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RESEARCH ARTICLE

Inhibitory effects of isatin Mannich bases on carbonic anhydrases, acetylcholinesterase, and butyrylcholinesterase

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Abstract

The effects of isatin Mannich bases incorporating (1-[piperidin-1-yl (**P1**)/morpholin-4-yl (**P2**)/*N*-methylpiperazin-1-yl (**P3**)]methyl)-1*H*-indole-2,3-dione) moieties against human (h) carbonic anhydrase (CA, EC 4.2.1.1) isoenzymes hCA I and hCA II, acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) enzymes were evaluated. **P1**–**P3** demonstrated impressive inhibition profiles against AChE and BChE and also inhibited both CAs at nanomolar level. These inhibitory effects were more powerful in all cases than the reference compounds used for all these enzymes. This study suggests that isatin Mannich bases **P1–P3** are good candidate compounds especially for the development of new cholinesterase inhibitors since they were 2.2–5.9 times better inhibitors than clinically used drug Tacrine.

Keywords

Acetylcholinesterase, butyrylcholinesterase, carbonic anhydrase, isatin, Mannich bases

History

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Introduction

Alzheimer's disease (AD) is characterised by a progressive decline of memory and cognition. Based on the world Alzheimer's reports, there were 36 million people in 2010, predicted to increase to 115 million by 2050[1]. One of the therapeutic strategies is based on the cholinergic hypothesis targeting cholinesterase enzymes.

Cholinesterases (ChE) are an enzyme family that catalyse the hydrolysis of acetylcholine (ACh) into choline and acetic acid, an essential process for the restoration of the cholinergic neurotransmission. There are two cholinesterase types: acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8). AChE is known to be abundant in the muscle, brain and erythrocyte membrane, whereas BChE has a higher activity in liver, intestine, heart, kidney and lung. They have similar molecular forms and active sites despite being products of different genes on the human chromosomes. Inhibition of hydrolyses of acetylcholine (ACh) and butrylcholine (BCh) by using cholinesterase inhibitors have been considered to increase the level of the ACh and BCh in synapses. Although there are many ongoing research activities for treatment of AD, only some drugs were approved by the Food and Drug Administration (FDA), such as Tacrine, Donepezil and Rivastigmin[2]. The 1*H*-indol motif which is also available in isatin structure was also used to design some new compounds which can be good candidate for the treatment of neurodegenerative diseases[3]. In addition, some isatin derivatives were considered to develop new cholinesterase inhibitors[1,4].

Carbonic anhydrases (CAs, EC 4.2.1.1) catalyse a very simple but physiologically essential reaction in all life kingdoms, the hydration of carbon dioxide (CO_2) to bicarbonate (HCO_3^-) and protons (H^+), with a high efficiency. Up to now, six distinct genetic CA families (α-, β-, γ-, δ-, ζ- and η-CAs) were discovered. Mammals including humans possess 16 different α-CA isoforms, which are involved in many crucial physiological or pathological processes connected with respiration and transport of CO_2/HCO_3^-, pH and CO_2 homeostasis, chemosensing, electrolyte secretion in a variety of tissues and organs, biosynthetic reactions, bone resorption, calcification, tumorigenicity, etc. CA inhibitors (CAIs) have found applications as important therapeutic agents. The most well known clinically established sulphonamide CA inhibitors include acetazolamide, dorzolamide, methazolamide, brinzolamide, dichlorophenamide and ethoxzolamide. Dorzolamide and brinzolamide are topically acting drugs, whereas the others are administered systemically and therefore have many undesirable side effects associated with them due to lack of sufficient selectivity towards CA isozymes[5,6].

Isatin (1*H*-Indole-2,3-dion) and its derivatives are biologically active compounds and have significant importance in medicinal chemistry. Isatin moiety has many biological activities, such as anticonvulsant, MAO-A and MAO-B inhibitory, antipsychotic, sedative, antianxiety, antimicrobial, antiviral, anti-inflammatory,

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analgesic, antioxidant, anticancer, and cholinesterase inhibitory activities[1–3,7]. On the other hand, Schiff bases of isatin have been reported as selective carbonic anhydrase inhibitors[8].

Mannich bases are an important group of compounds in medicinal chemistry and they are synthesised by Mannich reaction[9,10]. Mannich bases have wide range of biological activities, such as cytotoxic[11–13], antiinflammatory[14] and anticonvulsant[15] activities. Several types of phenolic Mannich bases were also reported with cytotoxic[13,16] and CA inhibitory[17] activities. The reported mechanism of Mannich bases are thiol alkylation[18,19], interaction with some enzymes which are important for antioxidant mechanisms, inhibition of mitochondrial respiration[20,21], topoisomerase enzyme inhibition[22,23] and tubulin polimerisation[24].

Isatin derivatives, which are carrying aminomethyl moiety, i.e. Mannich bases have important biological activities including anticancer[25] and anti-HIV[26] activities. Mannich bases of isatin were generally used as starting compounds or as intermediate compounds for the synthesis of various chemical designs. There is a study reporting the cytotoxicities of Mannich bases having the isatin motif and their corresponding Schiff base analogues. In this study, Manich bases had higher cytotoxicity than their corresponding Schiff bases[27].

In this study, it was aimed to investigate the CA, AChE and BChE inhibitory activities of some newly prepared isatin Mannich bases.

Experimental

Materials

^{1}H NMR (400 MHz) spectra were taken using a Varian Mercury Plus spectrometer (Varian Inc., Palo Alto, CA). Chemical shifts (δ) were reported in ppm. Melting points were determined using an Electrothermal 9100 (IA9100, Bibby Scientific Limited, Staffordshire, UK) instrument and are uncorrected.

Methods

Synthesis of mono Mannich bases, ***P1–P3***

A mixture of secondary amine compound (3.4 mmol) and paraformaldehyde (3.4 mmol) in ethanol (5 mL) was added into the solution of isatin (3.4 mmol) in ethanol (10 mL) (Scheme 1). The reaction mixture was heated for 4 h and then kept at +4 °C for 36 h to form crystals. The crystalline products were separated by filtration and dried at room temperature. Recrystallisation from ethanol provided pure compounds (**P1–P3**). Chemical structures of the compounds were confirmed by ^{1}H NMR and melting point.

1-(Piperidin-1-yl)methyl)-1H-indole-2,3-dione, ***P1***

Orange coloured solid (65%); m.p. 134–136 °C, 126–128 [27]. ^{1}H NMR (400 MHz, $CDCl_3$,) δ: 7.59–7.57 (m, 2H, Ar-H), 7.12–7.09 (m, 2H, Ar-H), 4.43 (s, 2H), 2.58–2.55 (m, 4H), 1.59–1.42 (m, 6H).

1-(Morpholino-4-yl)methyl)-1H-indole-2,3-dione, ***P2***

Orange coloured solid (68%); m.p. 199–200 °C, 194–196 [27]. ^{1}H NMR (400 MHz, $CDCl_3$,) δ: 7.62 (d, $J = 7.4$ Hz, 1H, Ar-H), 7.59 (d, $J = 7.7$ Hz, 1H, Ar-H), 7.15 (t, $J = 7.4$ Hz, 1H, Ar-H), 7.08 (d, $J = 7.7$ Hz, 1H, Ar-H), 4.44 (s, 2H), 3.70–3.68 (m, 4H), 2.64–2.61 (m, 4H).

1-[(4-Methylpiperazin-1-yl)methyl]-1H-indole-2,3-dione, ***P3***

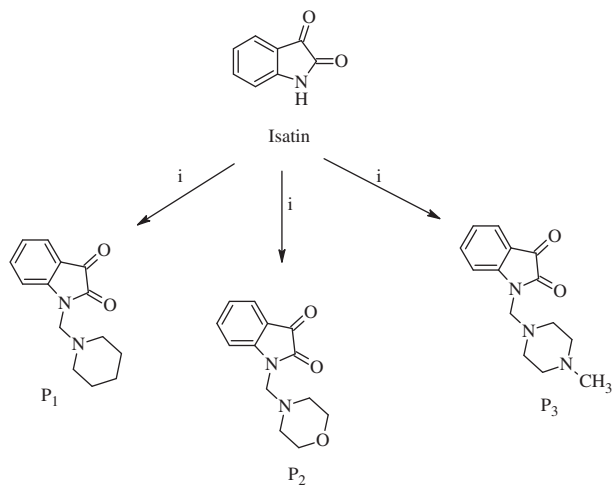
Orange red coloured solid (72%); m.p. 138–140 °C. ^{1}H NMR (400 MHz, $CDCl_3$,) δ: 7.61–7.56 (m, 2H, Ar-H), 7.12 (t, $J = 7.5$ Hz, 1H), 7.07 (d, $J = 8.1$ Hz, 1H), 4.47 (s, 2H), 2.66 (brs, 4H), 2.41 (brs, 4H), 2.26 (s, 3H).

Biological activity

Carbonic anhydrase inhibition assay

The purification of cytosolic CA isoenzymes (CA I and CA II) were previously described with a simple one-step method by a Sepharose-4B-L tyrosine-sulphanilamide affinity chromatoghraphy[28]. The protein quantity in the column effluents was determined spectrophotometrically at 280 nm. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was applied with a Bio-Rad Mini Gel system Mini-PROTEIN® system, Bio-Rad Laboratories, Inc., China after purification of both CA isoenzymes. Briefly, it was performed in acrylamide for the running (10%) and the stacking gel (3%) contained SDS (0.1%), respectively.

Activities of CA isoenzymes were determined according to a method by Verporte et al.[29] The increase in absorbance of reaction medium was spectrophotometrically recorded at 348 nm. Also, the quantity of protein was determined at 595 nm according to the Bradford method[30]. Bovine serum albumin was used as standard protein. For determination of inhibition effect of **P1–P3**



i = Paraformaldehyde, amine (P_1:Piperidine, P_2:Morfoline, P_3:*N*-Methylpiperazine), ethanol, reflux.

Scheme 1. Synthesis of isatin Mannich bases, **P1–P3**.

Table 1. Inhibitory effects of isatin Mannich bases **P1–P3** human carbonic anhydrase isoenzymes I and II (hCA I and II), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE).

	IC_{50} (nM)								K_i (nM)			
Compounds	hCA I	r^2	hCA II	r^2	AChE	r^2	BChE	r^2	hCA I	hCA II	AChE	BChE
P1	9.63	0.9711	7.29	0.9800	1.66	0.9868	2.15	0.9921	8.57 ± 1.65	5.02 ± 1.07	0.79 ± 0.27	0.86 ± 0.41
P2	8.67	0.9717	7.45	0.9812	1.86	0.9711	3.11	0.9914	9.43 ± 3.57	8.08 ± 1.98	0.75 ± 0.30	1.11 ± 0.43
P3	14.44	0.9805	7.37	0.9822	2.34	0.9946	1.53	0.9896	8.88 ± 2.22	5.71 ± 0.91	1.01 ± 0.24	0.42 ± 0.21
AZA*	13.08	0.9735	10.19	0.9658	–	–	–	–	10.16 ± 2.46	8.29 ± 1.38	–	–
TAC**	–	–	–	–	4.36	0.9871	5.29	0.9883	–	–	2.69 ± 0.56	2.49 ± 0.23

*Acetazolamide (AZA) was used as a standard inhibitor for both hCA I and II.
**Tacrine (TAC) was used as a standard inhibitor for AChE and BChE enzymes.

on both hCA isoenzymes, an activity (%)-[**P1–P3**] graph was drawn. The IC_{50} values were obtained from activity (%) versus compounds plots[31]. For calculation of K_i values, three different concentrations were used. The Lineweaver–Burk curves were drawn and calculations were realised[32].

Cholinesterase inhibition assay

The inhibitory effects of freshly synthesized isatin Mannich bases (**P1–P3**) on AChE and BChE activities were measured by slightly modifying the spectrophotometric Ellman method[33]. Acetylthiocholine iodide (AChI) and Butyrylcholine iodide (BChI) were used as a substrate of the reaction. 5,5′-Dithio-bis(2-nitro-benzoic acid) (DTNB) was used for the determination of the AChE/BChE activities. Briefly, 100 mL of Tris/HCl buffer (1 M, pH 8.0) and 10 mL of sample solution dissolved in ultra pure water at different concentrations and 50 mL AChE/BChE solution were mixed, incubated for 10 min at 25 °C. Then 50 mL of DTNB (0.5 mM) was added. The reaction was then started by the addition of 50 mL of AChI/BChI (10 mM). The hydrolysis of these substrates was observed spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, determined by the enzymatic hydrolysis of AChI/BChI, at a wavelength of 412 nm[34]. For the determination of inhibition effect of **P1–P3** on AChE and BChE, an activity (%)-[**P1–P3**] graph was drawn. The IC_{50} values were obtained from activity (%) versus compounds plots. For calculation of K_i values, three different concentrations were used. The Lineweaver–Burk curves were drawn and calculations were realised[32].

Results and discussion

The inhibition data of the isatin Mannich bases **P1–P3** are shown in Table 1. Our results indicate that **P1–P3** had active inhibition profiles against slow cytosolic isoform hCA I, and cytosolic dominant rapid isoenzyme hCA II. The cytosolic hCA I isoenzyme was effectively inhibited by **P1–P3** with inhibition constants in the low nanomolar range as $8.57 \pm 1.65 - 9.43 \pm 3.57$ nM. On the other hand, acetazolamide (AZA) which is used as clinical drug had given a K_i value of 10.16 ± 2.46 nM.

The data showed that **P1–P3** were more effective than AZA in terms of hCA II inhibitory properties. The compounds **P1–P3** showed similar inhibition profile on cytosolic hCA II isoenzyme, with K_i values in the range of 5.02 ± 1.07–8.08 ± 1.98 nM. The reference drug AZA showed a K_i value of 8.29 ± 1.38 nM. It means that the compounds were more powerful inhibitors against hCA II than reference compound.

AChE was effectively inhibited by **P1–P3** with K_i values in the range 0.75 ± 0.30–1.01 ± 0.24 nM. Similarly, these compounds inhibited BChE with K_i values in the range 0.42 ± 0.21–1.11 ± 0.43 nM. On the other hand, Tacrine (TAC), which is used for the treatment of AD had been shown to lower AChE and BChE inhibition profiles, K_i values are in the range 2.69 ± 0.56 and 2.49 ± 0.23 nM, respectively. When the results were carefully considered it can be easily noticed that Mannich bases **P1** with piperidine (3.4 times), **P2** with morpholine (3.6 times), **P3** with *N*-methylpiperazine (2.7 times) were 2.7–3.6 times more potent than reference drug Tacrine towards AchE. On the other hand, **P1** (2.7 times), **P2** (2.2 times) and **P3** (5.9 times) were 2.2–5.9 times more effective than the clinically used reference drug Tacrine towards BChE.

Conclusion

The effects of isatin Mannich bases **P1–P3** against hCA I/hCA II isoenzymes and AChE/BChE enzymes were evaluated. **P1–P3** demonstrated very impressive inhibition profile on cholinesterases enzymes (AChE and BChE) and they also inhibited both CA isoenzymes (hCA I and hCA II) at the nanomolar level. These inhibitory effects were more powerful in all cases than the references used for CA experiment and cholinesterases experiments.

This pilot study suggests that isatin Mannich bases **P1–P3** are good candidate compounds especially for the development of new cholinesterase inhibitors. Since they had 2.2–5.9 times powerful inhibition potency than clinical used drug, Tacrine. They are also useful candidates for the development of new CAIs, since they showed slightly more potent inhibition than the clinically used drug, Acetazolamide. It should be, however, mentioned that these compounds do not possess a chemical structure normally associated with CA inhibition[35–42] and as thus probably possess an unknown mechanism of inhibition which should be investigated in more detail[43].

Declaration of interest

The authors report no conflict of interest and are responsible for the contents and writing of the paper.

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