MÜSBED 2012;2(3):119-123

Araştırma / Original Paper

Studies on Imidazopyridine Derivatives as Acetylcholinesterase Inhibitors

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Kabul tarihi / Date of acceptance: 6 Ağustos 2012 / August 6, 2012

ÖZET

Asetilkolinesteraz inhibitörleri olarak imidazopiridin türevleri üzerine çalışmalar

Amaç: Bu çalışmada, imidazo[1,2-a]piridinin bazı hidrazid türevlerinin sentezlenmesi ve antikolinesteraz aktivitelerinin araştırılması amaçlandı.
Yöntem: İmidazo[1,2-a]piridin-2-karboksilik asid hidrazidi ile çeşitli benzaldehit türevlerinin reaksiyonu ile hedef bileşikler olan N-(benziliden)imidazo[1,2-a]piridin-2-karboksilik asid hidrazid türevlerine ulaşıldı. Bileşiklerin kimyasal yapıları, IR, ^{1}H-NMR ve FAB$^+$-MS spektral verileri ve elementel analiz verileri ile aydınlatıldı. Modifiye edilmiş Ellman spektrofotometrik metodu kullanılarak, elde edilen tüm bileşiklerin asetilkolinesteraz (AChE) inhibisyonları incelendi.
Bulgular: Donepezil (IC_{50}=0.058±0.002 µM) ile kıyaslandığında, sentezlenen bileşiklerin üç tanesinin (2, 3 ve 4), IC_{50} değerleri sırasıyla 74.42±4.29, 43.26±7.28 ve 18.29±2.31 µM olarak ölçülmüş ve AChE üzerinde ümit verici inhibisyonları gözlenmiştir.
Sonuçlar: Fenil halkası üzerindeki halojen sübstitisyonunun, antikolinesteraz etki üzerinde önemli katkısı vardır.
Anahtar sözcükler: İmidazo[1,2-a]piridin, hidrazon, kolinesteraz inhibitörleri

ABSTRACT

Studies on imidazopyridine derivatives as acetylcholinesterase inhibitors

Objective: In this study we aimed to synthesize some hydrazide derivatives of imidazo[1,2-a]pyridine and to evaluate their anticholinesterase activities.
Method: The reaction of imidazo[1,2-a]pyridine-2-carboxylic acid hydrazides with various benzaldehydes gave N-(benzylidene) imidazo[1,2-a]pyridine-2-carboxylic acid hydrazide derivatives. The chemical structures of the compounds were elucidated by IR, ^{1}H-NMR, FAB$^+$-MS spectral data and elemental analysis. In the pharmacological study, anti-cholinesterase activities of these compounds have been evaluated by using modified Ellman's spectrophotometric method.
Results: Three of the synthesized compounds (2, 3 and 4) can be identified as promising anticholinesterase agents due to their inhibitory effect on AChE with IC_{50} value of 74.42±4.29, 43.26±7.28 and 18.29±2.31 µM, respectively when compared with Donepezil (IC_{50}=0.058±0.002 µM).
Conclusion: The halogen substitutions on phenyl ring have a crucial influence on anticholinesterase activity.
Key words: Imidazo[1,2-a]pyridine, hydrazone, cholinesterase inhibitors

INTRODUCTION

It is well-known that acetylcholine (ACh) acts both as a pre-ganglionic and a postganglionic transmitter in the parasympathetic nervous system of vertebrates and invertebrates, and also as an excitatory neurotransmitter for voluntary muscles in the somatic nervous system (1,2). Acting at the cholinergic synapses, by quick hydrolysis of ACh to choline and acetate, acetylcholinesterase (AChE) is a terminator enzyme of nerve impulse transmission. Inhibition of AChE evolves a strategy for the treatment of several diseases such as Alzheimer's disease (AD), senile dementia, ataxia, myasthenia gravis and Parkinson's disease (3). AD is a chronic neurodegenerative disorder which is characterized by a loss of cognitive ability, severe behavioral abnormalities and ultimately death. AD is the most common cause of dementia among the elderly and there are currently 2.5 to 4.0 million estimated AD patients in the United States and some 17 to 25 million worldwide (4,5). In AD, increasing numbers of nerve cells degenerate and die along with loss in synapse, which permits a neuron to pass signal to another cell in central nervous system. As a result,

Table 1: Some characteristics of the compounds

Compound*	R	M.P. (°C)	Yield (%)	Molecular Formula
1	H	241-243	66	$C_{18}H_{16}N_6O_4$
2	Cl	274-276	73	$C_{18}H_{15}ClN_6O_4$
3	Br	278-279	80	$C_{18}H_{15}BrN_6O_4$
4	F	264-265	75	$C_{18}H_{15}FN_6O_4$
5	CH_3	260-261	70	$C_{19}H_{18}N_6O_4$
6	OCH_3	258-260	74	$C_{19}H_{18}N_6O_5$
7	NO_2	271-272	75	$C_{18}H_{15}N_7O_6$
8	$N(CH_3)_2$	246-248	68	$C_{20}H_{21}N_7O_4$

*please refer to the text for the properties of the compounds.

cognitive impairment and dementia occur (6). The neuropathology of AD is generally characterized by the presence of numerous amyloid 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, which plays an important role in the cognitive impairment associated with AD. Attempts to correct ACh deficiency in the brain of affected individuals produced the licensed medications for the symptomatic treatment of AD in the form of acetylcholinesterase inhibitors (AChEIs). A cholinesterase inhibitor reduces the breakdown of ACh levels, the drug may help counterweigh for the loss of functioning brain cells (7).

The literature survey reveals that hydrazide-hydrazone group plays an important role for anticholinesterase activity (8-15). In the present study, prompted by these observations, the synthesis and anti-cholinesterase activity screening of some hydrazide-hydrazone derivatives of imidazopyridines were aimed.

METHODS

Chemistry

All melting points (m.p.) were determined in open capillaries on a Gallenkamp apparatus and were uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck). Spectroscopic data were recorded by the following instruments: IR: Shimadzu IR-435 spectrophotometer; ^{1}H-NMR: Bruker 250 MHz spectrometer, MS-FAB: VG Quattro Mass spectrometer. Elemental analyses were recorded on Perkin Elmer EAL 240 spectrometer.

General procedure for synthesis of the compounds

3-Nitro-5-acetamido-6-methylimidazo[1,2-a]pyridine-2-carboxylic acid hydrazide (A)

This compound was prepared according to the previously reported method, by reacting ethyl 3-nitro-5-acetamido-6-methylimidazo[1,2-a]pyridine-2-carboxylate with hydrazine hydrate (16,17).

N-(Benzylidene)-3-nitro-5-acetamido-6-methylimidazo[1,2-a]pyridine-2-carboxylic acid hydrazide derivatives (1-8)

Equimolar quantities of acid hydrazide (A) (30 mmol) and appropriate benzaldehydes in 25 ml of absolute ethanol were refluxed for 3-5 h. The resulting solid was filtered and recrystallized from ethanol (Table 1) (18).

1: IR (KBr, cm^{-1}): 3390 and 3141 (NH), 1689 and 1676 (C=O), 1525–1345 (C=C and C=N).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.25 (3H, s, CH_3), 2.55 (3H, s, $COCH_3$), 6.90-8.30 (7H, m, aromatic protons), 9.60 (1H, s, NH), 9.75 (1H, d [J=6.20 Hz], CH), 12.25 (1H, bs, N-NH). MS (FAB) [M+1]: m/z 381. Anal. Calc. for $C_{18}H_{16}N_6O_4$: C, 56.84; H, 4.24; N, 22.09. Found: C, 56.80; H, 4.20; N, 22.11.

2: IR (KBr, cm^{-1}): 3409 and 3165 (NH), 1705 and 1685 (C=O), 1540–1355 (C=C and C=N).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.20 (3H, s, CH_3), 2.50 (3H, s, $COCH_3$), 7.40-8.30 (6H, m, aromatic protons), 9.70 (1H, s, NH), 9.80 (1H, d [J=6.23 Hz], CH), 12.30 (1H, bs, N-NH). MS (FAB) [M+1]: m/z 415. Anal. Calc. for $C_{18}H_{15}ClN_6O_4$: C, 52.12; H, 3.64; N, 20.26. Found: C, 52.14; H, 3.68; N, 20.30.

3: IR (KBr, cm^{-1}): 3375 and 3140 (NH), 1690 and 1665 (C=O), 1500–1301 (C=C and C=N).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.25 (3H, s, CH_3),

2.45 (3H, s, $COCH_3$), 7.30-8.20 (6H, m, aromatic protons), 9.65 (1H, s, NH), 9.70 (1H, d [J=5.91 Hz], CH), 12.10 (1H, bs, N-NH). MS (FAB) [M+2]: m/z 461. Anal. Calc. for $C_{18}H_{15}BrN_6O_4$: C, 47.08; H, 3.29; N, 18.30. Found: C, 47.10; H, 3.33; N, 18.26.

4: IR (KBr, cm^{-1}): 3445 and 3129(NH), 1690 and 1670 (C=O), 1541–1301(C=C and C=N).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.20 (3H, s, CH_3), 2.45 (3H, s, $COCH_3$), 7.35-8.25 (6H, m, aromatic protons), 9.70 (1H, s, NH), 9.75 (1H, d [J=6.12 Hz], CH), 12.10 and 12.30 (1H, two s, N-NH). MS (FAB) [M+1]: m/z 399. Anal. Calc. for $C_{18}H_{15}FN_6O_4$: C, 54.27; H, 3.80; N, 21.10. Found: C, 54.28; H, 3.79; N, 21.08.

5: IR (KBr, cm^{-1}): 3501 and 3126(NH), 1710 and 1696(C=O), 1539–1398(C=C and C=N).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.25 (3H, s, CH_3), 2.40(3H, s, phenyl-CH_3), 2.65 (3H, s, $COCH_3$), 7.10-8.30 (6H, m, aromatic protons), 9.70 (1H, br s, NH), 9.80 (1H, d [J=6.96 Hz], CH), 12.15 (1H, bs, N-NH). MS (FAB) [M+1]: m/z 395. Anal. Calc. for $C_{19}H_{18}N_6O_4$: C, 57.86; H, 4.60; N, 21.31. Found: C, 57.82; H, 4.59; N, 21.33.

6: IR (KBr, cm^{-1}): 3428 and 3129(NH), 1699 and 1671(C=O), 1555–1378(C=C and C=N).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.20 (3H, s, CH_3), 2.60 (3H, s, $COCH_3$), 3.75 and 3.90 (3H, two s, OCH3), 6.85-8.25 (6H, m, aromatic protons), 9.65 (1H, s, NH), 9.80 (1H, d [J=5.51 Hz], CH), 12.00 and 12.20 (1H, two s, N-NH). MS (FAB) [M+1]: m/z 411. Anal. Calc. for $C_{19}H_{18}N_6O_5$: C, 55.61; H, 4.42; N, 20.48. Found: C, 55.63; H, 4.40; N, 20.51.

7: IR (KBr, cm^{-1}): 3362 and 3111(NH), 1701 and 1675(C=O), 1522–1332(C=C and C=N).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.20 (3H, s, CH_3), 2.50 (3H, s, $COCH_3$), 6.95-8.20 (6H, m, aromatic protons), 9.55 (1H, s, NH), 9.65 (1H, d [J=5.81 Hz], CH), 12.10 (1H, bs, N-NH). MS (FAB) [M+1]: m/z 426. Anal. Calc. for $C_{18}H_{15}N_7O_6$: C, 50.83; H, 3.55; N, 23.05. Found: C, 50.81; H, 3.51; N, 23.09.

8: IR (KBr, cm^{-1}): 3408 and 3151(NH), 1701 and 1691(C=O), 1588–1369(C=C and C=N).

^{1}H-NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.25 (3H, s, CH_3), 2.60 (3H, s, $COCH_3$), 2.85-2.95 (6H, m, $N(CH_3)_2$), 7.05-8.35 (6H, m, aromatic protons), 9.60 (1H, s, NH), 9.70 (1H, d [J=5.98 Hz], CH), 12.25 (1H, bs, N-NH). MS (FAB) [M+1]: m/z 424. Anal. Calc. for $C_{20}H_{21}N_7O_4$: C, 56.73; H, 5.00; N, 23.16. Found: C, 56.71; H, 4.96; N, 23.19.

Pharmacology

AChE Inhibition

All compounds were subjected to a slightly modified method of Ellman's test (19) in order to evaluate their potency to inhibit the AChE. The spectrophotometric method is based on the reaction of released thiocholine to give a colored product with a chromogenic reagent 5,5-dithio-bis(2-nitrobenzoic)acid (DTNB). AChE, (E.C.3.1.1.7 from Electric Eel, 500 units), and Donepezil hydrochloride were purchased from Sigma-Aldrich (Steinheim, Germany). Potassium dihydrogen phosphate, DTNB, potassium hydroxide, sodium hydrogen carbonate, gelatine and acetylthiocholine iodide (ATC) were obtained from Fluka (Buchs, Switzerland). Spectrophotometric measurements were performed on a 1700 Shimadzu UV-1700 UV–Vis spectrophotometer. Cholinesterase activity of the compounds (1-8) was measured in 100 mM phosphate buffer (pH 8.0) at 25 °C, using ATC as substrates, respectively. DTNB (10 mM) was used in order to observe absorbance changes at 412 nm. Donepezil hydrochloride was used as a positive control (Table 2) (20).

Enzymatic assay

Enzyme solutions were prepared in gelatine solution (1%), at a concentration of 2.5 units/mL. AChE and compound solution (50 µL) which is prepared in 2% DMSO at a concentration range of 10^{-1}-10^{-6} mM were added to 3.0 mL phosphate buffer (pH 8±0.1) and incubated at 25°C for 5 min. The reaction was started by adding DTNB (50 µL) and ATC (10 µL) to the enzyme-inhibitor mixture. The production of the yellow anion was recorded for 10 min at 412 nm. As a control, an identical solution of the enzyme without the inhibitor was processed following the same protocol. The blank reading contained 3.0 mL buffer, 50 µL 2% DMSO, 50 µL DTNB and 10 µL substrate. All processes were assayed in triplicate. The inhibition rate (%) was calculated by the following equation:

Inhibition % = $(A_C - A_I) / A_C \times 100$

Table 2: AChE inbition (%) of the compounds and IC_{50} values

Compound*	AChE Inhibition (%)			
	100 µM	1 µM	0.01 µM	IC_{50} (µM)
1	29.07±4.02	17.26±1.91	5.97±0.91	>100
2	55.69±6.14	35.28±2.04	15.69±1.46	74.42±4.29
3	62.53±7.16	30.19±2.29	11.13±1.26	43.26±7.28
4	65.40±4.09	40.16±2.24	7.58±0.48	18.29±2.31
5	22.19±3.01	17.12±0.98	5.87±1.14	>100
6	39.05±5.09	21.75±1.09	10.07±0.92	>100
7	38.11±4.87	16.49±1.27	7.21±1.97	>100
8	41.23±1.84	18.16±1.96	4.93±0.89	>100
Donepezil	95.92±4.89	76.96±5.01	35.86±4.39	0.058±0.002

*please refer to the text for the properties of the compounds

Where A_I is the absorbance in the presence of the inhibitor, A_C is the absorbance of the control and both of the values are corrected with blank-reading value. SPSS for Windows 15.0 was used for statistical analysis. Data were expressed as Mean ± SD.

RESULTS

The structures of the compounds obtained were elucidated by spectral data. In the IR spectra, some significant stretching bands were observed due to N-H, C=O and C=N, C=C.

The ^{1}H-NMR spectra data were also consistent with the assigned structures. In the 250 MHz ^{1}H-NMR spectrum of compounds, we observed paired peaks for each of the protons N=CH (9.60-9.80 ppm), and N=NH (12.00-12.30 ppm) corresponding to (E) and (Z) forms of the compounds. For each compound, the intensities of these paired peaks differed from others, due to the variable amounts of (E) and (Z), which are usually unequal. The NH proton was observed at 9.55-9.70 ppm. All the other aromatic and aliphatic protons were observed at expected regions. The mass spectra (MS(FAB)) of compounds showed [M+1] peaks, in agreement with their molecular formula.

The anticholinesterase effects of the compounds (1-8) were determined by modified Ellman's spectrophotometric method (Table 2). Among these compounds (1-8), compounds 2, 3 and 4 can be identified as promising anticholinesterase agents due to their inhibitory effect on AChE with IC_{50} value of 74.42±4.29, 43.26±7.28 and 18.29±2.31 µM respectively when compared with Donepezil (IC_{50} =0.058±0.002 µM).

DISCUSSION

The compounds 2, 3 and 4 possess halogen substituent on phenyl ring, they showed different levels of anticholinesterase activity. The former bearing Br and F atoms exhibited the inhibitory effect on AChE with IC_{50} value of 43.26±7.28 and 18.29±2.31 µM, whereas the latter bearing Cl atom exhibited the inhibitory effect on AChE with an IC_{50} value of 74.42±4.29 µM. The other synthesized compounds (1,5-8) did not show notable inhibitory effect on AChE.

The results indicate that the halogen substitutions on phenyl ring have a crucial influence on anticholinesterase activity.

Acknowledgements

The author would like to thank the staff of Anadolu University Faculty of Pharmacy, Department of Pharmaceutical Chemistry for their valuable suggestions regarding the manuscript.

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