SYNTHESIS AND AChE INHIBITING ACTIVITY OF 2, 4 SUBSTITUTED 6-PHENYL PYRIMIDINES

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ABSTRACT

Novel substituted pyrimidines were synthesized from methyl 2,4-dioxo-4-phenyl-butan-2-one (I-A) and urea, followed by Mitsunobu coupling of I-A with benzyl or allyl alcohol to give the corresponding 2-hydroxy-6-phenyl-pyrimidine 4-carboxamides. AChE and BuChE assays revealed 2-hydroxy-6-phenyl-pyrimidine-4-carboxallylamide as the most active compound, IC50 = 90 μM, with no inhibition of BuChE.

Keywords: Pyrimidines; inhibition AChE; mitsunobu; TBTU

INTRODUCTION

Alzheimer’s disease (AD) is the most common age-related neurodegenerative disease, is a progressive neurodegenerative disorder that affects regions of the brain that control cognition, memory, language, speech and awareness to one’s physical surroundings1. Those alterations are associated with regional deficits in the cholinergic system. The development of acetylcholinesterase (AChE) inhibitor drugs has followed the finding that cholinergic pathways in the cerebral cortex and basal forebrain are compromised in AD2 and the resultant cholinergic deficit contributes to the cognitive impairment of these patients3. Cholinesterase inhibitors (ChEIs) are considered to be valuable as a therapeutic target and they have become the main approach to symptomatic treatment; Donepezil, galantamine and rivastigmine are the first line pharmacotherapy for mild to moderate Alzheimer’s disease4. The drugs have slightly different pharmacological properties, but they all work by inhibiting the breakdown of acetylcholine increasing the availability of acetylcholine in central synapses5. Acetylcholinesterase and butyrylcholinesterase (BuChE), the two forms of cholinesterase, co-exist ubiquitously throughout the body. They exhibit a high catalytic power and are very similar in structure and catalytic function. However, BuChE has a less defined role in biological processes, although it has been postulated that it acts as a detoxifying enzyme6.

Looking for new active compounds we have isolated several natural alkaloids from Aristotelia chilensis4, which have displayed broad range of pharmacological activities. Recently investigations have shown that substituted pyrimidines are potent ChE inhibitors7 of Acetyl and Butyrylcholine. Therefore it seems interesting to synthesize 6-phenylpyrimidines as specific ChE, substituted at position 2 and 4 and evaluated their enzymatic activity.

EXPERIMENTAL

Melting points were determined on a Melting Point SMP10 (Stuart) and are uncorrected. The 1H NMR spectra were determined using a Bruker ARX 300 instrument, operating at 300.1 MHz (¹H) and 75.5 MHz (¹C) or Bruker ARX 500 operating at 500 MHz (¹H) and 125 MHz (¹C). High-Resolution ESI mass spectra (HR-MS) were measured on a Q-TOF mass spectrometer Micromass (Manchester). El low-resolution MS spectra were measured on Trace DQSI GC/MS-system Axel Semrau GmbH & Co. Column chromatography was performed using Merck silica gel 60 (0.063–0.200 mm). TLC was carried out on a Merck silica gel 60 PF254. Solvents used in this study were distilled prior to use and dried over appropriate drying agents.

Enzyme assays

The in vitro measurement of AChE and BuChE inhibition were carried out using a colorimetric method adapted to 96-well microtiter plates8. The AChE was obtained from Electrophorus electricus (C3389-500UN), type VI-S, Sigma Chemical Co., St. Louis, MO), and BuChE from equine serum (C1057-1KU, Sigma Chemical Co., St. Louis, MO). Fifty μL of test sample dissolved in 100 mM phosphate buffer pH 7.6, and 50 μL, of AChE or BuChE solution (final concentration of 0.03 U/mL and 0.01 U/mL, respectively) were added to each well, and the plates were pre-incubated for 30 min at room temperature. After the pre-incubation period, acetylthiocholine iodide (or butyrylthiocholine iodide) was added to a final concentration of 200 μM. 5,5'-Dithio-bis(2-nitrobenzoic acid) (DTNB) was used for the measurement of cholinesterase activity. The hydrolysis of ACh or BCh was monitored by following the formation of the yellow 5-thio-2-nitrobenzoate anion. The absorbance was read in a Thermo Multiskan Ex Instrument microplate reader at 405 nm after 3 min. The enzyme activity was calculated as a percentage compared to a control using only the buffer and enzyme solution. The compounds were assessed in the dilution interval of 500-15.63 μg/mL, and the alkaloid galanthamine was used as the reference compound. The ChEs inhibitory activity of each compounds was expressed in terms of the IC50 value (μg/mL and μM) required to inhibit the hydrolysis of the substrate by 50%, as calculated from the dose-response curve.

RESULTS AND DISCUSSION

2, 4-Dioxobutanones are interesting starting materials for the synthesis of ligands and organometallic complexes of anti-HIV-1 integrase9. Amides and hydrazides of 2, 4-dioxobutanon acid possess antimicrobial and analgesic activity10. The β diketone I-A has been synthesized in 84% yield from the acetonophene and dimethylxolate in dimethoxethane, using NaH as base, scheme 1. Compound I-A shows an interchangebale proton at 15.83 ppm suggesting that I-A occurs only as the β-hydroxyketone.

Hydrolysis of I-A in aqueous NaOH for 10-15 min gave the corresponding acid II-B in 80% yield. II-B is known to possess promising activity against HIV-1 integrase11 and slow-binding inhibition of KDPG aldolase12 which is a target for new bacteriostatic or bactericidal drugs. Other reaction conditions, such as NaOH in MeOH, afforded II-B in a yield of ca. 50%, together with unreacted starting material.

Scheme 1

Pyrimidine I-A1 was synthesized from β-hydroxy ketone I-A in 78% yield, using an excess of urea in MeOH under reflux in the presence of catalytic amounts of H2SO4. Ethers I-A2 and I-A3 were obtained from I-A1 under Mitsunobu conditions, using disisopropylazodicarboxylate (DIAD) and Triphenylphosphine in dimethylformamide. Column chromatography afforded the products in ca. 87% yield, scheme 2.

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Table 1. Activity of 6-phenyl pyrimidine 2,4 substituted toward the enzyme AChE.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>AChE IC₅₀ mg/mL</th>
<th>μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I-A1</td>
<td>0.024</td>
<td>104.3</td>
</tr>
<tr>
<td>2</td>
<td>I-A2</td>
<td>0.089</td>
<td>278.0</td>
</tr>
<tr>
<td>3</td>
<td>I-A3</td>
<td>0.032</td>
<td>118.0</td>
</tr>
<tr>
<td>4</td>
<td>II-B1</td>
<td>0.081</td>
<td>375.0</td>
</tr>
<tr>
<td>5</td>
<td>II-B2</td>
<td>0.059</td>
<td>184.0</td>
</tr>
<tr>
<td>6</td>
<td>II-B3</td>
<td>0.023</td>
<td>90.1</td>
</tr>
<tr>
<td>7</td>
<td>II-B4</td>
<td>0.063</td>
<td>185.8</td>
</tr>
<tr>
<td>*</td>
<td>Galantamine</td>
<td>1.1x10⁴</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* Internal control, n=3.

(Z)-Methyl 2-hydroxy-4-oxo-4-phenylbut-2-enone (I-A): A mixture of acetoephene (5g, 41.7 mmol) and dimethoxalate (5g, 50 mmol) were stirred in 150mL of anhydrous dimethoxyethane, then sodium hydride (1.2g, 50 mmol) was added. The mixture was stirred under reflux for 4h. The reaction was cooled and quenched with water 100mL and HCl until pH 2. The mixture was extracted 3 times with EtOAc, and the organic layer was evaporated in vacuo. The compound was precipitated as a white solid (7.2g, 84%). 1H-NMR (CDCl₃, 300 MHz) ppm: 3.94 (3H, s); 7.10 (1H, s); 7.50 (2H, m); 7.61 (1H, m); 8.00 (2H, m), 15.83 (1H, s, interchangeable). 13C-NMR (CDCl₃, 75 MHz) ppm: 55.41; 99.45; 127.55; 129.16; 130.19; 135.16; 162.61; 169.09; 190.81. EI-MS: m/z 206.

Methyl 2-hydroxy-6-phenylpyrimidine-4-carboxylate (I-A1): A mixture of compound I-A (3.0g, 14.6 mmol) and urea (2.8g, 47 mmol) were dissolved in 100mL of methanol and ca. 100 μL of conc. sulfuric acid was added. The solution was stirred overnight. The product precipitated as a white solid (2.8g, 87%) mp = 195-196°C. 1H-NMR (DMSO-d₆, 500 MHz) ppm: 3.91 (3H, s); 7.59 (3H, m); 7.72 (1H, s); 8.13 (2H, d, J = 7.2 Hz); 12.47 (1H, s, br). 13C-NMR (DMSO-d₆, 125 MHz) ppm: 53.50; 105.98; 127.92; 129.51; 132.40; 135.23; 153.43; 162.54; 163.78; 168.22.

Scheme 2

Reaction of 4-phenyl-2,4-dioxobutanoic acid (I-B) with an excess of urea and catalytic amounts of H₂SO₄ in refluxing toluene gave 2-hydroxy-6-phenylpyrimidine-4-carboxylic acid (I-BII) in yields of ca. 55%. II-BI precipitated after the reaction as a white solid, soluble in dimethylsulfoxide or dimethylformamide. Amide coupling of II-B1 with benzyl or allyl amines with O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumtetrafluoroborate (TBTU) in DMF gave compounds II-B2, II-B3, and II-B4, respectively, in ca. 40% yield, scheme 3. Attempts to prepare secondary amides with diethyl- or dibenzylamine were unsuccessful, as only traces of product were detected.

Scheme 3

Compounds I-A2; I-A3; II-B2; II-B3; II-B4, as well as pyrimidines I-A1 and II-B1 were evaluated as inhibitors of AChE (Table 1). All amides showed higher activity than pyrimidine carboxylic acid, II-B1. The most active compound was the allyl amide II-B3 with an IC₅₀ of 90.1 μM. All compounds were also tested with BuChE, but did not show significant inhibition (IC₅₀ > 1000 μM).

(Z)-2-Hydroxy-4-oxo-4-phenylbut-2-enic acid (II-B): Hydrolysis of 1-A (5.5g, 26.7mmol) was carried out with NaOH (1.1g, 26.7mmol) in 20mL of water and stirring at 50°C for 15 min. The reaction was filtered and the reaction mixture was extracted three times with 10 mL of ethyl acetate. The water layer was acidified with HCl until pH 2. The product was precipitated as a white solid, it was filtered off and dried (4.1g, 80%) mp = 198-199°C. 1H-NMR (CDCl₃, 300 MHz) ppm: 6.75 (1H, br); 7.52 (2H, m); 7.53 (1H, d, J = 4.2 Hz); 7.87 (2H, d, J = 4.5 Hz). 13C-NMR (CDCl₃, 75 MHz) ppm: 43.14; 52.92; 103.29; 117.69; 127.74; 128.69; 131.95; 132.44; 135.13; 148.32; 157.10; 161.52; 171.68. ESI-MS: m/z = 271.1389.
solid (200mg, 43%), decomposes at ca. 205 °C. \( ^1 \)H-NMR (DMSO d6, 300 MHz) ppm: 4.58 (2H, d, J= 3 Hz); 7.29-7.35 (2H, m); 7.42 (1H, t, J= 3Hz); 7.53 – 7.60 (4H, m); 7.73 (1H, d, J= 6 Hz); 8.00 (1H, d, J= 3 Hz); 8.07 (1H, d, J= 3 Hz); 9.38 (1H, s). \( ^{13} \)C-NMR (DMSO d6, 75 MHz) ppm: 41.01; 110.08; 119.60; 125.00; 125.67; 127.79; 128.29; 129.08; 129.18; 129.55; 129.61; 133.00; 136.19; 143.30; 163.37. ESI-MS: M+1: 340.1096

**CONCLUSIONS**

The investigation described in this communication reports the synthesis of substituted pyrimidines in few steps with goods yields and simplicity of operations. Separately we modified the pyrimidine substituent at position 2 through Mitsunobu reaction and the carboxy group at position 4 by amidation. All compounds were evaluated as AChE inhibitors showing that position 4 is the most important for enzyme-inhibition. N-Allyl-2-hydroxy-6-phenylpyrimidine-4-carboxamide (II-B3) is the most active compound against AChE with IC\(_{50}\) 90.1μM. It is highly selective for AChE, not showing activity against BuChE.

**REFERENCES**


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