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Synthesis and biological evaluation of new oxopyrrolidine derivatives as inhibitors of acetyl cholinesterase and β amyloid protein as anti – Alzheimer's agents

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ABSTRACT

A new series of oxopyrrolidines was synthesized and evaluated for their effect on Alzheimer's disease by measuring their inhibitory activity against acetyl cholinesterase enzyme and amyloid β 42 protein. Most of the compounds showed good inhibitory activity with ethyl 2-(2-(2, 6-dimethylphenylcarbamoyl)- 5-oxopyrrolidin-1-yl) acetate (**V**) having the highest activity against acetyl cholinesterase with IC_{50} value 1.84 ng/g tissue compared to standard donepezil 3.34 ng/g tissue. Furthermore, compound 1-((4-(4-chlorophenyl) piperazin-1-yl) methyl)-N-(2,6-dimethylphenyl)-5- oxopyrrolidine- 2-carboxamide **(IIIe)** displayed the highest activity against β 42 protein with IC_{50} value of 11.3 Pg/g tissue compared to 18.4 Pg/g tissue of donepezil.

1. Introduction

Alzheimer's disease (AD), is one of the most severe conditions affecting elderly people. It is estimated that around 24 million people worldwide are suffering from AD. The figure is expected to increase significantly over the next 50 years due to increasing life expectancy [1]. Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by abnormal accumulation of β amyloid (A β) peptide, neurofibrillary tangles and cholinergic neurons loss [2]. Among many pharmacological agents, acetyl cholinesterase inhibitors AChEI) are the only class of compounds that have consistently proven to be efficacious in treating the cognitive and functional symptoms of Alzheimer's disease [3,4]. In patients with Alzheimer's disease, large numbers of senile plaques are found throughout the cerebral cortex and hippocampus the principal proteinaceous component of the amyloid deposited is β amyloid protein A β) [5]. According to the amyloid hypothesis, the neuronal loss observed in AD is caused by deposition of extracellular aggregates of the A β protein [6]. Strong evidence has been obtained that the deposition of A β in senile plaques plays a seminal role in AD pathogenesis [7]. The current pharmacological treatment of Alzheimer's disease (AD) comes down to four marketed drugs (tacrine, donepezil, rivastigmine and galantamine) (Fig. 1) all of which are acetyl cholinesterase inhibitors, conforming to the cholinergic hypothesis [8]. Donepezil, an acetyl cholinesterase inhibitor, is an approved drug for the treatment of Alzheimer's disease AD) [9] and is the current first choice drug for AD since it is a very potent, low toxic and well tolerated. [11] donepezil was found also to significantly improve Aβ induced memory impairment [12].

Several researchers also debated piperazine derivatives as potential neuroprotective agents [13].

Donepezil, which is a benzylpiperidine derivative, was chosen as a reference standard drug it is thought to mimic the binding mode of Ach by structural similarity and therefore, is a competitive inhibitor of AChE [17] the main features of donepezil were taken into consideration while designing the new derivatives. The four main parts essential for its activity, the indanone moiety (a), a spacer (b), positive charge center (c) and a phenyl moiety (d) [18] Fig. 2.

From the previous we designed and synthesized new oxopyrrolidine derivatives with structural resemblance to donepezil bearing substituted piperazine to investigate structure modification of the

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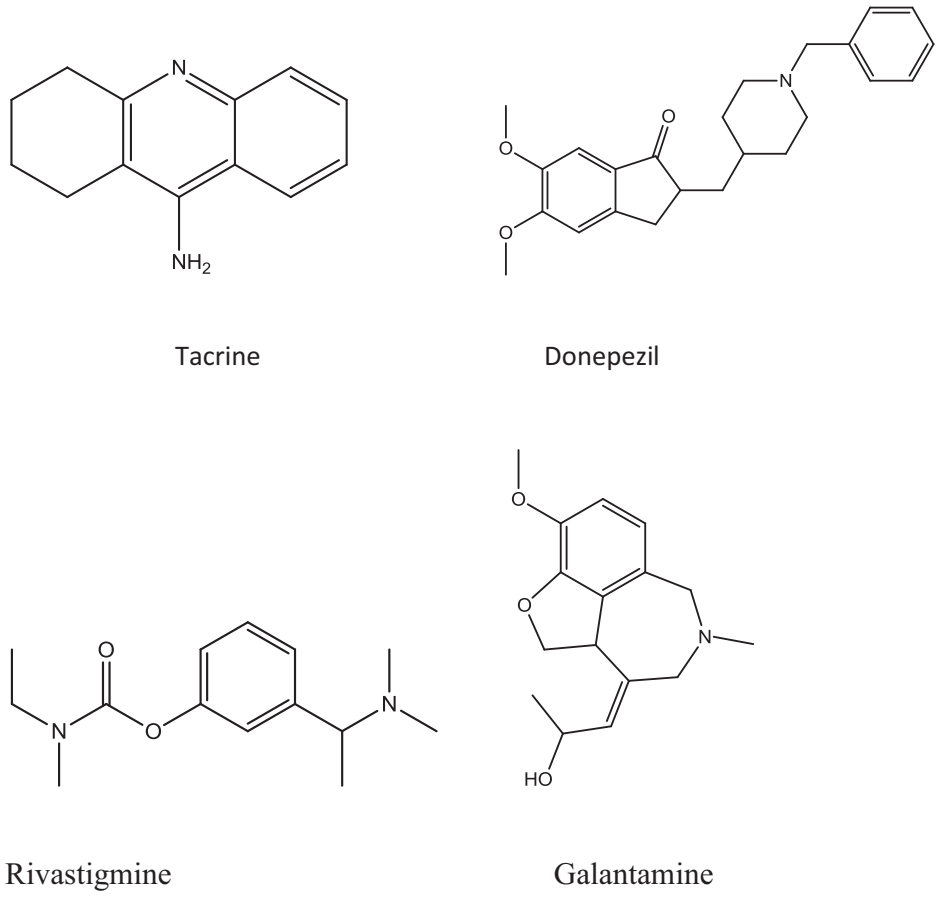


Fig. 1. Structure of tacrine, donepezil, rivastigmine and galantamine.

new derivatives. The new derivatives were tested for inhibition of acetyl cholinesterase enzyme and amyloid β 42 protein.

2. Materials and methods

2.1. Chemistry

All melting points were determined in open glass capillaries on a Gallenkamp melting point apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer spectrophotometer using potassium bromide discs. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini 300 MHz and Bruker 400 MHz using DMSO-d_6 as solvent. The chemical shifts were reported as parts per-million δ ppm, tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a Jeol-SX-102 instrument. Elemental analysis was performed on a Perkin-Elmer 2400 C, H, N analyzer and values were within ±0.4% of theoretical percentages. The progress of the reaction was monitored on readymade Silica –gel plates fluorescent (Merck) using $CHCl_3$/CH_3OH (9.5:0.5) as solvent using, UV lamp.

Pyroglutamic acid **I** was purchased from Aldrich chemicals,1-(2-ethoxy-2-oxoethyl)-5-oxopyrrolidine-2-carboxylic acid **IV** and ethyl 2-(2,3-dioxoindolin-1-yl)acetate **VIII** were synthesized according to reported procedures [15,10].

2.2. General procedure for the synthesis of (IIa-d)

To a solution of **(I)** (0.1 mol, 1.29 gm) in ethanol (5 ml) was added a solution of formalin (0.1 mol, 0.03 gm) and the appropriate piperazine derivative (0.1 mol) in ethanol (5 ml), the reaction was then heated under reflux for 2 h. The reaction was allowed to cool, filtered, the separated solid was dried and finally crystallized from ethanol.

2.2.1. 5-Oxo-1-((4-phenylpiperazin-1-yl) methyl) pyrrolidine-2-carboxylic acid (IIa)

Mp 102 °C, yield 85%, IR(KBr, cm^{-1}): 3406(OH), 3087 (CH aromatic), 2970 (CH alphatic), 1710, 1680 (CO). ^{1}H NMR 400 MHz (DMSO-d_6): 1.94–2.35 (m,4H, 2CH $_2$), 2.59(d, *j = 6* Hz, 4H, piperazine), 3.05 (d, *j = 5* Hz, 4H, piperazine),4.28(s, 2H, CH_2),6.74(t, 1H, Ar-H),6.89 (d, *j = 6* Hz, 2H, Ar-H), 6.92 (s,1H,CHCO)7.18((d, *j = 7.2* Hz, 2H, Ar-H), 8.10 (s,1H,OH, D_2O exchangeable). MS: *m/z* (% abundance): *m/z* 302 (M^+−3, 28.16%). Anal. Calcd for $C_{16}H_{21}N_3O_3$: C, 63.35; H, 6.98; N, 13.85. Found: C, 63.02; H, 6.99; N, 13.80.

2.2.2. 1-((4-(4-Methoxyphenyl) piperazin-1-yl) methyl)-5-oxopyrrolidine-2-carboxylic acid (IIb)

Mp 115 °C, yield 80%, IR (KBr, cm^{-1}): 3400(OH), 3080 (CH aromatic), 2980 (CH alphatic), 1710,1680 (CO). ^{1}H NMR 300 MHz (DMSO-d_6): 1.95–2.23 (m,4H, 2CH_2), 2.33(d, *j = 5.1* Hz, 4H, piperazine), 3.10 (d, *j = 6.5* Hz, 4H, piperazine),3.67 (s, 3H, OCH_3),4.18 (s, 2H, CH_2),6.80(d, *j = 9* Hz, 2H, Ar-H),6.84 (s, 1H,CHCO), 6.85 (d, *j = 8.4* Hz, 2H, Ar-H), 7.9 (s,1H,OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm): 22.75 (CH_2 Pyrrolidine), 29.38 (CH_2 Pyrrolidine), 49.26 (2CH_2Piperzine), 50.96(2CH_2Piperzine), 55.12 (CH Pyrrolidine), 59.79 (OCH_3), 63.11 (NCH_2), 114.20 (2 C Ar) 117.35 (2 C Ar), 118.02 (C-Ar), 145.26 (C Ar), 173.88 (CO), 175.30 (CO). MS: *m/z* (% abundance): *m/z* 333(M^+, 0.01%), Anal.Calcd for $C_{17}H_{23}N_3O_4$; C, 61.25; H, 6.95; N, 12.60. Found: C, 61.22; H, 6.96; N, 12.60.

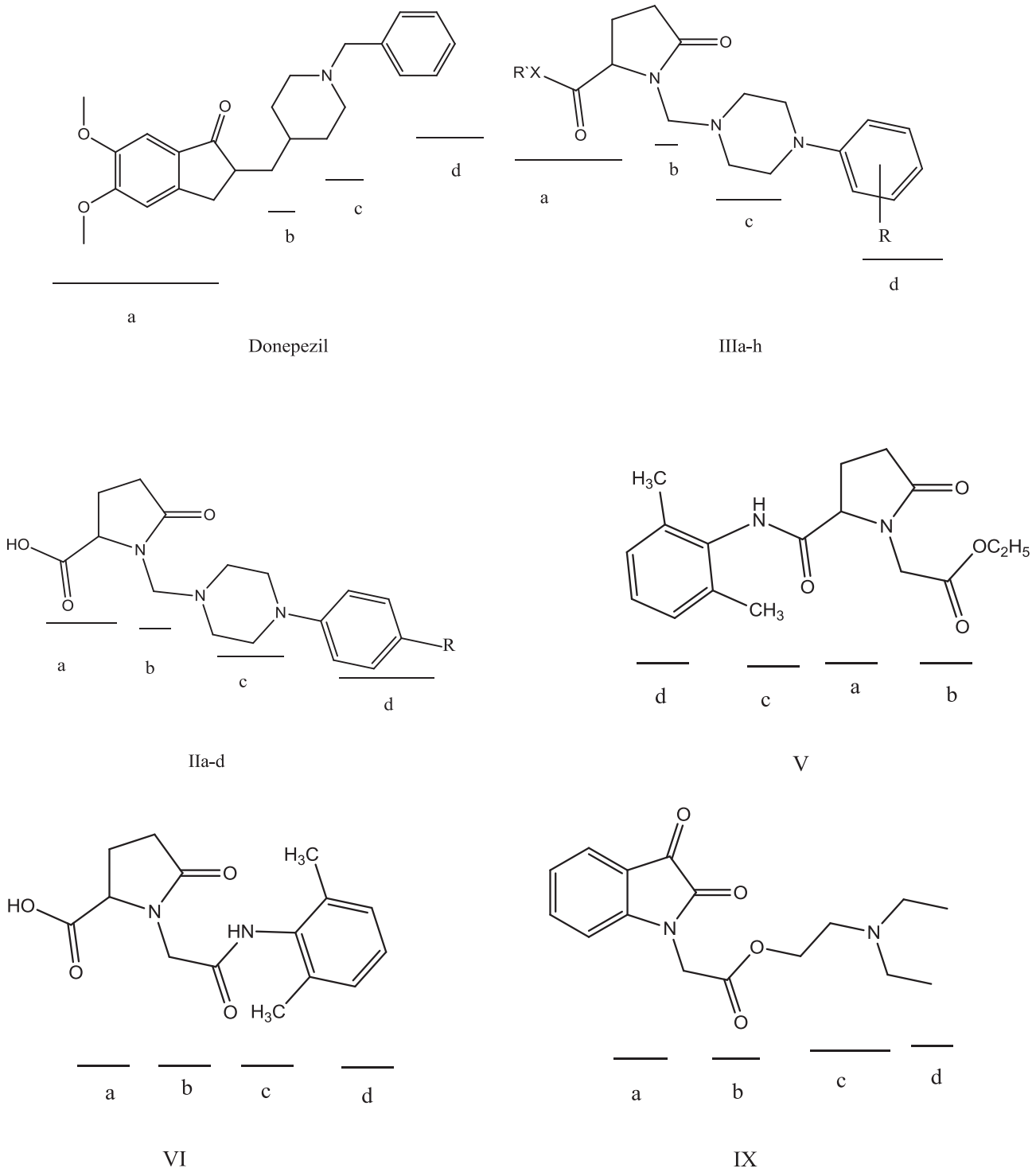


Fig. 2. Resemblances between donepezil and synthesized derivatives, showing the four main parts important for biological activity.

2.2.3. 1-((4-(4-chlorophenyl) piperazin-1-yl) methyl)-5-oxopyrrolidine-2-carboxylic acid (IIc)

Mp 107 °C, yield 70%, IR (KBr, cm^{-1}): 3405(OH), 3082 (CH aromatic), 2990 (CH alphatic), 1710, 1680 (CO). ^{1}H NMR 300 MHz (DMSO-d_6): 1.95–2.12 (m,4H, $2CH_2$), 2.39(d, *j* = *8*, 4H, piperazine), 3.08 (d, *j* = *7.24*,4H, piperazine), 3.93(s, 2H,CH_2), 6.98(d, *j* = *8.9* Hz, 2H, Ar-H),), 7.05(s,1H, CHCO),7.09(d, *j* = *8.7* Hz, 2H, Ar-H),), 7.9 (s,1H,OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm): 23.21 (CH_2 Pyrrolidine), 25.11 (CH_2 Pyrrolidine), 42.96 ($2CH_2$Piperzine), 46.61($2CH_2$Piperzine), 55.30 (CH Pyrrolidine), 63.20 (NCH_2), 115.60 (2 C aromatic), 115.82 (2 C Ar), 117.68 (C Ar), 117.75 (C Ar), 174. 25 (CO), 175.83 (CO). MS: *m*/*z* (% abundance): *m*/*z* 337 (M^+, 0.33%), 339(M^+, 1.22%). Anal.Calcd for $C_{16}H_{20}ClN_3O_3$: C, 56.89; H, 5.97; N, 12.44. Found: C, 56.88; H, 5.96; N, 12.42.

2.2.4. 1-((4-(2-fluorophenyl) piperazin-1-yl) methyl)-5-oxopyrrolidine-2-carboxylic acid (IId)

Mp 105 C, yield 43%, IR(KBr, cm^{-1}): 3404(OH), 3066 (CH aromatic), 2941 (CH alphatic), 1710,1670 (CO). ^{1}H NMR 300 MHz (DMSO d_6): 1.93–2.27 (m,4H, 2CH $_2$), 2.99(d, *j = 8.49* Hz, 4H, piperazine), 3.12 (d, *j = 7.80* Hz, 4H, piperazine),4.20 (s,2H,CH_2),6.98(m, 2H, Ar-H), 7.02(s,1H, CHCO),7.2(m,2H, Ar-H), 7.82 (s,1H,OH, D_2O exchangeable). Anal. Calcd for $C_{16}H_{20}FN_3O_3$; C, 59.80; H, 6.27; N, 13.08. Found: C, 59.83; H, 6.26; N, 13.02.

2.3. General procedure for the synthesis of (IIIa-h)

A mixture of IIa-d (0.01 mol) and thionyl chloride was heated under reflux for 1 h. Excess thionyl chloride was distilled off under reduced pressure, the remaining residue was dissolved in dry benzene, the appropriate amine (0.01 mol) was added, finally triethylamine (0.005 mol) was added and the reaction mixture was heated under reflux for 5 h. The reaction mixture was allowed to cool, filtered, the separated solid was dried and crystallized from ethanol.

2.3.1. N-(2,6-dimethylphenyl)-5-oxo-1-((4-phenylpiperazin-1-yl)methyl) pyrrolidine-2- carboxamide (IIIa)

Mp > 300 °C, yield 77%, IR (KBr, cm^{-1}): 3067(CH aromatic), 2981 (CH aliphatic), 1710,1680 (CO). ^{1}H NMR 300 MHz (DMSO d_6): 1.19 (s, 6H, CH_3), 2.13–2.19 (m,4H, $2CH_2$), 2.25(d, 4H, piperazine), 3.05 (d, 4H, piperazine), 3.72 (s,2H,CH_2), 6.87–7.35(m, 8H, Ar-H), 7.05 (s,1H, CHCO), 10.19 (s,1H,NH, D_2O exchangeable). Anal. Calcd for $C_{24}H_{30}N_4O_2$: C, 70.91; H, 7.44; N,13.78. Found: C, 71.02; H, 7.50; N, 13.77.

2.3.2. 2-(Diethylamino)ethyl 5-oxo- 1-((4-phenylpiperazin-1-yl) methyl) pyrrolidine- 2-carboxylate (IIIb)

Mp > 300 °C, yield 45%, IR (KBr, cm^{-1}): 3051(CH aromatic), 2958–2924 (CH aliphatic), 1710,1697 (CO). ^{1}H NMR 300 MHz (DMSO d_6): 1.21 (t, 6H, $2CH_3$), 1.93–1.96 (m, 4H, $2CH_2$) 2.13 (t, 4H, $2CH_2$), 2.29–2.36 (m, 4H, piperazine) 3.15–3.20(m,4H, piperazine), 4.06(q,4H, $2CH_2$) 4.20 (s,2H,CH_2),7.35(s,1H, CHCO), 7.4–7.8 (m,5H, Ar-H). MS: *m/z* (% abundance): *m/z* 402 (M^+, 0.11%). Anal. Calcd for $C_{22}H_{34}N_4O_3$: C, 65.64; H, 8.51; N, 13.92. Found: C, 65.65; H, 8.50; N, 13.88.

2.3.3. 1-((4-(4-Methoxyphenyl)piperazin-1-yl)methyl)- N-(2,6-dimethylphenyl)-5 - oxopyrrolidine- 2-carboxamide (IIIc)

Mp > 300 °C, yield 78%, IR (KBr, cm^{-1}): 3089(CH aromatic), 2978 (CH alphatic), 1710,1680 (CO). ^{1}H NMR 300 MHz (DMSO d_6):1.22 (s,6H, 2CH3), 1.91–2.02 (m,4H, $2CH_2$), 2.29–2.35(m, 4H, piperazine), 3.07–3.69 (m, 4H, piperazine), 3.70 (s,2H,CH_2), 3.83 (s, 3H, OCH3), 6.87–6.95 (m, 3H, Ar-H), 7.05 (s,1H, CHCO), 7.27–7.95(m, 4H,Ar-H), 10.19 (s,1H,NH, D_2O exchangeable). Anal. Calcd for $C_{25}H_{32}N_4O_3$: C, 68.78; H, 7.39; N, 12.83. Found: C, 68.76; H, 7.40; N, 12.79.

2.3.4. 2-(Diethylamino)ethyl 1-((4-(4-methoxyphenyl)piperazin-1-yl) methyl) -5- oxopyrrolidine -2-carboxylate (IIId)

Mp > 300 °C, yield 42%, IR (KBr, cm^{-1}): 3063(CH aromatic), 2978–2983 (CH aliphatic), 1700,1690 (CO). ^{1}H NMR 300 MHz (DMSO-d_6): 1.23 (t,6H,$2CH_3$), 2.11–2.18 (m, 4H, $2CH_2$) 2.27–2.35 (m, 4H, $2CH_2$), 3.06 (m, 4H, piperazine) 3.22(d,4H, piperazine), 3.60(s, 3H, OCH_3),3.80 (q,4H, $2CH_2$) 4.18 (s,2H,CH_2),7.06(s,1H, CHCO), 7.70–7.99(m,4H, Ar-H).^{13}C NMR (DMSO-d_6, δ ppm): 8.91 ($2CH_3$), 22.80(CH_2 pyrroldine), 30.01(CH_2 pyrroldine),40.37 ($2CH_2$ ethyl), 43.77 ($2CH_2$ piperazine), 49.56 ($2CH_2$ piperazine), 55.20 (CH_2 ethylene), 55.74 (OCH_3), 56.44(CH pyrrolidine), 57.25 (CH_2 ethylene),64.65 (NCH_2N), 114.83 (2 C Ar), 118.58 (2C Ar), 128.21 (C Ar), 135 (CH Ar), 173.87 (CO), 177.47 (CO). Anal. Calcd for $C_{23}H_{36}N_4O_4$: C, 63.86; H, 8.39; N,12.95. Found: C, 63.85; H, 8.44; N, 12.89.

2.3.5. 1-((4-(4-Chlorophenyl) piperazin-1-yl) methyl)-N-(2,6-dimethylphenyl)-5- oxopyrrolidine- 2-carboxamide (IIIe)

Mp > 300 °C, yield 66%, IR (KBr, cm^{-1}): 3091 (CH aromatic), 2999–2870(CH alphatic), 1680,1705 (CO). ^{1}H NMR 300 MHz (DMSO-d_6): 1.15(s,6H,$2CH_3$), 2.05–2.17 (m,4H, $2CH_2$), 2.42(d, 4H, piperazine),2.96–3.03 (m, 4H, piperazine), 4,04 (s,2H,CH_2), 6.39 (m, 3H,Ar-H), 6.77–6.79 (m,4H,Ar-H), 7.06 (s,1H, CHCO), 9.1 (s,1H,NH, D_2O exchangeable), Anal. Calcd for $C_{24}H_{29}ClN_4O_2$: C, 65.37; H, 6.63; N, 12.71. Found: C, 65.36; H, 6.60; N, 12.72.

2.3.6. 2-(Diethylamino) ethyl 1-((4-(4-chlorophenyl)piperazin-1-yl) methyl)-5- oxopyrrolidine-2-carboxylate (IIIf)

Mp > 300 °C, yield 32%, IR (KBr, cm-1): 3063(CH aromatic), 2976–2941 (CH aliphatic), 1690,1680 (CO). ^{1}H NMR 300 MHz (DMSO-d_6): 1.17 (t,6H,$2CH_3$), 1.96–2.10 (m, 4H, $2CH_2$) 2.28 (t, 4H, $2CH_2$), 2.29–2.37 (m, 4H, piperazine) 3.15–3.30(m,4H, piperazine), 4.06(q,4H, $2CH_2$) 4.20 (s,2H,CH_2),6.93(d, *j = 4.50* Hz, 2H, Ar-H) 0.7.02(d, *j = 3.00*, 2H, Ar-H), 7.00(s,1H, CHCO). Anal. Calcd for $C_{22}H_{33}ClN_4O_3$: C, 60.47; H, 7.61; N,12.82. Found: C, 60.45; H, 7.64; N, 12.80.

2.3.7. 1-((4-(2-Fluorophenyl)piperazin-1-yl)methyl)-N-(2,6-dimethylphenyl)-5-oxopyrrolidine-2-carboxamide (IIIg)

Mp > 300 °C, yield 34%, IR (KBr, cm^{-1}): 3054(CH aromatic), 2980–2888(CH alphatic), 1680,1700 (CO). ^{1}H NMR 300 MHz (DMSO-d_6): 1.15(s, 6H,$2CH_3$), 2.04–2.08 (m,4H, $2CH_2$), 2.12–2.22 (m, 4H, piperazine), 3.06–3.09 (m, 4H, piperazine), 3.60 (s,2H, CH_2), 6.39–6.42(m, 3H, Ar-H), 6.77–6.79 (m,4H,Ar-H), 7.06 (s,1H, CHCO), 8.9 (s,1H,NH, D_2O exchangeable). Anal. Calcd for $C_{24}H_{29}FN_4O_2$: C, 67.90; H, 6.89; N, 13.20. Found: C, 67.88; H, 6.87; N, 13.17.

2.3.8. 2-(Diethylamino)ethyl1-((4-(2-fluorophenyl)piperazin-1-yl) methyl)- 5-oxopyrrolidine - 2-carboxylate (IIIh)

Mp > 300 °C, yield 32%, IR (KBr, cm-1): 3063(CH aromatic), 2976–2941 (CH aliphatic), 1690,1680 (CO). ^{1}H NMR 300 MHz (DMSO-d_6): 1.20 (t,6H,$2CH_3$), 1.95–2.20 (m, 4H, $2CH_2$) 2.27 (m, 4H, piperazine), 3.04–3.12 (m, 4H, $2CH_2$) 3.38–3.64(m,4H, piperazine), 3.74 (q,4H, $2CH_2$) 4.30 (s,2H,CH_2),6.43–7.72 (m, 4H, Ar-H), 7.16(s,1H, CHCO). Anal.Calcd for $C_{22}H_{33}FN_4O_3$: C, 62.48; H, 7.91; N,13.32. Found: C, 62.46; H, 7.84; N, 13.00.

2.4. Procedure for the synthesis of ethyl 2-(2-(2, 6-dimethylphenylcarbamoyl)- 5-oxopyrrolidin-1-yl) acetate (V)

Excess thionyl chloride (0.02 mol., 1.0 gm) was added to **(IV)** (0.01 mol, 2.15 gm) then the reaction was heated under reflux for 1 h .The excess thionyl chloride was distilled off under reduced pressure and the residue formed was dissolved in dry benzene (20 ml), 2, 6- dimethyl aniline (0.01 mol, 1.21 gm) was added and the reaction was further heated under reflux in the presence of triethylamine for 2 h. The reaction mixture was allowed to cool, filtered, the separated solid was dried and crystallized from ethanol.

Mp 123 °C, yield 87%, IR(KBr, cm-1): 3375(NH), 3025 (CH aromatic), 2926 (CH alphatic), 1714,1685,1666 (3CO). ^{1}H NMR 300 MHz (DMSO-d_6): 1.20 (s,6H, $2CH_3$), 1.99–2.21 (m, 4H,2 CH_2), 2.9 (t, 3H, CH_3), 4.0 (q,2H, CH_2), 4.20(s, 2H,CH_2), 6.74(d, *j = 7.2* Hz, 2H, Ar-H), 6.87(t, 1H, Ar-H), 6.90 (s, 1H, CHCO), 8.0 (s, 1H,NH, D_2O exchangeable).^{13}C NMR (DMSO-d_6, δ ppm): 18,35 (CH_3 ethyl), 25.07($2CH_3$),26.11 (CH_2 ethyl) 29.53 (CH_2 Pyrrolidine), 29.81 (CH_2 Pyrrolidine), 45.78 (NCH_2),56.28 (CH Pyrrolidine),125.55 (C Ar), 126.94 (2C Ar),133.01 (2C Ar), 136.18 (C Ar),171.47 (CO), 174.84

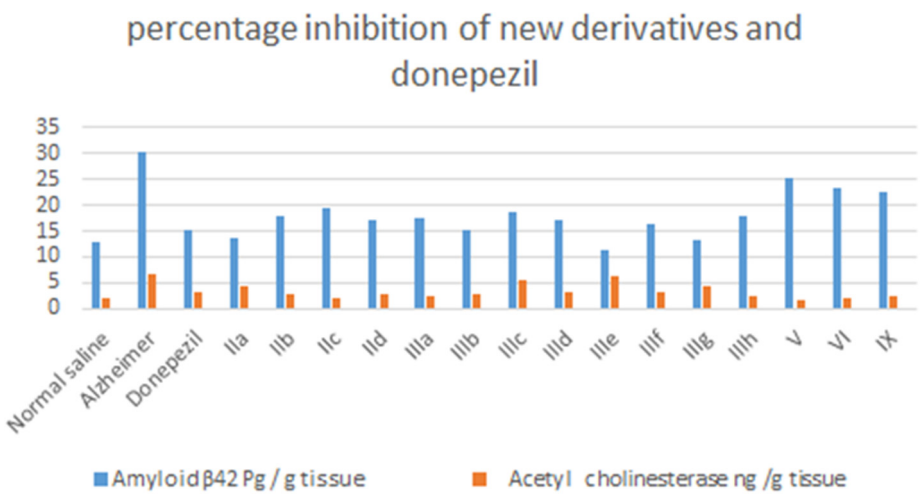


Fig. 3. Bar chart representation of acetyl cholinesterase and β 42 IC_{50} of Normal saline, Alzheimer's disease, donepezil and the newly synthesized compounds.

Table 1
IC_{50} results of the effect of normal saline, Alzheimer's disease, the synthesized compounds and donepezil on acetyl cholinesterase and amyloid β42.

	Amyloid β42 Pg/g tissue	Acetyl cholinesterase ng/g tissue
Normal saline	12.7	1.96
Alzheimer	30.1	6.68
Donepezil	18.4	3.34
IIa	13.5	4.18
IIb	17.9	2.66
IIc	19.6	2.17
IId	17.3	2.96
IIIa	17.5	2.24
IIIb	15.3	3.0
IIIc	18.5	5.36
IIId	17.1	3.06
IIIe	11.3	6.37
IIIf	16.3	3.08
IIIg	13.4	4.46
IIIh	17.9	2.36
V	25.3	1.84
VI	23.2	2.11
IX	22.6	2.28

(CO), 177.48(CO). MS: *m/z* (% abundance): *m/z* 318 (M^+, 0.14%). Anal. Calcd for $C_{17}H_{22}N_2O_4$: C, 64.13; H, 6.97; N,8.80. Found: C, 64.15; H, 6.96; N, 8.80.

2.5. Procedure for the synthesis of 1-((2,6-dimethylphenylcarbamoyl) methyl)- 5-oxopyrrolidine-2-carboxylic acid (VI)

Compound **(IV)** (0.01 mol., 2.15 gm) was added to a solution of 2,6- dimethyl aniline (0.01 mol, 1.21 gm) in absolute ethanol (20 ml) and the reaction mixture was heated under reflux for 12 h. It was then cooled, filtered, the separated solid was dried and crystallized from ethanol.

Mp 140 °C, yield 66%, IR (KBr, cm-1): 3350(OH),3278 (NH), 3050 (CH Ar), 2993 (CH alphatic), 1710,1685,1670 (3CO). ^{1}H NMR 300 MHz (DMSO-d_6): 1.87 (s,6H, $2CH_3$), 2.091 (m, 4H,2 CH_2), 4.28(s, 2H,CH_2), 5.6 (s, 1H, OH, D_2O exchangeable), 7.68(d, 2H, *j* = *6* Hz, Ar-H), 7.72(t,1H, Ar-H), 7.9 (s, 1H, CHCO), 8.0 (s, 1H,NH, D_2O exchangeable).^{13}C NMR (DMSO-d_6, δ ppm): 25.52($2CH_3$), 30.12 (CH_2 Pyrrolidine), 39.29 (CH_2 Pyrrolidine), 40.55 (NCH_2),56.75 (CH Pyrrolidine),124.2 (C Ar), 126.3 (2C Ar),135.3 (2C Ar), 140.2 (C Ar),175.94 (CO), 177.45 (2CO). Anal. Calcd for $C_{15}H_{18}N_2O_4$: C, 62.06; H, 6.25; N, 9.65. Found: C, 62.04; H, 6.26; N, 9.61.

2.5.1. 2-(Diethylamino) ethyl 2-(2,3-dioxoindolin-1-yl)acetate (IX)

To A solution of **(VIII)** (0.01 mol, 2.3 gm) in ethanol (20 ml) was added N^1,N^1-diethyl ethylene diamine (0.01 mol, 1.1 gm) and the reaction mixture was heated under reflux for 7 h. The reaction mixture was then cooled, filtered, the separated solid was dried and crystallized from ethanol.

Mp 227 °C, yield 62%, IR (KBr, cm-1): 3092 (CH aromatic), 2974 (CH alphatic), 1734, 1728, 1716 (CO). ^{1}H NMR 300 MHz (DMSO-d_6): 1.15 (t,6H, $2CH_3$), 2.71–2.80(m,4H, $2CH_2$), 4.14 (s, 2H, CH_2), 4.53(q,4H,$2CH_2$),6.96(d, *j* = *6.9* Hz, 2H, Ar-H),7.13(d, *j* = *6.9* Hz, 2H, Ar-H). Anal.Calcd for $C_{16}H_{20}N_2O_4$: C, 63.14; H, 6.62; N,9.20. Found: C, 63.12; H, 6.60; N, 9.17.

2.6. Pharmacology

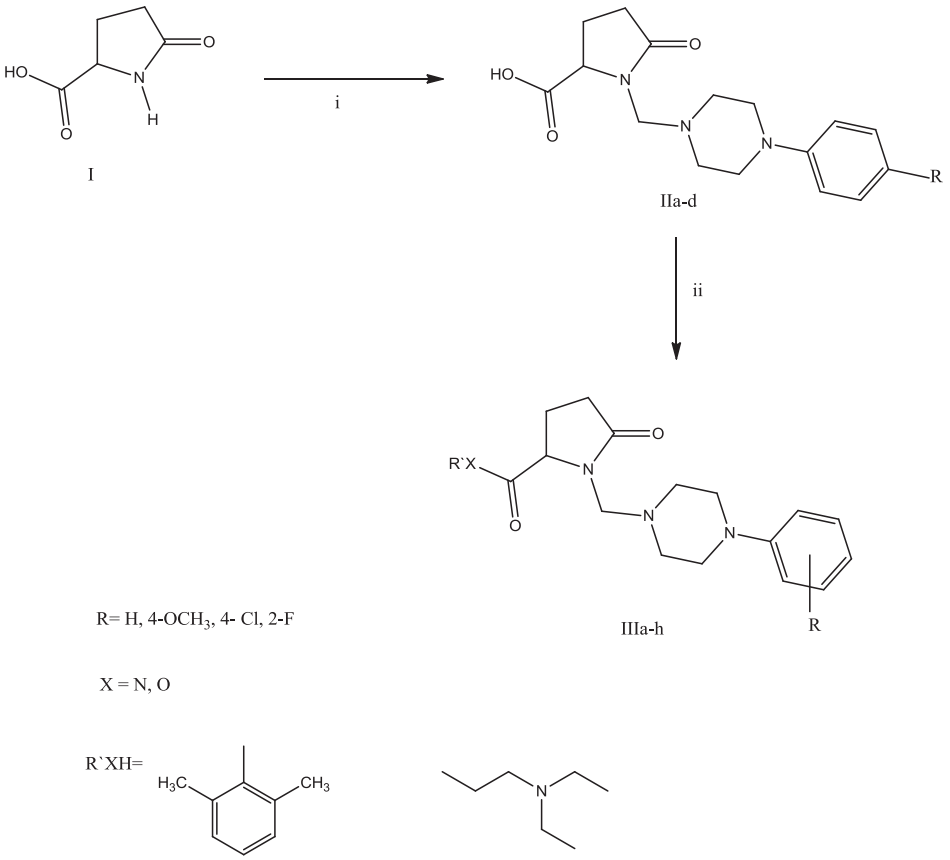
Male albino Wistar rats (200–250 g) were used in the present experiment. Animals were kept under standard laboratory conditions, maintained on a 12/12-h light/dark cycle. Food and water were available *ad libitum* until the beginning of the experiment. Animals were randomly assigned to 17 treatment groups (each group of 6 rats) divided as follows; Group **I**: Rats received vehicle (1% tween 80, orally) and served as normal control group. Group **II**: Rats received $AlCl_3$ (100 mg/kg, orally) and served as Alzheimer's control group. Group **III**: Rats received donepezil (200, 100, 50, 25 mg/kg, orally) and served as standard control group, Groups **IV- XVII**: Rats received new compounds (200, 100, 50,25 mg/kg, orally).

Alzheimer's disease was induced in all groups except for the normal control group by oral administration of $AlCl_3$ daily for 30 consecutive days. Treatments started on the 31 st day and for 21 days thereafter. One hour after the last drug administration, animals were euthanized under deep ether anesthesia by decapitation. Brains were removed and homogenized immediately in ice cold saline to obtain 10% (w/v) homogenate using glass homogenizer (glas-Col homogenizer). The homogenate was centrifuged at 15,000 rpm for 20 min and the supernatant was used for estimation of acetyl cholinesterase activity (AChE) and amyloid beta peptide 1–42 (Aβ1-42). The parameters were measured using enzyme linked immuno sorbent (ELISA) Kit and according to the manufacturer's instructions. Fig. 3 (see Table 1).

3. Results and discussion

3.1. Chemistry

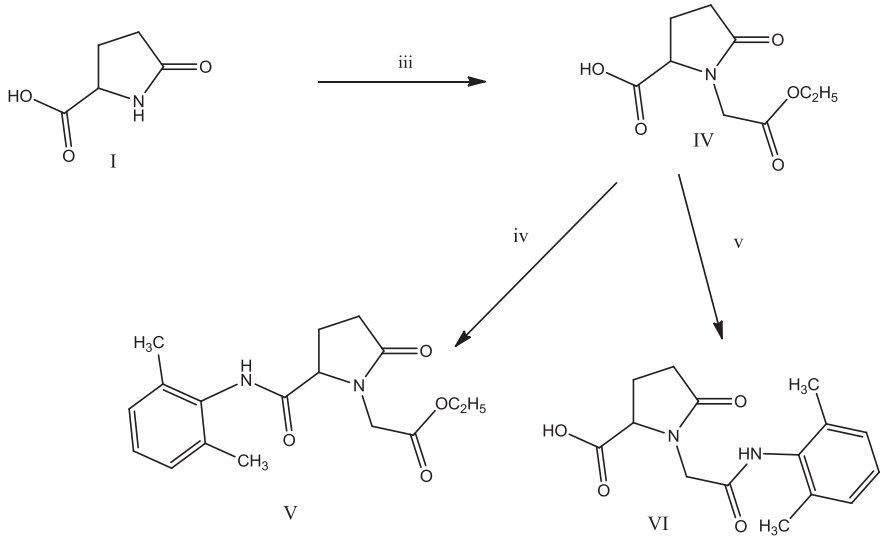
The newly synthesized compounds were presented in Schemes 1–3 the synthesis of mannich derivatives of pyroglutamic acid



Reagents and conditions: i= formalin, ethanol and piperazine derivatives/ reflux

ii= $SOCl_2$ / HXR` / Dry benzene/ reflux

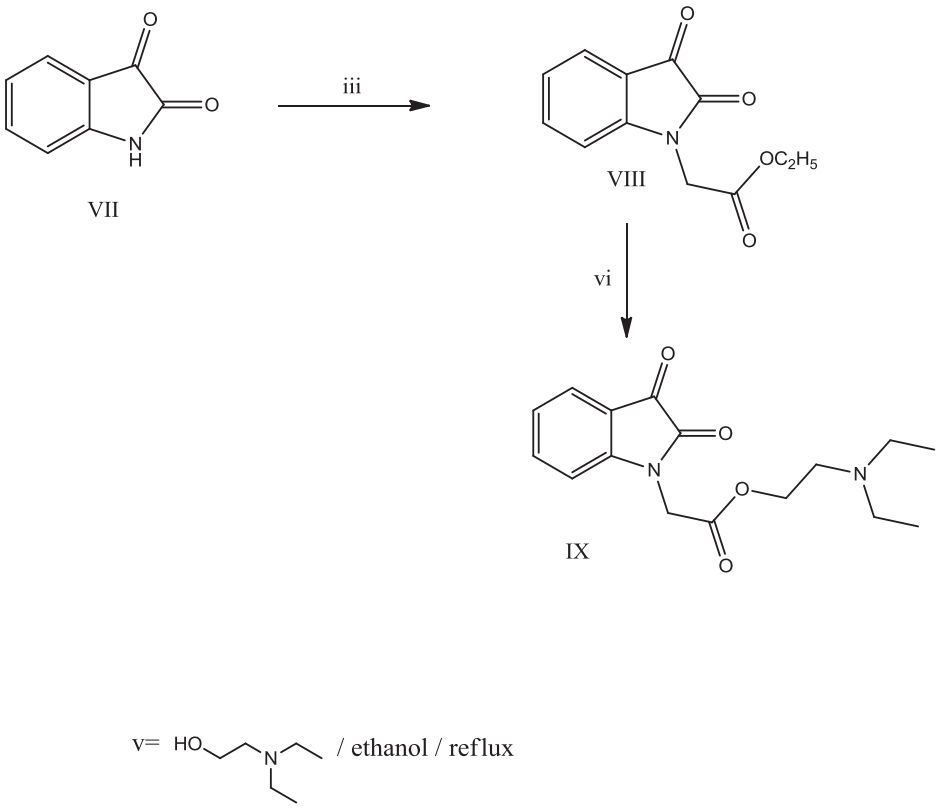
Scheme 1. Synthesis protocol. General synthesis of compounds **IIa-d and IIIa-h**.



Reagents and conditions: iii= $ClCH_2COOC_2H_5$ / ethanol/ reflux

iv) $SOCl_2$ 2) 2,6 -dimethyl aniline / dry benzene/ reflux v= 2,6 -dimethyl aniline/ ethanol/ reflux

Scheme 2. Synthesis protocol. General synthesis of compounds **V and VI**.



Scheme 3. Synthesis protocol. General synthesis of compound **IX**.

were not previously reported so the synthesis performed in Scheme 1 was unavailable through previously described methods. Literature survey revealed the procedure for such synthesis can be performed by the reaction of the secondary amine and formalin in ethanol with the starting material which in this case is pyroglutamic acid [14]. The substituted aryl piperazines chosen namely phenyl, p-methoxy, p-chloro and 2-flouro were dissolved in ethanol then after the addition of formalin the mixture was added to a solution of pyroglutamic acid in ethanol. The produced mannich derivatives **IIa-d** structure was confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy, they showed distinctive singlet signal in ^{1}H NMR at 4.28, 4.18, 4.22 and 4.20 ppm respectively equivalent to the CH_2 group in addition to the phenyl protons and piperazine protons ^{13}C NMR proved the structure by the distinct CH_2 at 63.11 ppm in addition to the molecular ion peak in mass spectra. **IIa-d** derivatives were further reacted with 2,6-dimethyl aniline and N,N-diethyl ethanolamine after their conversion into the corresponding acid chloride using thionyl chloride following reported procedure [15],the amide thus formed in the presence of tri-ethyl amine showed the D_2O exchangeable NH proton at 10.19, 10.19, 9.1and 8.9 ppm in case of 2,6-dimethyl aniline or the $2CH_2$ protons at 2.13,2.27,2.28 and 3.04 ppm and the ethyl protons when N,N-diethyl ethanolamine was used. Scheme 2 utilizes the reaction of pyroglutamic acid with ethyl chloroacetate as reported [10] which was followed by reaction with 2,6-dimethyl aniline either by substitution of the ester functional group producing compound **VI** or by reaction with the acid chloride of **IV** producing compound **V.** The produced amide derivatives showed distinct aromatic protons in addition to the D_2O exchangeable NH protons at 8.0 ppm, furthermore indol -2,3-dione (isatin) **VII** was used as a structure analogue to pyroglutamic acid. **VII** was reacted with ethyl chloroacetate following reported procedure [10] producing compound **VIII** which was further reacted with N,N-diethyl ethanol amine producing compound **IX** which spectral data was consistent with the proposed structure. ^{1}H NMR revealed the ethyl protons and the adjacent two CH_2 groups at 4.52 ppm and the distinct singlet signal of CH_2 at 4.14 ppm.

3.2. Pharmacology

The fifteen new compounds **IIa-c**, **IIIa-h, V, VI and IX** were subjected to pharmacological screening and were tested for their acetyl cholinesterase inhibitory activity and β amyloid protein content against donepezil as reference *in vivo*, following reported procedure [16,17]. First, The normal values when only saline was given to rats were measured, then the values after Alzheimer's disease has been forced into rats were measured, finally the values after injecting the tested compounds were measured. Male rats were treated with the compounds and with donepezil, at first, the level of acetyl cholinesterase enzyme and β amyloid protein was measured. All the compounds, degraded the level of acetyl cholinesterase enzyme effectively and decreased the level of β amyloid protein. Eleven of the fifteen tested compounds showed inhibitory effects on acetyl cholinesterase Cenzyme better than donepezil namely compounds **V, VI, IIc, IIIa, IX, IIIh**, **IIb, IId, IIIb, IIId and IIIf,** with IC_{50} values 1.84, 2.11, 2.17, 2.24, 2.28, 2.36, 2.66, 2.96, 3.0, 3.06 and 3.08 ng/g tissue where donepezil IC_{50} value 3.34 ng/g tissue.

On the other hand, most of the tested compounds degraded the level of β amyloid protein better than donepezil Compounds **IIIe, IIIg, IIa, IIIb, IIIf, IIId, IId, IIIa, IIb** and **IIIh** showed the highest inhibitory activity of values 11.3, 13.4, 13.5,15.3, 16.3, 17.1, 17.3, 17.5, 17.9 and 17.9 pg /g tissue better than donepezil 18.4 pg/ tissue. Moreover, compounds **IIIf**, **IIId, IIIa, IIb IIIh, IIIc and IIc** showed inhibitory activity values of 16.3, 17.1, 17.5, 17.9, 17.9, 18.5 and 19.6 Pg/ml on amyloid β 42 which appear to be close values to the standard donepezil.

Finally compounds **IIIc and IIIe** showed the least activity on acetyl cholinesterase enzyme with values 5.36 and 6.37 ng/g tissue but still slightly better than the disease value of 6.68 ng/g tissue the amyloid β 42 inhibitory activity of **IX, VI and V** was the least of the tested compounds with values 22.6, 23.2 and 25.3 Pg/ml but still of better value than the disease of 30.1 Pg/ml.

From the above acetyl cholinesterase inhibition showed better with the 2,6-dimethyl aniline derivative used **(V,VI)** even at different positions of the ring, followed by the use of *p*-chloro phenyl piperazine derivative where its activity remained better than donepezil when an amide linkage has been introduced bearing the diethyl ethylene diamine. On the other hand, the *p*-methoxy phenyl piperazine activity was retained only in case of the 2,6-dimethyl aniline derivative as well as its acid derivative. The *o*- fluoro phenyl piperazine showed good activity with the acid and the diethyl ethylene diamine. Although the phenyl piperazine showed the least activity when acid derivative but its activity increased when an amide linkage has been introduced.

The β amyloid protein inhibitory activity was presented by the new derivatives mainly the phenyl piperazine when an acid or both amide derivatives were used. Followed by the *p*- methoxy phenyl piperazine as an acid or the diethyl ethylene diamine derivative, then the *o*-flouro phenyl piperazine as an acid or its two amide derivatives. Finally the *p*-chloro phenyl amides showed better activity than the acid derivative.

4. Conclusion

The main objective of this paper was to synthesize new derivatives bearing the 2-oxopyrrolidine ring resembling indanone moiety of donepezil and test their pharmacological activity on Alzheimer‘s disease, where two main criteria were taken into consideration, the inhibition of acetyl cholinesterase enzyme and amyloid β 42 in the brain tissues.

Most of the derivatives were found effective as anti-Alzheimer‘s disease specifically compounds **V&VI** which displayed the best activity, the 2,6-dimethyl phenyl group at two different positions to test its activity which showed similar potency of the two derivatives, but being attached to the carboxylic acid in case of **V** showed better activity on the tested enzymes.

The duel activity was best presented by compounds **IIIb and IIa** having their inhibitory activity on both acetyl cholinesterase enzyme and amyloid β 42 protein with values better than donepezil.

Conflict of interest

The authors have declared no conflict of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bioorg.2017.11.008. These data include MOL files and InChiKeys of the most important compounds described in this article.

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