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,