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Research Article

RATIONAL DESIGN AND SYNTHESIS OF SOME NOVEL SUBSTITUTED ACETAMIDES BEARING 2-[3-ACETYL-(1H-INDOL-1-YL/5-METHOXY-1H-INDOL-1-YL)] MOTIFS AND THEIR STUDY FOR ACETYLCHOLINESTERASE INHIBITORY ACTIVITY

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ABSTRACT

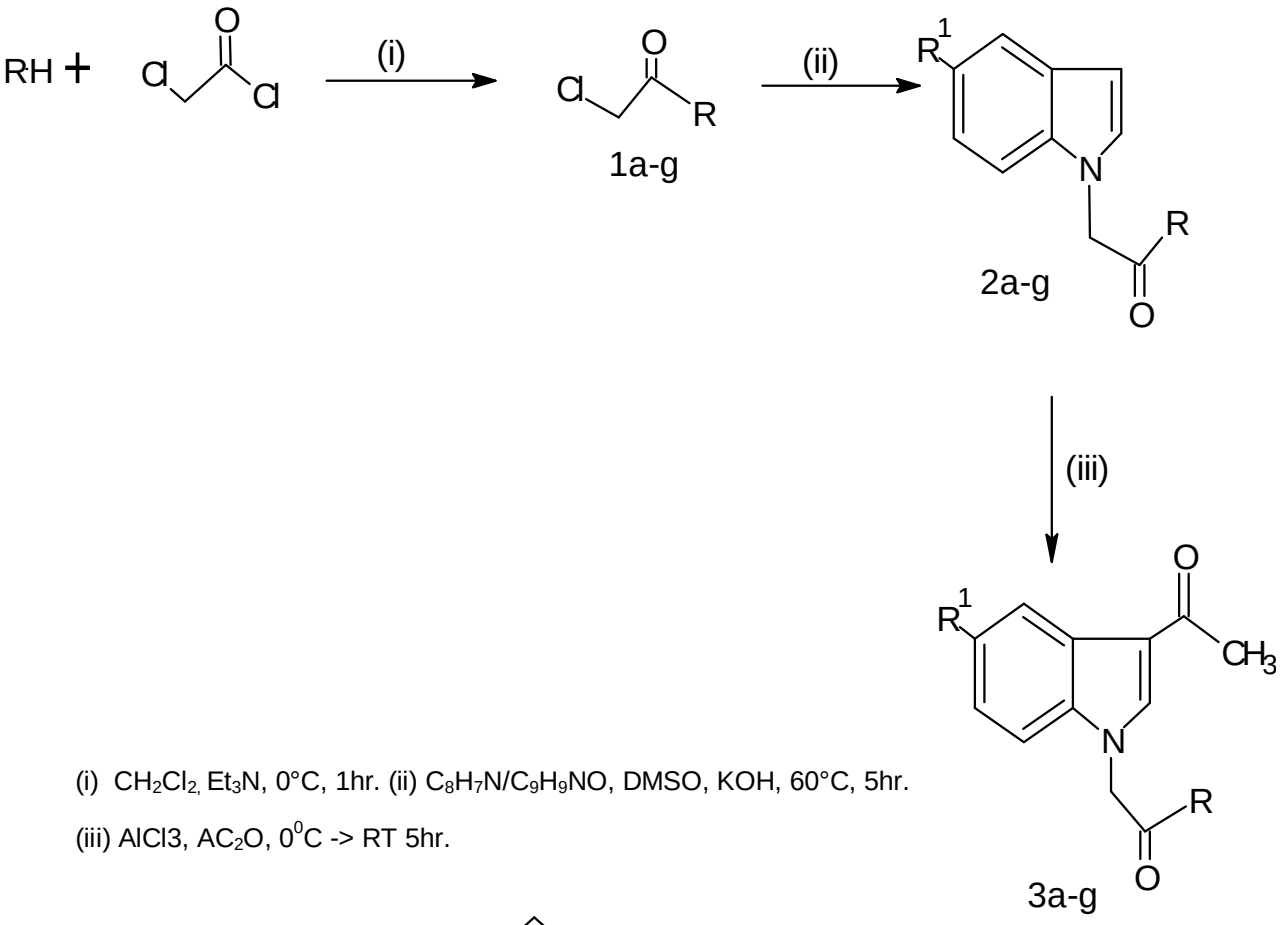
Currently about 20 million people are suffering from Alzheimer's disease (AD) and this figure is projected to touch 34 million in another 10 years. One of the presently used important drug to treat AD is rivastigmine. But it has short half-life and limited usage. So some new compounds, substituted acetamides bearing indole moiety which are related to rivastigmine were designed and synthesized. 2-chlorocycloaminoethanones and 2-chloro N-alkyl acetamides were synthesized by adding chloroacetyl chloride dropwise to a mixture of cycloamine/N-alkylamines, dichoromethane and triethylamine and stirring for 1 hour at 0°C. These compounds were stirred at 60°C for 5 hours with a solution of indole/5-methoxy indole in DMSO in presence of potassium hydroxide to obtain 2-[(1H-indol/5-methoxy-1H-indol-1-yl)-1-cyclo amino/N-alkylamino]-ethanones.Titled compounds were synthesized by acetylating the above compounds with acetic anhydride in presence of anhydrous aluminum chloride and dichloromethane at 0°C. Synthesized compounds were characterized on the basis of physical and spectroscopic data. Anticholinesterase inhibitory activity of these compounds was determined by Ellman's esterase assay and was found to be active in 371.5µM to1025µM range. These compounds were also found to be stable when subjected to forced alkaline degradation.

Keywords: Indole, Rivastigmine, acetamide and acetyl cholinesterase.

INTRODUCTION

Many thousands of people are suffering from Alzheimer's disease (AD) and the number may double in another decade. It is a neurological disorder and is clinically characterized by loss of memory. It affects cholinergic neurons in the basal forebrain. AD may be due to the massive deposition of amyloid plaques[1]. Acetyl cholinesterase (AChE) plays a crucial role in the pathogenesis of AD by mediating the hydrolysis of acetylcholine. Moreover, the neurotoxicity of amyloid components is increased by the presence of AChE[2]. So AChE inhibition has been broadly explored in AD treatment. Rivastigmine and other presently used drugs have short half-life and limited usage[3-4].In the present study some novel substituted acetamides bearing 2-(3-acetyl-1H-indol-1-yl) motifs, which are structurally related to rivastigmine were synthesized for better safety, efficacy and longer half-life.

SCHEME



MATERIALS AND METHODS

Representative method for preparation of 2-chloro-cycloamine-ethanones and 2-chloro-N-alkyl-acetamides (1a-g)

To a mixture of cycloamine/N-alkyl amine, dichloromethane (20mL) and triethylamine (1 equivalent), chloroacetylchloride (1.5 equivalent) was added in a drop wise manner and stirred for 1 hour at 0^0c in an ice bath. The completion of the reaction was confirmed by TLC using hexane: ethyl acetate (6:4) as the solvent system. Distilled water was added to the reaction mixture and dichloromethane fraction was collected. The combined extract was dried over anhydrous sodium sulphate and concentrated in vacuo to afford titled compounds (1a-g) as yellow coloured oily compounds[5].

Representative method for preparation of 2-[(1H-indol/5-methoxy-1H-indol-1-yl)-1-(cycloamino/N-alkyl amino)]-ethanone (2a-g)

To a solution of indole/5-methoxy indole (1 equivalent) in DMSO (4 mL), Potassium hydroxide (2 equivalents) was added and stirred at room temperature for 1 hour. To these, compounds (1a-g) (2 equivalents) were added correspondingly and the reaction mixture was stirred at 60^0c for 5 hours. The completion of reaction was confirmed by TLC using Hexane: Ethyl acetate (2:8) as solvent system. Reaction mixture was then poured into ice cold water and neutralized with dil.HCl. Separated solid was filtered, dried and recrystallized from methanol[6].

Method of preparation of 2-[3-acetyl(1H-indol/5-methoxy-1H-indol-1 yl-)]-1-(cycloamino/N-alkylamino)-ethanone (3a-g)

Anhydrous aluminum chloride (3.5 equivalents) was added to dichloromethane (100 mL) and stirred at 0^0c till a suspension was formed. Acetic anhydride (1.5 equivalents) was added drop by drop at 0^0c and the stirred reaction mixture till a clear solution was obtained. Compounds (2a-g) (1equivalent) which were dissolved in 5mL DCM were added drop by drop to the reaction mixture and stirred vigorously for 5 hours at 0^0c to room temperature. Completion of the reaction mixture was confirmed by TLC using Chloroform: Methanol (9:1). The reaction mixture was extracted with brine, sodium bicarbonate, brine successively. DCM fraction was collected and evaporated in vacuo. Titled compounds were obtained in pure form by column chromatography using solvent system chloroform: methanol.

Ellman Esterase Assay

The assay is based on the measurement of changes in absorbance at 412 nm. The assay uses thiol ester of acetylcholine instead of oxy ester acetylcholine. AChE hydrolyses the acetylcholine to produce thiolcholineand acetate. Thiocholine produced in the reaction reacts with 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) andreleases 5-thio-2-nitrobenzoic acid (yellow colour complex) which absorbs light in the range of 400-420 nm with maximal absorption at 412 nm. The anticholinesterase activities were determined according to Ellman's Esterase assay method against freshly prepared AChE from rat plasma using rivastigmine asreference compound[7].

RESULTS AND DISCUSSION

Synthesized compounds were characterized by physical data such as melting point, different Rf value etc. and spectral data such I.R, H¹NMR and mass. According to Ellman esterase assay[7] compounds were found to be active in 371.5µM to1024µM range. Among the synthesized compounds 3C was found to be more potent with IC_{50} of 371.5 µM. These compounds were also found to be stable when subjected to forced alkaline degradation.

IR Spectra of compound 3a

IR (KBr, cm^{-1}): 2926 (-CH_3 Str); 2854 (-CH_2 Str); 1647 (-CO- Str); 1469-1392 (Ar-C=C Str.); 1222 (Asymm.-C-O-C Str.); 1136 (sym. –C-O-C Str.).

1 H NMR spectra of compound 3a

1H NMR (400 MHz, DMSO-d_6): δ 8.24(s, 1H); 8.91-8.16(m,1H);7.42-7.41 (dd ,J=8.52Hz and 6.32Hz,1H); 7.22-7.19 (m, 2H); 5.25 (s, 2H); 3.52 (s, 2H); 3.43 (t,J=3.19Hz,2H); 2.42 (s,3H); 1,62 (s, 4H); 1.46 (s, 2H).

GC-MS spectra of compound 3a

GC-MS (IM): m/z 284 $(M)^+$; 269[M-15, -CH_3]$^+$; 241(M-43, -$COCH_3$)$^+$; 172[M-$C_6H_{10}NO$]$^+$; 84[M-172,-$C_{12}H_{10}NO_2$]$^+$; 69[M6-215,-$C_{12}H_7NO_2$]$^+$.

IR Spectra of compound 3c

IR (KBr, cm^{-1}): 2966 (-CH_3 Str); 2856 (-CH_2 Str); 1666 (-CO Str); 1473-1392 (Ar-C=C Str); 1230 (Asymm.-C-O-C Str.); 1111 (sym. –C-O-C Str.).

1 H NMR spectra of compound 3c

1H NMR (300 MHz, DMSO-d_6): δ 8.23(s, 1H); 8.19-8.16 (dd ,J=6 Hz and 3 Hz, 1H); 7.48-7.45 (dd, J=6Hz and 3 Hz,1H); 7.26-7.17 (m, 2H); 5.30 (s, 2H); 3.71(t, J=2.43 Hz, 2H); 3.60 (t, J=2.5 Hz, 4H); 3.45 (t, J=2.61 Hz, 2H); 2.43 (s, 3H).

GC-MS spectra of compound 3c:

GC-MS (IM): m/z 286 $(M1)^+$; M1-15(271, -CH_3)$^+$; M1-43(243, -$COCH_3$)$^+$; M1-114(172, -C_5H_8NO)$^+$; M1-200(86, -$C_{12}H_{12}NO_2$)$^+$; M6-16(70,-NH_3)$^+$.

CONCLUSION

Titled compounds were found to have interaction with acetyl cholinesterase receptor as determined by Ellman's Esterase assay. Synthesized compounds inhibited the enzyme at a range of 375.5 µM to 1025 µM. Since these compounds were found to be resistant to forced alkaline degradation, they could be resistant to AChE mediated hydrolysis. All the

compounds were found to be drugable under Lipinski rule.

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Table 1: Physical data of synthesized compound-(3a-g)

S. No.	Molecular formula	Mol. Wt.	Melting point (°C)	% Yield	R_f*	λmax	Predicted Log P[s]
3a	$C_{17}H_{20}N_2O_2$	284.35	176-178	51	0.84	211	0.94
3b	$C_{16}H_{18}N_2O_2$	270.33	168-170	30	0.73	211	0.53
3c	$C_{16}H_{18}N_2O_3$	286.33	218-220	59	0.79	211	-0.19
3d	$C_{18}H_{22}N_2O_3$	314.38	158-160	26	0.84	277	0.82
3e	$C_{17}H_{20}N_2O_3$	300.35	160-162	30	0.75	206	0.4
3f	$C_{17}H_{20}N_2O_3$	316.35	162-164	43	0.80	278	-0.32
3g	$C_{15}H_{18}N_2O_2$	258.32	146-148	61	0.68	212	0.55

*Solvent system-Chloroform: Methanol (9:1); [#]Solvent-Methanol ; [$]Chemdraw software Ultra11

Table 2: Inhibition of AChE activities of the Compound (3a-g)

Compound code	IC50±SEM;AChE (µM)
Rivastigmine	2.88
3a	903±4
3b	977±14
3c	371..5±3.5
3d	644±44
3e	708.5±25.5
3f	723.5±17.5
3g	1025±25

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