide **1** in presence 10 ml of glacial acetic acid reacted under neat conditions resulting in the formation of *N*-(3-oxobutanoyl)pyrazine-2-carboxamide **3** with the yield of 74%. The *N*-(3-oxobutanoyl)pyrazine-2-carboxamide was further taken for the Biginelli condensation reaction by reacting it with urea/thiourea and appropriate aldehyde in the presence of catalytic amount of laboratory made *p*-toluenesulphonic acid [30]. The advantages of the catalyst were better yields and do not require dry solvents. The first step in the mechanism of the Biginelli reaction is the acid-catalyzed condensation of the urea with the aldehyde. This reaction begins with protonation of the aldehyde by the acid and is followed by an attack of the amine from urea. Proton transfer steps, then result in a protonated alcohol which leaves as water to form an *N*-acyliminium ion intermediate [31], subsequently enol form of the β-keto ester attacks the *N*-acyliminium ion to generate an open chain ureide which readily cyclizes to a tetrahydropyrimidines. The reaction times were found to be 12 min. The IR spectra of compounds (4a–l) showed strong absorption bands for the amine group (3233–3373 cm^{-1}), amide

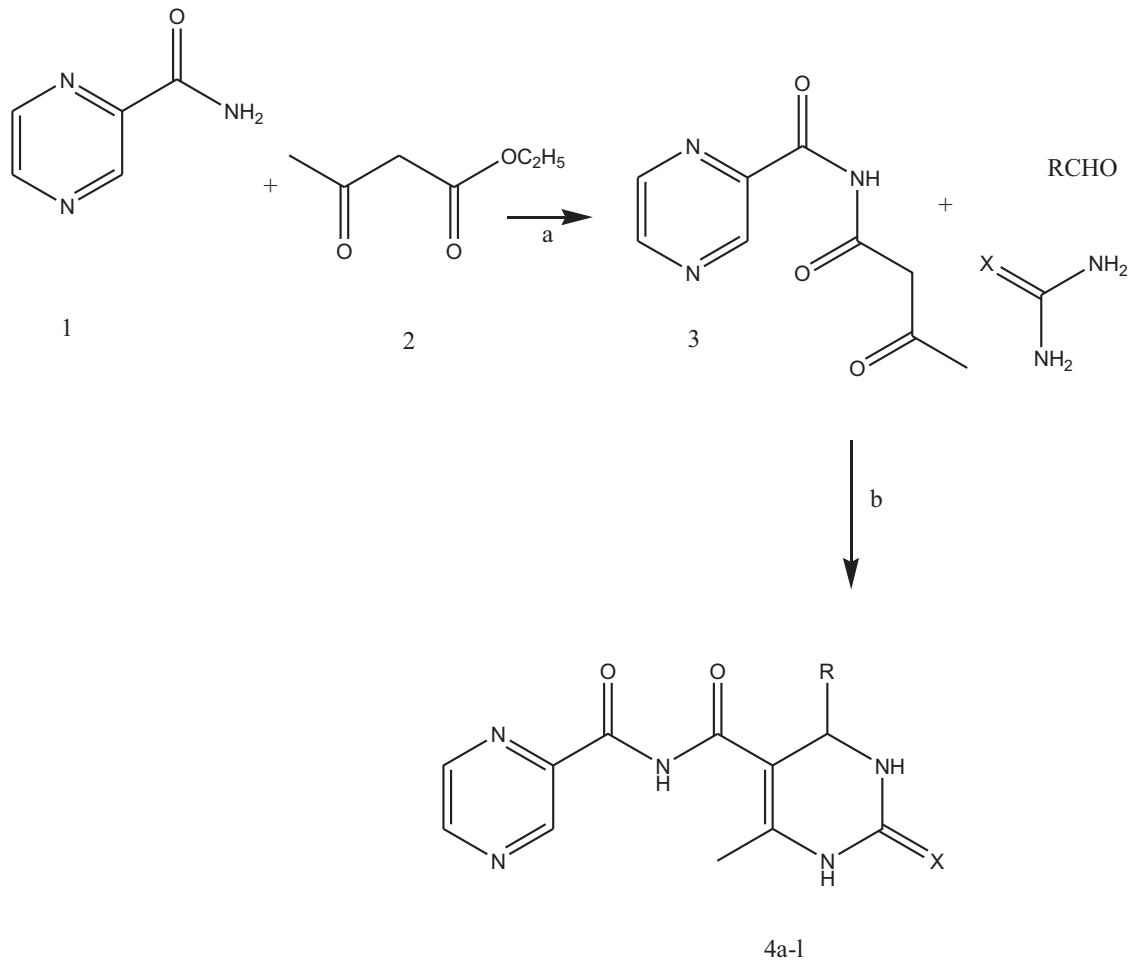


Fig. 1. Synthesis of compounds (4a–l). Reagents and conditions: (a) reflux 3.0 h, CH_3COOH; (b) C_2H_5OH, *p*-toluenesulphonic acid, and microwave irradiation (300 W) for 12 min.

group (1672–1684 cm^{-1}), aliphatic C—H stretching (2926–2994 cm^{-1}), aromatic C—H stretching (3134–3212 cm^{-1}) and aromatic C=C stretching (1539–1591 cm^{-1}). ^1H NMR spectrum of compounds 4a–l showed a methyl group protons singlet at (2.01–2.09 ppm), CH-R protons singlet at (5.34–5.52 ppm), aromatic protons triplet at (6.84–7.30 ppm) and amine protons singlet at (9.07–10.18 ppm). The mass spectra and elemental analysis results were within ±0.6% of the theoretical values. Totally, twelve compounds (4a–l) various substituted 1,2,3,4-tetrahydropyrimidines, were synthesized with the yield ranging from 70% to 83%. These conditions enable this method to be applicable for the synthesis of 1,2,3,4-tetrahydropyrimidines based heterocyclic compounds. The present protocol best describes the synthesis of 1,2,3,4-tetrahydropyrimidines. All the reported 1,2,3,4-tetrahydropyrimidines compounds were found to be novel and not reported elsewhere.

3.2. Acetylcholinesterase inhibitory activity

Among the novel substituted 1,2,3,4-tetrahydropyrimidine derivatives for treating AD, their anti-cholinesterase activities (compounds 4a–l) was assayed according to Ellman's method on acetyl cholinesterase (AChE) from electric eel using commercial donepezil HCl as the reference standard [32,33]. The butyls cholinesterase's (BuChE) inhibitory on equine serum BuChE were also examined by the same method. Inhibition of AChE activities of the synthesized compounds is shown in Fig. 2 and Table 1. The data listed in Fig. 2 and Table 1 clearly shows that most of the designed compounds exhibited good to moderate inhibitory activities toward the AChE and BuChE inhibition are summarized in Fig. 2 and Table 1. All the synthesized 1,2,3,4-tetrahydropyrimidine derivatives were potent inhibitors of AChE, with IC_{50} values ranging from micro molar to sub-micro molar. Especially, compound 4l showed the best AChE and BuChE inhibitory activity of all the 1,2,3,4-tetrahydropyrimidine derivatives, with an IC_{50} value of 0.11 μM and 3.4 μM. Among the compounds reported herein, compound 4l is arguably the most potent.

4. Structural activity relationship

Analyzing the activities of the synthesized compounds, the following structure activity relationships (SAR) were obtained. The fifth position of 1,2,3,4-tetrahydropyrimidines contain *N*-(3-oxobutanoyl)pyrazine-2-carboxamide group contributed toward acetyl and butyl cholinesterase inhibitor activity, and fourth positions of 1,2,3,4-tetrahydropyrimidines contain substituted phenyl and hetero aromatic ring responsible acetyl and butyl cholinesterase inhibitor activity [26]. Heteroaryl substituted compounds at 4th position it enhance the potency of the compounds when compare with the unsubstituted or substituted aryl containing compounds. Substituted atom or group of atom must be the strong electron withdrawing nature of potent activity because it decreases electron density in the ring due to inductive effect. Fluoride and chloride substitution at fourth position of

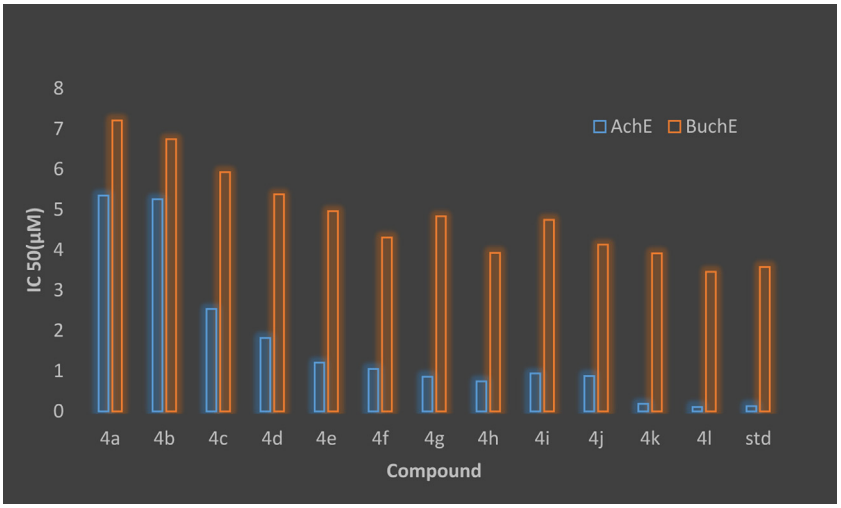
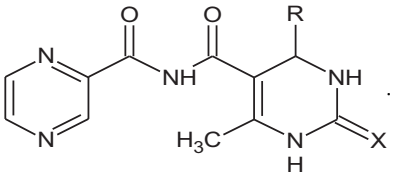


Fig. 2. *In vitro* acetyl and butyl cholinesterase inhibitor activity of compounds (4a–l) and Donepezil HCl (std.).

Table 1
Synthesized 1,2,3,4-tetrahydropyrimidines: *in vitro* acetyl and butyl cholinesterase inhibitor activity



Serial no.	Compound	*R*	*X*	AChE IC_{50} (μM) ± SEM	BuChE IC_{50} (μM) ± SEM
1	4a	Phenyl	O	5.35 ± 0.01	7.21 ± 0.01
2	4b	Phenyl	S	5.26 ± 0.01	6.75 ± 0.01
3	4c	3-Nitorophenyl	O	2.54 ± 0.01	5.93 ± 0.01
4	4d	3-Nitorophenyl	S	1.82 ± 0.01	5.38 ± 0.01
5	4e	3-Chlorophenyl	O	1.21 ± 0.01	4.96 ± 0.01
6	4f	3-Chlorophenyl	S	1.05 ± 0.01	4.31 ± 0.01
7	4g	4-Flurophenyl	O	0.86 ± 0.01	4.84 ± 0.01
8	4h	4-Flurophenyl	S	0.75 ± 0.01	3.93 ± 0.01
9	4i	4-Chlorophenyl	O	0.94 ± 0.01	4.75 ± 0.01
10	4j	4-Chlorophenyl	S	0.88 ± 0.01	4.13 ± 0.01
11	4k	4-Pyridyl	O	0.19 ± 0.01	3.92 ± 0.01
12	4l	4-Pyridyl	S	0.11 ± 0.01	3.46 ± 0.01
13	Donepezil HCl	Standard	–	0.13 ± 0.01	3.58 ± 0.01

phenyl ring showed potent action because of strong electron withdrawing nature due to inductive effect. Substitution of fluro, chloro group at third and fourth position of phenyl ring showed potent action when compare with nitro atom. The second position sulfur substituted derivatives most potent when compare with oxygen atoms. Among the compounds reported herein, compound 4l is arguably the most potent when compared with current therapeutic agent donepezil HCl because heteroaryl ring present at 4th position of 1,2,3,4-tetrahydropyrimidines it enhances the acetyl and butyl cholinesterase inhibitor activity (Fig. 2 and Table 1).

5. Conclusion

In summary, a series of novel 1,2,3,4-tetrahydropyrimidines of biological interest were synthesized and analyzed for their structures. The libraries of compounds were prepared by using laboratory made *p*-toluenesulfonic acid as an efficient catalyst when compare with Lewis acid. The importance of substitutions at the fourth positions of 1,2,3,4-tetrahydropyrimidines was studied toward the acetyl and butyl cholinesterase inhibitor activity. The acetyl and butyl cholinesterase inhibitor activity data revealed that the all synthesized compounds proved to be active against acetyl and butyl cholinesterase enzymes. Almost all of the titled compounds exhibited weak, moderate, or high acetyl and butyl cholinesterase inhibitor activity. Compound 4l showed potent acetyl and butyl cholinesterase inhibitor activity when compare with the donepezil HCl, our present study makes it an interesting compound when compared to the current therapeutic agents and are considered the candidates to investigate further for the same.

Acknowledgments

The authors wish to thank the Sunrise University for research support. Also, thank the Molecules Research Laboratory for *in vitro* cholinesterase enzyme inhibitor activity, Chennai, India.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.btre.2014.10.007.

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