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Highlights on mechanisms of newly synthesized compounds targeting multiple systems provide a novel perspective on Alzheimer's disease treatment

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ABSTRACT

*Alzheimer's disease (AD) is a multi-factorial disease, thus the successful treatment will likely require the development of hybrid structures that target multiple systems. This was achieved through combination of two molecules to obtain new drug candidate. Although curcumin was believed to have high biological activity, it has drawbacks that affect its biological activity. Modification of curcumin moiety by synthesizing new derivatives has a great attention to improve its bioavailability. Moreover, the addition of heterocyclic rings to steroids molecules often leads to a change of their biological activity and appearance of new interesting pharmacological properties. Based on these evidences, our goal was to synthesize new hybrid drugs and to elucidate the efficacy of these novel synthesized compounds in the recession of AD induced in adult female albino rats. This goal was achieved by reacting curcumin with different reagent to get new heterocyclic derivatives. Besides that, steroid moiety was also modified by submitting it to react it with various reagent to form new hybrid molecules. The resulting compounds **7a** and **13c** were tested as anti-Alzheimer's disease in vivo through targeting multiple pathways implicated in the pathogenesis of AD. The results showed the anti-Alzheimer's disease of the two compounds with different degrees depending on their structure where compound **13c** showed strongest activity than compound **7a**. In conclusion, the addition or fusion of heterocyclic rings in curcumin or steroid molecule could improve their biological activity in regression of AD in the experimental model. The biological study provides clear evidence for the anti-Alzheimer's disease potency of the newly synthesized derivatives through their anti-cholinesterase activity, antioxidant property and antiapoptotic potential.*

Keywords: Alzheimer's disease, Curcumin derivative, Heterosteroids, Rats.

INTRODUCTION

Development of hybrid structures, in which pharmacologically crucial structural elements from two molecules are combined to produce a non-identical twin drug, is a rational approach to obtain therapeutically useful molecules [1]. Alzheimer's disease (AD), the most common form of dementia in elderly people, is a complex neurodegenerative disorder of the central nervous system, characterized by progressive impairment in memory, cognitive functions and behavioral disturbances [2]. AD syndrome is associated with a severe deficit in the cholinergic neurotransmission due to a progressive degeneration in basal forebrain [3], with the loss of neuronal projections to the cortex paralleled by a reduction in the levels of the acetylcholine (ACh), and its biosynthetic enzyme choline acetyl transferase (ChAT) , and an elevation of acetylcholinesterase (AChE) [4].The amyloid hypothesis suggests that the progression of AD is attributed to the accelerated accumulation of toxic forms of self-induced and/or AChE-promoted toxic aggregates of Aβ peptides.[5, 6]. These multiple factors in AD pathology mandate the need to therapies that exhibit dual AChE inhibition as well as reduce the formation of neurotoxic Aβ-aggregates.

Due to the multi-pathogenesis of AD, the classical approach modulating at one target may be inadequate in this complex disease. Therefore, searching the candidates acting at multiple sites of pathologic cascade has become a new strategy to design new drugs for AD. Thus, associations of AChEIs with compounds acting at different sites of the pathologic pathway provide additional benefits [7].

Curcumin has demonstrated therapeutic effects in transgenic mouse models of AD [8]. The absorption, biodistribution, metabolism, and elimination studies on curcumin have, unfortunately, shown only poor absorption, rapid metabolism, and elimination which represented as common reasons for poor bioavailability of this interesting polyphenolic compound. Researchers hope to achieve improved biological activity of curcumin by structural modifications [9].

It has long been believed that small alterations in the structure of certain steroids can greatly affect their receptor binding affinity and biological activity [10]. Several studies have demonstrated that the introduction of various heterocyclic rings to ring A or ring D of various steroids was effective in the production of variety of compounds with potential biological activities [11]. Heterosteroids have been accredited with a great amount of attention over the years by medicinal chemists for discovery of new chemical entities with potential to afford some promising drugs in the future. The incorporation of a heterocyclic ring or a heteroatom in the steroid backbone affects the chemical properties of a steroid and often results in useful alterations in its biological activities. Heterosteroids encompass a wide range of compounds such as GABA receptor antagonists, [12], aromatase inhibitors such as (formestane and exemestane) [13], and neuromuscular junction blocking agents like pipecuronium [14].

The focus of our interest was to synthesize novel efficient curcumin derivatives containing promising heterocyclic nucleus. Moreover, hybrid candidates were synthesized through the combination of the steroid molecule with heterocyclic nucleus. Furthermore, the study was extended to investigate the potential anti-Alzheimer's disease effect of the two newly synthesized compounds in the experimental model.

EXPERIMENTAL SECTION

Chemistry

Starting steroids and curcumin were purchased from Sigma Company, USA. All solvents were anhydrated by distillation prior to using. All melting points were measured using an Electrothermal apparatus and are uncorrected. The IR spectra were recorded in (KBr discs) on a Shimadzu FT-IR 8201 PC spectrometer and expressed in cm^{-1}. The ^{1}H NMR and ^{13}C NMR spectra were recorded with Jeol instrument (Japan), at 270 and 125 MHz respectively, in DMSO-d6 as solvent and chemical shifts were recorded in ppm relative to TMS. The spin multiplicities were abbreviated by the letters: s-singlet, d-doublet, t-triplet, qquartet and m (multiplet, more than quartet). Mass spectra were recorded on a GCMS-QP 1000 ex spectra mass spectrometer operating at 70 ev. Elemental analyses were carried by the Microanalytical Data Unit at the National Research Centre, Giza, Egypt and the Microanalytical Data Unit at Cairo University, Giza, Egypt. The reactions were monitored by thin layer chromatography (TLC) which was carried out using Merck 60 F254 aluminum sheets and visualized by UV light (254 nm). The mixtures were separated by preparative TLC and gravity chromatography. All steroid derivatives showed the characteristic spectral data of cyclopentanoperhydrophenanthrene nuclei of androstane series were similar to those reported in literature [15]. Compounds **2** and **8** were prepared according to published procedures [16, 17].

Synthesis of compound (3a) and (3b):-

General procedure

Dissolve 3-amino-2,3,4,5,6,7-hexahydrobenzo[b]thiophene-2-carbonitrile **2a** or ethyl3-amino-2,3,4,5,6,7-hexahydrobenzo[b]thiophene-2-carboxylate **2b** [16] (1mmol) in hydrochloric (6mL, 6N) and cooled at 0-5^{o}C. Sodium nitrite (0.69gm, 1mmol) in water (10 mL) was added drop wise with stirring for 10min. The result diazonium salt was added with stirring to a solution of curcumin (**1**) (0.36gm, 1mmol) in acetic acid (40 mL) at 0-5^{o}C and the reaction mixture was stirred at 0-5^{o}C for 3 hours. The solid product formed was collected by filtration and crystallized from appropriate solvent to give compounds **3a**, **3b** respectively.

2-((Z)-((1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-7,6-dien-4-yl)diazenyl)-4,5,6,7-tetra hydrobenzo[b]thiophene-3-carbonitrile (3a).

Dark brown powder, from absolute ethanol. Yield = (0.42gm) 77 %, mp 138-140^{o} C. IR (kBr, cm^{-1}): ν 3450 (OH), 2972 (CH_3), 2870 (CH_2), 2215 (CN), 1700, 1690 (2C=O). ^{1}H NMR (DMSO-d6, ppm): δ = 1.65 (q, 4H, 2 CH_2), 3.72 (s, 6H, 2OCH_3), 6.65-7.50 (m, 6H, aromatic protons), 6.81 (d, 2H, 2CH aliphatic), 7.54 (d, 2H, 2CH aliphatic), 9.10 (s, H, OH, D_2O exchangeable), 10.03 (s, H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 19.50, 33.50, 116.60, 119.00 (4CH2), 63.00 (C-N), 119.80 (CN), 113.20(CH), 56.10(2OCH_3), 188.90, 115.50, 145.60, 120.00 (aromatic C), 125.00, 135.00, 142.00 (thiophene), 190.00 (2C=O). MS (EI) m/z = 557.160, 150 (100%), 177 (46%),

190 (25%), 433(18%), 506 (1%)[M+˙].Calc for $C_{30}H_{27}N_3O_6S$ (557.620): C, 64.62; H, 4.88; N, 7.54; S, 5.75%, found: C, 64.35; H, 4.63; N, 7.28; S, 5.49%.

2-((Z)-((1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-3,5-dioxhepta-1,6-dien-4-yl)diazenyl)-4,5,6,7-tetra hydrobenzo[b]thiophene (3b).

Brown powder, from absolute ethanol. Yield= (0.44gm) 73 %, mp 100-102^{o}C. IR (kBr, cm^{-1}): ν 3440 (OH), 2930 (CH_3), 2875 (CH_2), 1720, 1715, 1700 (3C=O). ^{1}H NMR (DMSO-d6, ppm): δ = 1.30 (t, 3H, CH_3), 1.62(m, 2H, CH_2), 3.81(s, 6H, 2OCH_3), 6.57-7.52 (m, 6H, aromatic protons), 7.54 (d, 4H, 4CH aliphatic), 9.80 (s, H, OH, D_2O exchangeable), 10.04 (s, H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 13.60 (CH3)17.60 (CH2), 23.50, 35.20, 116.00, 119.00 (4CH), 56.20 (2OCH_3), 112.80, 116.80, 128.70, 144.00 (aromatic C), 125.00, 135.00, 142.00 (thiophene), 126.60, 140.50 (2C=C), 190.00 (2C=O). MS (EI) m/z = 604.190, 135(100%), 181(55%), 208 (25%) [M+˙].Calc for $C_{32}H_{32}N_2O_8S$ (604.670): C, 63.56; H, 5.33; N, 4.63; S, 5.30%, found: C, 63.29; H, 5. 10; N, 4.35; S, 5.52%.

General procedure for the synthesis of pyrazole derivatives (4a-c) and (5a-c):-

To a solution of **3a** and/or **3b** (1mmol) in absolute ethanol (30 mL) containing a catalytic amount of triethylamine (0.5mL) added equimolar amount of hydrazines namely, hydrazine hydrate (0.05gm, 1 mmol), phenyl hydrazine (0.10 gm, 1 mmol)and or thiosemicarbazide (0.09 gm, 1 mmol) and or cyanoacetic acid hydrazide (0.09 gm, 1 mmol) were added. The reaction mixture was heated under reflux for 3-5 hours until all the starting material had disappeared as indicated by TLC. The reaction mixture was poured into ice/water mixture, and neutralized with dilute HCl. The formed solid product in each case was collected by filtration, dried and crystallized from appropriate solvent.

2-((E)-(3,5-bis((E)-4-hydroxy-3-methoxystyryl)-1H-pyrazol-4-yl)diazenyl)-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carbonitrile (4a).

Dark brown powder from absolute ethanol. Yield (0.28gm) 52 %, mp=132-134^{o}C. IR (kBr, cm^{-1}): ν 3400-3320 (OH, NH), 2870 (CH_2), 2210 (CN). ^{1}H NMR (DMSO-d6, ppm): δ = 1.62 (q, 2H, CH_2), 2.55(m, 2H, CH_2), 3.73 (s, 6H, 2OCH_3), 6.65-6.75 (m, 7H, aromatic protons and NH), 6.99(s, 4H, 4CH aliphatic), 9.74 (s, 1H, OH, D_2O exchangeable), 10.20 (s, 1H, OH, D2O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 17.10, 35.90, 116.60, 119.90 (4CH_2), 114.40, 128.60, 119.90, 115.90 (aromatic carbon), 114.80 (CN), 130.00, 135.10, 136.00, 142.00 (thiophene). MS (EI) m/z = 553.190, 86(65%), 135(100%), 138 (83%), 178 (26%), 227 (27%), 256 (31%), 312 (25%), 553(4%) [M+˙].Calc for $C_{30}H_{27}N_5O_4S$ (553.630): C, 65.08; H, 4.92; N, 12.65; S, 5.79%, found: C, 64.78; H, 4.72; N, 12.35; S, 5.48%.

2-((E)-(3,5-bis((E)-4-hydroxy-3-methoxystyryl)-1-phenyl-1H-pyrazol-4-yl)diazenyl)-4,5,6,7-tetrahydrobenzo [b]thiophene-3-carbonitrile (4b).

Brown powder from absolute ethanol. Yield = (0.28gm) 45 %, mp 154-156^{o}C. IR (kBr, cm^{-1}): ν 3450 (OH), 2870 (CH_2), 2210 (CN). ^{1}H NMR (DMSO-d6, ppm): δ 1.62 (q, 2H, CH_2), 2.55(s, 2H, CH_2), 3.73 (s, 6H, 2OCH_3), 6.57-7.04 (m, 15H, aromatic protons and 4CH aliphatic), 9.10 (s, 1H, OH, D_2O exchangeable), 9.74 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ 19.40, 33.50, 116.59, 119.82 (4CH_2), 56.35(2OCH_3), 104.72, 126.00, 141.30 (pyrazole), 113.20, 136.00, 135.00, 145.20 (thiophene), 115.50, 120.00, 145.60, 188.90 (aromatic C), 119.80 (CN), 124.80, 103.80 (2C=C). MS (EI) m/z = 629.210, 55.05 (100%), 98 (30%), 132 (36%), 171 (26%), 212 (31%), 321 (78%), 404 (46%), 587 (53%) [M+˙].Calc for $C_{36}H_{31}N_5O_4S$ (629.73): C, 68.66; H, 4.96; N, 11.12; S, 5.09%, found: C, 68.36; H, 4.63; N, 10.82; S, 4.79%.

4-((E)-(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)diazenyl)-3,5-bis((E)-4-hydroxy-3-methoxystyryl)-1H-pyrazole-1-carbothioamide (4c).

Brown powder from absolute ethanol. Yield= (0.49gm) 81 %, mp=150-152^{o}c. IR (kBr, cm^{-1}): ν 3450- 3320((OH, NH_2), 2972 (CH_3), 2870 (CH_2), 2210 (CN), 1230 (C=S). 1H NMR (DMSO-d6,ppm): δ = 1.65 (q, 4H, 2 CH_2), 1.96 (q, 4H, 2CH_2), 3.72 (s, 6H, 2OCH_3), 6.65-7.75 (m, 12H, aromatic protons , NH_2 and 4CH aliphatic), 9.10 (s, 1H, OH, D_2O exchangeable), 10.02 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 19.40, 33.50, 116.60, 119.90 (4CH_2), 56.20 (2OCH_3), 105.70, 13.00 (pyrazole), 113.70, 135.60, 136.70, 145.60 (thiophene), 115.50, 120.01, 45.60, 188.90 (aromatic C), 119.80 (CN), 124.80, 130.80 (2C=C), 190.00(C=S). MS (EI) m/z = 614.160, 73 (100%), 105 (74%), 164 (88%), 183 (43%), 211 (26%), 255 (36%), 358 (33%), 408 (35%), 423(52%), 553 (19%) [M+˙].Calc for $C_{31}H_{28}N_6O_4S_2$ (612.720): C, 60.77; H, 4.61; N, 13.72; S, 10.47%, found: C, 60. 57; H, 4.30; N, 13.45; S, 10.33%.

2-((E)-(1-(2-cyanoacetyl)-3,5-bis((E)-4-hydroxy-3-methoxystyryl)-1H-pyrazol-4-yl)diazenyl)-4,5,6,7-tetra hydrobenzo[b]thiophene-3-carbonitrile (4d).
Dark brown powder from absolute ethanol. Yield = (0.53gm) 86 %, mp=140-142^{o}C.IR (kBr, cm^{-1}): ν 3450 (OH), 2870 (CH_2), 2210 (CN), 1720 (C=O). ^{1}H NMR (DMSO-d6, ppm): δ = 1.62 (q, 4H, $2CH_2$), 2.55(q, 4H, $2CH_2$), 3.52 (s, 2H, CH_2), 3.72 (s, 6H, $2OCH_3$), 6.57-7.10 (m, 10H, aromatic protons and 4 CH aliphatic), 9.90 (s, 1H, OH, D_2O exchangeable), 10.02 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 17.1, 25.70, 35.30, 116.60, 119.80 ($5CH_2$), 56.30 ($2OCH_3$), 105.70, 133.03(pyrazole), 114.90, 174.80 (2CN), 113.70, 135.60, 136.70, 145.00 (thiophene), 124.80, 130.80 (2C=C), 113.00, 116.60, 119.90, 122.60 (aromatic C), 171.00 (C=O). MS (EI) m/z = 620.180, 52 (75%), 65 (60%), 150 (18%), 226 (5%), 561 (4%) [M+˙].Calc for $C_{33}H_{28}N_6O_5S$ (620.680): C, 63.86; H, 4.55; N, 13.54; S, 5.17%, found: C, 63.44; H, 4.85; N, 13.24; S, 5.37%.

Ethyl2-((E)-(3,5-bis((E)-4-hydroxy-3-methoxystyryl)-1H-pyrazol-4-yl)diazenyl)-4,5,6,7-tetrahydrobenzo [b]thiophene-3-carboxylate(5a).
Dark brown powder from absolute ethanol. Yield= (0.5gm) 84 %, mp=117-119^{o}C.IR (kBr, cm^{-1}): ν 3440-3320 (OH, NH), 2930 (CH_3), 2875 (CH_2), 1720 (C=O). ^{1}H NMR (DMSO-d6, ppm): δ = 1.30 (t, 3H, CH_3), 1.62 (m, 4H, $2CH_2$), 2.55(q, 4H, $2CH_2$), 3.81(s, 6H, $2OCH_3$), 4.20 (q, 2H,CH_2), 6.57-7.52 (m, 7H, aromatic protons, NH and 4CH aliphatic), 9.82 (s, 1H, OH, D_2O exchangeable), 10.04 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 13.60(CH_3), 59.20 (CH2), 17.60, 35.39, 116.60, 119.80 ($4CH_2$), 23.50 (CH), 56.20 ($2OCH_3$), 105.21, 133.30 (pyrazole), 124.78, 130.0 (2C=C), 112.80, 116.80, 128.70, 144.00 (aromatic C), 135.00, 136.00, 142.01, 130.00 (thiophene), 190.00 (C=O). MS (EI) m/z = 600.200, 136(87%0,237(51%),363(25%0, 391(19%),446(35%) [M+˙].Calc for $C_{32}H_{32}N_4O_6S$ (600.680): C, 63.98; H, 5.37; N, 9.33; S, 5.34%, found: C, 64.08; H, 5.07; N, 9. 00; S, 5.00%.

Ethyl2-((E)-(3,5-bis((E)-4-hydroxy-3-methoxystyryl)-1-phenyl-1H-pyrazol-4-yl)diazenyl)-4,5,6,7-tetrahydro benzo[b]thiophene-3-carboxylate (5b).
Brown powder from absolute ethanol. Yield= (0.54gm) 81 %, mp 122-124^{o}C .IR (kBr, cm^{-1}): ν 3440 (OH), 2930 (CH_3), 2875 (CH_2), 1720 (C=O). ^{1}H NMR (DMSO-d6, ppm): δ = 1.30 (t, 3H, CH_3), 1.62(m, 4H, $2CH_2$), 2.55(q, 4H, $2CH_2$), 3.81(s, 6H, $2OCH_3$), 4.20 (q, 2H,CH_2), 6.57-7.52 (m, 15H, aromatic protons and 4CH aliphatic), 9.41(s, H, OH, D_2O exchangeable), 10.41(s, H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 13.60(CH3), 19.90, 35.30, 116.59, 119.90 ($4CH_2$), 56.20 ($2OCH_3$), 59.09 (CH_2), 112.80, 116.80, 128.70, 144.00 (aromatic C), 105.09, 133.00 (pyrazole), 124.80, 130.80(2C=C), 130.00, 135.03, 136.00, 142.09 (thiophene), 190.00 (C=O). MS (EI) m/z = 676.780, 77(100%), 123(40%), 209(38%), 467(30%),599(15%) [M+˙].Calc for $C_{38}H_{36}N_4O_6S$ (676.780): C, 67.44; H, 5.36; N, 8.28; S, 4.74%, found: C, 67.04; H, 5.06; N, 7.98; S, 4.44%.

Ethyl2-((E)-(1-carbamothioyl-3,5-bis((E)-4-hydroxyl-3-methoxtstyryl)-1H-pyrazol-4-yl)diazenyl)-4,5,6,7–tetrahydrobenzo[b] thiophene-3-carboxylate (5c).
Brown powder from ethanol. Yield= (0.4gm) 61 %, mp=148-150^{o}c. IR (kBr, cm^{-1}): ν 3440- 3320 (OH, NH_2), 2930 (CH_3), 2875 (CH_2), 1720 (C=O), 1230 (C=S). ^{1}H NMR (DMSO-d6, ppm): δ = 1.30 (t, 3H, CH_3), 1.62 (m, 4H, $2CH_2$), 2.55 (q, 4H, $2CH_2$), 3.81(s, 6H, $2OCH_3$), 4.20 (q, 2H,CH_2), 6.57-7.52 (m, 12H, aromatic protons, NH2 and 4CH aliphatic), 9.78 (s, 1H, OH, D_2O exchangeable), 10.20 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 13.60 (CH_3), 17.60, 115.30, 116.60, 119.90 ($4CH_2$), 56.20 ($2OCH_3$), 59.10(CH_2), 104.72, 126.00, 141.30 (pyrazole), 112.80, 116.80, 128.70, 144.00 (aromatic C), 124.80, 130.80 (2C=C), 130.00, 135.00, 136.00, 142.00 (thiophene), 176.80(C=S), 190.00 (C=O). MS (EI) m/z = 659.190, 59 (75%), 123 (42%), 209 (18%), 450 (36%), 536 (32%), 599 (20%) [M+˙].Calc for $C_{33}H_{33}N_5O_6S_2$ (659.780): C, 60.07; H, 5.04; N, 10.61; S, 9.72%, found: C, 60.37; H, 4.74; N, 10.90; S, 9.63%.

Ethyl2-((E)-(1-(2-cyanoacetyl)-3,5-bis((E)-4-hydroxy-3-methoxystyryl)-1H-pyrazol-4-yl)diazenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (5d).
Brown powder from absolute ethanol. Yield= (0.57gm) 86 %, mp=169-172^{o}C.IR (kBr, cm^{-1}): ν 3440 (OH), 2930 (CH_3), 2875 (CH_2), 1720, 1700 (2C=O). ^{1}H NMR (DMSO-d6, ppm): δ = 1.3 (t, 3H, CH_3), 1.62 (q, 2H, CH_2), 2.55(m, 2H, CH_2), 3.52 (s, 2H, CH_2), 3.72(s, 6H, $2OCH_3$), 4.20 (q, 2H, CH_2),6.57-7.10 (m, 10H, aromatic protons and 4 CH aliphatic), 9.90 (s, 1H, OH, D_2O exchangeable), 10.20 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 13.60 (CH_3), 19.10, 35.30, 116.60, 119.90 ($4CH_2$), 56.30 ($2OCH_3$), 59.20 (CH_2), 105.00, 133.00 (pyrazole), 124.80, 130.80 (2C=C), 130.00, 135.00, 136.00, 142.00 (thiophene), 174.80 (C=N), 118.70, 160.00 (2C=O), 113.00, 116.60, 119.90, 122.60 (aromatic C). MS (EI) m/z = 667.210, 68(90%0, 136(40), 209(18%), 58(32%), 531 (28%), 599 (20%) [M+˙].Calc for $C_{35}H_{33}N_5O_7S$ (667.730): C, 62.96; H, 4.98; N, 10.49; S, 4.80%, found: C, 63.06; H, 5.00; N, 10.09; S, 4.90%.

Synthesis of compounds (7a) and (7b).

General procedure*:*

To a solution of compound 8 (0.55, 1mmol) in absolute ethanol (25mL) containing a catalytic amount of piperidine (0.5mL) either malononitrile (0.07g, 1mmol) or ethyl cyanoacetate (0.11g, 1mmol) was added. The reaction mixture was heated under reflux for 4-6 hours until all the reactants had disappeared as indicated by TLC. The reaction mixture after cooling at room temperature was poured into ice /water mixture and neutralized with dil.HCl. The formed solid product, in each case, was filtered off, dried and crystallized from appropriate solvent.

2,4-diamino-1-(((1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dien-4-ylidene)amino)-1, 2,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-b]pyridine-3-carbonitrile (7a).

Green powder from ethanol. Yield= (0.48gm) 77 %, mp=148-150^{o}C. IR (kBr, cm^{-1}): ν 3440 - 3400 (OH, NH and NH_2), 2930 (CH_3), 2875 (CH_2), 2215 (CN), 1720, 1700 (2C=O). ^{1}H NMR (DMSO-d6, ppm): δ = 1.62(m, 4H, $2CH_2$), 2.19(t, 2H, α-methylene proton), 3.81(S, 6H, $2OCH_3$), 6.57-7.54 (m, 13H, Aromatic proton, 4CH aliphatic, NH and NH_2), 9.80 (s, 1H, OH, D_2O exchangeable), 10.02 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 20.90, 23.90, 25.40, 35.30 ($4CH_2$), 56.30 ($2OCH_3$), 112.00, 118.20, 128.20, 144.00 (aromatic C), 118.00, 134.00, 137.00, 142.00 (thiophene), 123.40, 152.70(2C=C), 154.00, 117.20 (CN), 190.00 (2C=O). MS (EI) m/z = 625.200, 136 (70%), 177 (56%), 259 (35%), 366 (60%), 448 (32%), 489 (15%) [M+˙].Calc for $C_{33}H_{31}N_5O_6S$ (625.690): C, 63.35; H, 4.99; N, 11.19; S, 5.12 %, found: C, 63.64; H, 5.01; N, 10.89; S, 4.82 %.

Ethyl-2,4-diamino-1-(((1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dien-4-ylidene) amino)-1,2,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3-b]pyridine-3-carboxylate (7b).

Brown powder from ethanol. Yield= (0.53gm) 79 %, mp=128-130^{o}C. IR (kBr, cm^{-1}): ν 3440 -3400 (OH, NH and NH_2), 2930 (CH_3), 2875 cm^{-1} (CH_2), 1720, 1710, 1700 (3C=O). ^{1}H NMR (DMSO-d6, ppm): δ =1.3 (t, 3H, CH_3), 1.63 (m, 4H, $2CH_2$), 2.59 (t, 4H, α-methylene proton), 3.38(s, 6H, $2OCH_3$), 4.20 (q, 2H,CH_2), 6.57-7.54 (m, 13H, Aromatic protons, 4CH aliphatic, NH and NH_2), 9.80 (s, 1H, OH, D_2O exchangeable), 10.02 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 13.70 (CH_3), 20.90, 23.90, 25.4, 35.30 ($4CH_2$), 56.30 ($2OCH_3$), 59.90 (CH_2), 112.00, 118.20, 128.20, 144.00 (aromatic C), 118.00, 134.00, 137.00, 142.00 (thiophene), 167.80, 190.00 (3C=O), 123.40, 152.70 (2C=C). MS (EI) m/z = 672.230, 73(75%), 136(70%), 306(40%), 366(60%), 536(20%), 599(15%) [M+˙].Calc for $C_{35}H_{36}N_4O_8S$ (672.230): C, 62.49; H, 5.39; N, 8.33; S, 4.77 %, found: C, 62.19; H, 5.09; N, 8. 03; S, 4.47%.

Synthesis of compounds (10a) and (10b):

A mixture of α-Bromoandrostanalone **8** [17] (3.69gm, 10mmol) potassium hydroxide (0.56 gm, 10 mmol) and phenylisothiocyanate (1.35gm,1mmol) in dry N,N-dimethylformamide (10 mL) was stirred at room temperature for 2 hours .The appropriate amount of malononitrile (0.66 gm, 10mmol) or ethylmalonate (1.30 gm, 10 mmol) was added and the reaction mixture was stirred for 2 hours after 24 hours the reaction mixture was diluted with water, the solid product that formed was collected by filtration, washed with water and crystallized from absolute ethanol to give compounds **10a** and **10b**.

2-((1S,12aS,14aS)-1-hydroxy-12a,14a-dimethyl-8-phenyl-3,3a,4,5,5a, 6,6a,7,11,11a,12,12a,12b,13,14,14a-hexa decahydro-1H-cyclopenta[3,4]tetrapheno[9,10-d]thiazol-9(2H,3bH,8H)-ylidene) malononitrile (10a).

Blue powder from absolute ethanol. Yield (0.42gm) 80 %; mp = 108-109^{o}C; IR (KBr cm^{-1}): ν 3429 (OH), 2933 (CH_3), 2860 (CH_2), 2205, 2200(2CN), 1660 (C=C). ^{1}H NMR (DMSO-d6, ppm): δ = 0.82 (s, 3H, Me-19), 0.9-1.10 (m, steroid moiety protons), 1.16 (s, 3H, Me-18), 6.46-7.01 (m, 5H, aromatic protons), 8.31(s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d6,ppm): δ = 11.90 (CH_3), 16.40 (CH_3), 31.95 (C-1), 31.27 (C-2), 57.70 (C-3), 123.90 (C-4), 140.03 (C-5), 33.0 (C-6), 32.1 (C-7), 35.12 (C-8), 49.98 (C-9), 36.80 (C-10), 22.51 (C-11), 35.05 (C-12), 44.36 (C-13), 53.42 (C-14), 27.1 (C-15), 22.50 (C-16), 79.80 (C-17), 11.18 (C-18), 16.89 (C-19), 113.40, 139.60, 179.80 (thiazole-C), 113.70 (2CN), 116.30, 118.80, 129.60, 191.30 (aromatic -c). MS (EI) m/z = 525.280, 65 (45%), 91 (25%), 101 (9%), 415 (15%) [M+˙].Calc for $C_{33}H_{39}N_3OS$ (525.750): C, 75.39; H, 7.48; N, 7.99; S, 6.10, found: C, 75.70; H, 7.08; N, 8.10; S, 5.70%.

(Z)-ethyl2-cyano-2-((1S,12aS,14aS)-1-hydroxy-12a,14a-dimethyl-8-phenyl-3,3a,4,5,5a,6,6a,7,11,11a,12,12a, 12b,13,14,14a-hexadecahydro-1H-cyclopenta[3,4]tetrapheno[9,10-d]thiazol-9(2H,3bH,8H)-ylidene) acetate (10b).

Dark blue powder. Yield (0.37gm) 65 %; mp = 113-115^{o}C; IR (KBr cm^{-1}): ν 3415 (OH), 2930 (CH_3), 2860 (CH_2), 2210 (CN), 1740 (C=O), 1622 (C=C). ^{1}H NMR (DMSO-d6, ppm): δ = 0.82 (s, 3H, Me-19), 0.9-1.10 (m, steroid moiety protons), 1.17 (s, 3H, Me-18), 1.30(t, 3H, CH_3), 4.19 (q, 2H, CH_2), 6.46-7.01(m, 5H, aromatic protons), 8.31(s, 1H, OH, D_2O exchangeable). ^{13} C NMR (DMSO-d6,ppm): δ = 11.90 ($2CH_3$ aliphatic), 60.90 (CH2), 31.95 (C-1), 31.27 (C-2), 57.70 (C-3), 123.90 (C-4), 140.03 (C-5), 33.0 (C-6), 32.1 (C-7), 35.12 (C-8), 49.98 (C-9), 36.80 (C-10), 22.51 (C-11), 35.05 (C-12), 44.36 (C-13), 53.42 (C-14), 27.1 (C-15), 22.50 (C-16), 79.80 (C-17), 11.18 (C-

18), 16.89 (C-19), 113.40, 139.60, 179.80 (Thiazole-C), 113.70 (C=N), 116.30, 118.80, 129.60, 191.30 (aromatic -c), 142.00 (CH3), 165.00 (C=O). MS (EI) m/z = 572.310, 57 (100%), 79 (32%), 415 (21%), 533 (5%) [M+˙].Calc for $C_{35}H_{44}N_2O_3S$ (572.800): C, 73.39; H, 7.74; N, 4.89; S, 5.60, found: C, 73.50; H, 7.94; N, 4.49; S, 5.30%.

Synthesis of compounds (11) and (12):
To a mixture of compound **10a** (0.52gm, 1mmol) with hydrazine hydrate (0.05gm, 1 mmol) or phenyl hydrazine in (0.10 gm, 1mmol) in 30 mL absolute ethanol, a catalytic amount of triethylamine was added (0.5mL).The reaction mixture was heated under reflux for 3hours until all the starting materials had disappeared as indicated by TLC. Then poured into ice/water mixture. The formed solid product was collected by filtration dried and crystallized from absolute ethanol to form compounds **11**, **12** respectively.

(1S,12aS,14aS)-9-(3,5-diamino-4H-pyrazol-4-ylidene)-12a,14a-dimethyl-8-phenyl-2,3,3a,3b,4,5,5a,6,6a, 7,8,9, 11,11a,12,12a,12b,13,14,14a-icosahydro-1H-cyclopenta[3,4]tetrapheno[9,10-d]thiazol-1-ol (11).
Gray powder. Yield (0.32gm) 58 %; mp = 134-135^{o}C; IR (KBr cm^{-1}): ν 3429-3415 (OH, $2NH_2$), 2930 (CH_3), 2860 (CH_2), 1660 (C=C), 1622 (C=N). ^{1}H NMR (DMSO-d6, ppm): δ = 0.82 (s, 3H, Me-19), 0.9-1.10 (m, steroid moiety protons), 1.17 (s, 3H, Me-18), 6.46-7.11 (m, 5H, aromatic), 8.30 (s, 4H, $2NH_2$, D_2O exchangeable), 11.50 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 11.90 ($2CH_3$ aliphatic), 31.95 (C-1), 31.27 (C-2), 57.70 (C-3), 123.90 (C-4), 140.03 (C-5), 33.0 (C-6), 32.1 (C-7), 35.12 (C-8), 49.98 (C-9), 36.80 (C-10), 22.51 (C-11), 35.05 (C-12), 44.36 (C-13), 53.42 (C-14), 27.1 (C-15), 22.50 (C-16), 79.80 (C-17), 11.18 (C-18), 16.89 (C-19), 85.00, 155.00, 164.00 (pyrazolo), 164.00 (2C-N), 116.00, 118.80, 128.90, 141.30(Aromatic). MS (EI) m/z = 557.320, 77 (100%), 86 (30%), 91(71%), 101 (34%), 172 (78%), 275 (54%), 378 (33%), 506 (17%)[M+˙].Calc for $C_{33}H_{43}N_5OS$ (557.790): C, 71.06; H, 7.77; N, 12.56; S, 5.75, found: C, 70.76; H, 7.90; N, 12.26; S, 5.45%.

(1S,12aS,14aS,E)-9-(3-Amino-5-imino-1-phenyl-1H-pyrazol-4(5H)-ylidene)-12a,14a-dimethyl-8-phenyl-2,3,3a, 3b,4,5,5a,6,6a,7,8,9,11,11a,12,12a,12b,13,14,14a-icosahydro-1H-cyclopenta[3,4]tetrapheno[9,10-d]thiazol-1-ol (12).
Dark brown crystals. Yield (0.38gm) 60 %; mp = 98-100^{o}C; IR (KBr cm^{-1}): ν 3429-3550 (OH, NH and NH_2), 2930 (CH_3), 2860 (CH_2), 1660 (C=C), 1622 (C=N). ^{1}H NMR (DMSO-d6, 300 MHz): δ = 0.82(s, 3H, Me-19),0.95-1.10 (m, steroid moiety protons), 1.17 (s, 3H, Me-18), 6.46-7.11 (m, 10H, aromatic H), 8.30 (s, 2H, NH_2, D_2O exchangeable), 11.2(s, 1H, OH, D2O exchangable),11.60 (s,1H, imino proton, D_2O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 11.90 (2CH3 aliphatic), 31.95 (C-1), 31.27 (C-2), 57.70 (C-3), 123.90 (C-4), 140.03 (C-5), 33.0 (C-6), 32.1 (C-7), 35.12 (C-8), 49.98 (C-9), 36.80 (C-10), 22.51 (C-11), 35.05 (C-12), 44.36 (C-13), 53.42 (C-14), 27.1 (C-15), 22.50 (C-16), 79.80 (C-17), 11.18 (C-18), 16.89 (C-19), 116.00, 118.80, 128.90, 141.30(Aromatic), 91.00, 155.00, 164.00 (pyrazole),113.40, 139.60, 146.70 (thiazole), 152.22 (C=N), 164.00(C-N),. MS (EI) m/z = 633.350, 77(100%), 172(32%), 461(25%), 556(20%) [M+˙].Calc for $C_{39}H_{47}N_5OS$ (633.890): C, 73.90; H, 7.47; N, 11.05; S, 5.06, found: C, 74.00; H, 7.67; N, 10.75; S, 4.76%.

2.1.7. Synthesis of compounds (13a), (13b) and (13c):
To a mixture of compound **10a** (0.52gm, 1mmol) urea (0.06 gm, 1 mmol), thiourea (0.07 gm, 1 mmol) and or guanidine hydrochloride (0.09 gm, 1mmol) in 30 mL absolute ethanol, a catalytic amount of triethylamine was added (0.5mL).The reaction mixture was heated under reflux for 3-5hours until all the starting materials had disappeared as indicated by TLC.Then poured over ice/water mixture. The formed solid product was collected by filtration dried and crystallized from absolute ethanol to form compounds **13a-c** respectively.

4,6-Diamino-5-((1S,12aS,14aS)-1-hydroxy-12a,14a-dimethyl-8-phenyl-3,3a,4,5,5a,6,6a,7,11,11a,12,12a,12b, 13,14,14a-hexadecahydro-1H-cyclopenta[3,4]tetrapheno[9,10-d]thiazol-9(2H,3bH,8H)-ylidene)pyrimidin-2(5H)-one(13a).
Brown powder, Yield (0.37gm) 64 %; mp = 168-170^{o}C; IR (KBr cm^{-1}): ν 3420-3415 (OH, 2 NH_2), 2976 (CH_3), 2845 (CH_2), 1652 (C=O), 1613 (C=C), 1622 (C=N). ^{1}H NMR (DMSO-d6, ppm): δ = 0.87 (s, 3H, Me-19), 0.95-1.10 (m, steroid moiety protons), 1.05 (s, 3H, Me-18), 8.31(s, 4H, $2NH_2$, D_2O exchangeable), 6.46-7.01 (m, 5H, aromatic), 11.50 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 11.90 ($2CH_3$ aliphatic), 31.95 (C-1), 31.27 (C-2), 57.70 (C-3), 123.90 (C-4), 140.03 (C-5), 33.00 (C-6), 32.10 (C-7), 35.12 (C-8), 49.98 (C-9), 36.80 (C-10), 22.51 (C-11), 35.05 (C-12), 44.36 (C-13), 53.42 (C-14), 27.1 (C-15), 22.50 (C-16), 79.80 (C-17), 11.18 (C-18), 16.89 (C-19), 85.00, 163.00, 164.00 (pyrimidine), 113.51, 139.31, 178.00 (thiazole) 116.30, 118.80, 129.60, 141.30 (C-phenyl), 154.50(C=O),. MS (EI) m/z = 585.310, 66 (100%), 170 (95%), 182 (98%), 226 (22%), 550 (25%) [M+˙].Calc for $C_{34}H_{43}N_5O_2S$ (585.800): C, 69.71; H, 7.40; N, 11.96; S, 5.47, found: C, 70.00; H, 7.20; N, 11.56; S, 5.07%.

4,6-Diamino-5-((1S,12aS,14aS)-1-hydroxy-12a,14a-dimethyl-8-phenyl-3,3a,4,5,5a,6,6a,7,11,11a,12,12a, 12b,13,14,14a-hexadecahydro-1H-cyclopenta[3,4]tetrapheno[9,10-d]thiazol-9(2H,3bH,8H)-ylidene) pyrimidine-2(5H)-thione (13b).

Dark gray powder from ethanol.Yield (0.37gm) 62 %; mp = 180-182^{o}C IR (KBr cm^{-1}): ν 3429-3415 (broad OH, 2NH$_2$), 2976 (CH$_3$), 2845 (CH$_2$), 1200 (C=S), 1613 (C=C), 1622 (C=N). ^{1}H NMR (DMSO-d6, ppm): δ = 0.95 (s, 3H, Me-19), 0.95-1.10 (m, steroid moiety protons), 1.08 (s, 3H, Me-18), 6.46-7.01 (m, 5H, aromatic), 8.31(s, 4H, 2NH$_2$, D$_2$O exchangeable), 11.50 (s, 1H, OH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 11.89 (2CH$_3$ aliphatic), 33.05 (C-1), 29.98 (C-2), 56.98 (C-3), 124.00 (C-4), 139.03 (C-5), 32.90 (C-6), 31.89 (C-7), 35.22 (C-8), 50.00 (C-9), 36.84 (C-10), 22.59 (C-11), 35.56 (C-12), 44.68 (C-13), 52.49 (C-14), 27.60 (C-15), 22.50 (C-16), 79.80 (C-17), 10.88 (C-18), 17.00 (C-19), 84.99, 162.97, 164.20 (pyrimidine), 112.91, 139.45, 178.00(thiazole) 116.30, 117.89, 130.00, 142.42(C-phenyl), 192.00(C=S). MS (EI) m/z = 601.290, 32 (42%), 140(70%), 460(33%), 570(19%) [M+˙].Calc for $C_{34}H_{43}N_5OS_2$ (601.870): C, 67.85; H, 7.20; N, 11.64; S, 10.66%, found: C, 67.45; H, 7.00; N, 12.00; S, 10.36%.

(1S,12aS,14aS,E)-9-(2,6-Diamino-4-iminopyrimidin-5(4H)-ylidene)-12a,14a-dimethyl-8-phenyl-2,3,3a,3b, 4,5,5a,6,6a,7,8,9,11,11a,12,12a,12b,13,14,14a-icosahydro-1H-cyclopenta[3,4]tetrapheno[9,10-d]thiazol-1-ol (13c).

Pale green powder. Yield (0.38gm) 66 %; mp = 180-182^{o}C; IR (KBr cm^{-1}): ν3420- 3415 (OH, NH and 2NH$_2$), 2970 (CH$_3$), 2840 (CH$_2$), 1622 (C=N). ^{1}H NMR (DMSO-d6, ppm): δ = 0.95 (s, 3H, Me-19), 0.95-1.10(m, steroid moiety protons), 1.08 (s, 3H, Me-18), 6.06-7.00 (m, 5H, aromatic), 8.30(s, 4H, 2NH$_2$, D$_2$O exchangeable), 11.50 (s, 2H, OH and NH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 12.00 (2CH$_3$ aliphatic), 32.00 (C-1), 31.27 (C-2), 57.90 (C-3), 124.92 (C-4), 141.33 (C-5), 33.00 (C-6), 31. 90 (C-7), 34.94 (C-8), 49.98 (C-9), 36.80 (C-10), 21.98 (C-11), 35.05 (C-12), 44.36 (C-13), 53.42 (C-14), 27.1 (C-15), 22.50 (C-16), 79.80 (C-17), 11.18 (C-18), 17.00 (C-19), 85.00, 163.00, 164.00 (pyrimidine), 112.92, 143.35, 176.20 (thiazole) 115.33, 120.85, 130.41, 141.45 (C-phenyl), 153.70(C=N).MS (EI) m/z = 584.330, 32(49%), 123(72%), 461(42%0, 553(38%) [M+˙].Calc for C34H44N6OS (584.820): C, 69.83; H, 7.58; N, 14.37; S, 5.48%, found: C, 69.43; H, 7.18; N, 14.70; S, 5.70%.

Synthesis of (14):

To a mixture of compound **10b** (0.57gm, 1mmol) guanidine hydrochloride (0.09 gm, 1mmol) in 30 mL absolute ethanol. A catalytic amount of triethylamine was added (0.5mL).The reaction mixture was heated under reflux for 3-5hours until all the starting materials had disappeared as indicated by TLC.Then poured on ice/water mixture. The formed solid product was collected by filtration dried and crystallized from absolute ethanol to form compound 24.

(Z)-2,6-Diamino-5-((1S,12aS,14aS)-1-hydroxy-12a,14a-dimethyl-8-phenyl-3,3a,4,5,5a,6,6a,7,11,11a,12,12a, 12b,13,14,14a-hexadecahydro-1H-cyclopenta[3,4]tetrapheno[9,10-d]thiazol-9(2H,3bH,8H)-ylidene)pyrimidin-4(5H)-one (14).

Dark green powder. Yield (0.38gm) 65 %; mp = 94-96^{o}C; IR (KBr cm^{-1}): ν 3420 (OH, NH$_2$&2NH), 2975(CH$_3$), 2830 (CH$_2$), 1652 (C=O), 1622 (C=N), 1613(C=C). ^{1}H NMR (DMSO-d6, ppm): δ = 0.88 (s, 3H, Me-19), 0.95-1.10(m, steroid moiety protons), 1.04 (s, 3H, Me-18), 8.00(s, 2H, 2NH,D$_2$O exchangeable), 6.32-7.02(m, 5H, aromatic), 8.30(s, 2H, NH$_2$, D$_2$O exchangeable), 11.50(s, 1H, OH, D$_2$O exchangeable). ^{13}C NMR (DMSO-d6, ppm): δ = 11.90 (2CH$_3$ aliphatic), 31.95 (C-1), 31.27 (C-2), 57.70 (C-3), 123.90 (C-4), 140.03 (C-5), 33.0 (C-6), 32.1 (C-7), 35.12 (C-8), 49.98 (C-9), 36.80 (C-10), 22.51 (C-11), 35.05 (C-12), 44.36 (C-13), 53.42 (C-14), 27.1 (C-15), 22.50 (C-16), 79.80 (C-17), 11.18 (C-18), 16.89 (C-19), 92.00, 163.00, 164.00 (pyrimidine), 113.40, 135.60, 146.70 (thiazole), 115.00, 129.30, 146.70, 118.50 (aromatic carbons), 153.70(C=N), 166.00(C=O), 168.00(C-N). MS (EI) m/z =585.310, 62 (100%), 179 (84%), 365 (35%), 413 (44%) [M+˙].Calc for $C_{34}H_{43}N_5O_2S$ (585.800): C, 69.71; H, 7.40; N, 11.96; S, 5.47%, found: C, 69.90; H, 7.10; N, 11.56; S, 5.00%.

Biological assays:

Chemical and drug

Aluminum chloride was purchased from Sigma Company, U.S.A. Rivastigmin , Exelon, 1.5 mg was purchased from Novartis company, Germany. All other chemicals and solvents were of analytical grade.

Experimental design

Female albino rats (56 rats), weighing 200–220 g, aged 16-18 weeks, were supplied by the Animal House Colony of the National Research Centre, Giza, Egypt, and acclimated for one week in a specific pathogen-free (SPF) barrier area where temperature is 25±1°C and humidity is 55%. Rats were controlled constantly with 12 h light/dark cycle at National Research Centre Animal Facility Breeding Colony. Rats were individually housed with *ad libitum* access to standard laboratory diet and tap water. All animal procedures were performed after approval from the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No. 85–23, revised 1985).

The animals in the present study were assigned into 7 main groups. (n=8) as follows: Group 1: Normal healthy rats administered DMSO in distilled water (1mL/rat) served as control group. Group 2: AD group in which the rats administered $AlCl_3$ in a dose of 17 mg/kg b.wt. for one month [18]. Group 3: Healthy rats administered compound **7a** in a dose of 50 mg/kg b.wt daily for two months. Group 4: Healthy rats administered compound **13c** in a dose of 50 mg/kg b.wt daily for two months so as to confirm the safety of these compounds. Group 5: AD- bearing rats treated with compound **7a** daily for two months in equimolar doses of 50 mg/kg b.wt. Group 6: AD- bearing rats treated with compound **13c** daily for two months in equimolar doses of 50 mg/kg b.wt. The tested compounds were dissolved in DMSO in distilled water and given to rats orally with oral agavage. The selected dose of these compounds was literature dependent according to the study Ahmed and Mannaa[19] Group 7: AD- bearing rats treated orally with rivastigmine dissolved in DMSO in distilled water in a dose of 0.3 mg/kg b.wt daily for two months [20]

Sample collection

After animal treatment was over, rats were kept in metabolic cages individually for collection of 24h urine samples for determination of 8-OHG and creatinine. Then, the rats were sacrificed by cervical dislocation following ether anesthesia and the whole brain of each rat was rapidly dissected, thoroughly washed in ice-cold isotonic saline, blotted dry and then weighed. One half of each brain was homogenized immediately to give 10% (w/v) homogenate in ice-cold medium containing 50 mM Tris-Hcl (pH 7.4) and 300 mM sucrose [21]. The homogenate was centrifuged at 1800 xg for 10 min at 4 ºC. The supernatant (10%) was stored at -20 pending for biochemical analysis. The second half of each brain was fixed in buffered formaline (10%) for immunohistochemical and histopathological examination.

Biochemical analyses

Colorimetric assay was used for the determination of brain AchE activity using butyrylthiocholineiodide as substrate using kit purchased from Centronic GmbH, Germany following the method described by Henry et al. [22]. Brain Ach level was measured by colorimetric method using choline/acetylcholine assay kit purchased from Biovision Research Products Co., Linda Vista Avenue, USA, according to the method of Oswald et al. [23]. Colorimetric assay for determination of brain GSH level was used by using the kit purchased from Bio-diagnostic Co., Egypt, according to the method described by Beutler [24]. Colorimetric assay was used for the determination of brain paroxenase activity following a modified method described by Watson et al [25]. Protocol for urinary 8-OHG analysis was modified from the method described by Kim et al. [26] using HPLC technique. Brain caspase3 level was estimated ELISA using Caspase3 assay kit purchased from Glory science co., Veterans Blvd.Suit, U.S.A.Brain P 53 level was assayed by ELISA using P 53 assay kit purchased from Glory science co., Veterans Blvd.Suit, U.S.A. Brain BCL2 level was quantified by ELISA using BCL2 assay kit purchased from Glory science co., Veterans Blvd.Suit, U.S.A..Urinary creatinine level was determined kinetically following the method of Larsen [27]. Quantitative estimation of total protein in the brain homogenate was carried out according to the method of Lowery et al. [28].

Immunohistochemistry

After fixation of brain samples of in 10% formalin buffer for 24 hours, washing was done in tap water. Then, ascending grade of ethyl alcohol was used for dehydration. Specimens were cleared in xylene and embedded in paraffin for 24 hours. Sections were cut into 4 µ thick by slidge microtome then, fixed on positive slides in 65^{o}C oven for 1 hr according to Bancroft and Gamble [29].Image analysis was performed using Image Analyzer J, 1.4 1a NIH, (USA) for determination of optical density (O.D) that reflects the intensity of immunostaining of ChAT positive cells.

Histopathology

Paraffin bees wax tissue blocks prepared for immunohistochemical analysis were used and sectioned at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin stain and then examined through the light electric microscope [30].

Statistical analysis

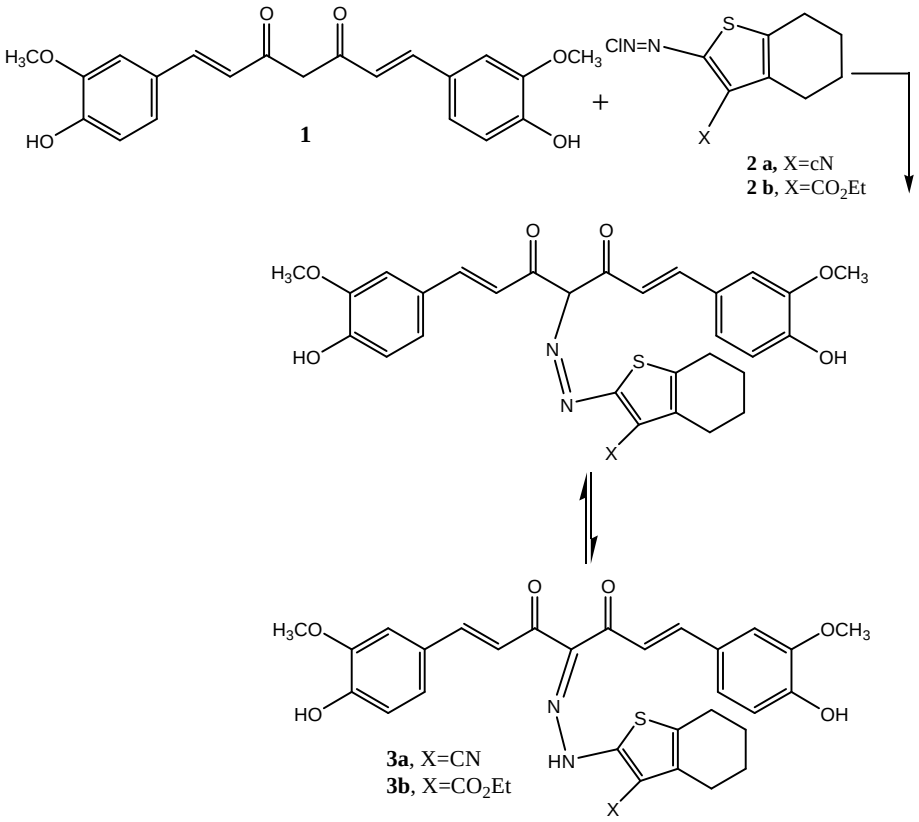
In the present study, all results were expressed as Mean + S.E of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 11 followed by least significant difference (LSD) to compare significance between groups [31]. Difference was considered significant when P value was < 0.05.

$$\% \text{ Difference} = \frac{\text{Treated value} - \text{Control value}}{\text{Control value}} \times 100$$

RESULTS AND DISCUSSION

Chemistry

In the aim of synthesis of structural modifications of curcumin the diazonium salt of 2-aminotetrahydrobenzothiophenecarbonitrile **2a** derivative (compounds were prepared, in a one-pot facile high yield reaction, following the Gewald synthesis [16]) was coupled with Curcumin **1** in acetic acid yielded the coupling product 2-((Z)-((1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-7,6-dien-4-yl)diazenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (**3a**) Similarly, when curcumin **1** was submitted to react with the diazonium salt of ethyl-2-aminobenzo[b]thiophene-3-carboxylate **2b** in acetic acid it yielded 2-((Z)-((1E,6E)-17-bis(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dien-4-yl)diazenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene (**3b**) (Scheme1).

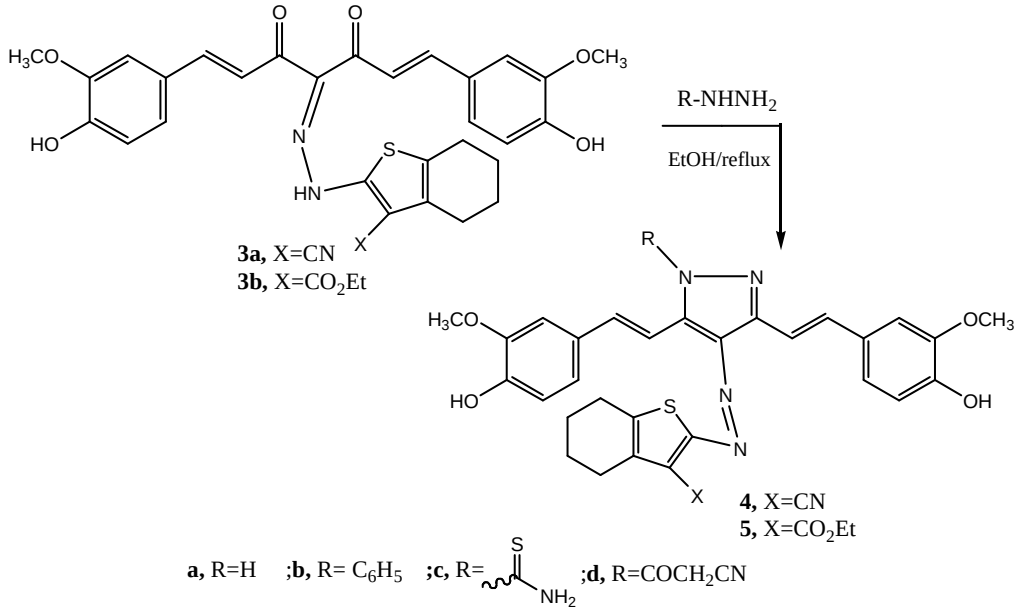


Scheme 1

The structure of compound **8** was inferred from its IR spectrum revealed strong absorption bands at 1700, 1690, 2215, 2870, 2972 and 3450 cm^{-1} attributable to ν 2C=O, ν CN, ν CH_2, ν CH_3 and ν OH respectively.^{1}HNMR, ^{13}CNMR and EIMS agreed well with the assigned structure of experimental part. The structure of compound **9** was inferred from correct microanalytical and spectral data.

The structure of compound **3a** and **3b** were verified chemically as follows:

When compound **3a** or **3b** were allowed to react with hydrazines namely, hydrazine hydrate, phenyl hydrazine, and or thiosemicarbazide or cyanoacetylhydrazide in boiling ethanol in presence of Et_3N it afforded the pyrazole derivatives **4a-d** or **5a-d**. The reaction possibly takes place *via* condensation of hydrazine derivative with β-dicarbonyl compound to yield the desired products.

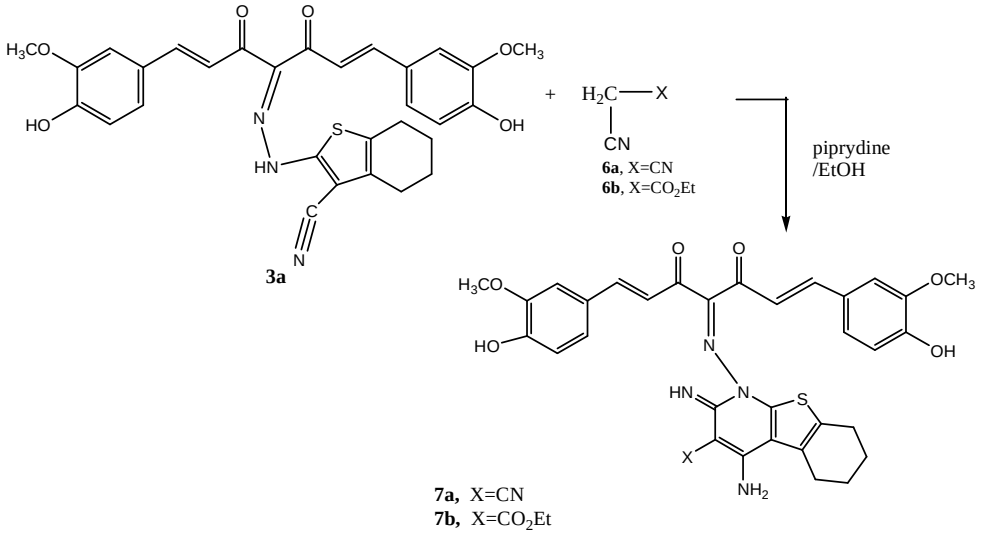


Scheme 2

The IR spectrum revealed strong absorption bands at 2210, 3320- 3400 cm^{-1} due to ν CN, and ν OH or ν NH and devoid any band for carbonyl groups for carbonyl **4a-c**. 1HNMR, ^{13}CNMR and EIMS are agreed well with the proposed structure of (*cf*. Materials and methods).

The IR spectra of compounds **5a-d** were revealed strong absorption bands in the region 1720, 2875, 2930 and 3320-3440 cm^{-1} attributable to ν C=O, ν CH_2, ν CH_3 and ν NH or ν OH. ^{1}HNMR, ^{13}CNMR and EIMS are agreed well with the proposed structure of (*cf*. Materials and methods).

When a solution of compound **3a** in boiling ethanol was allowed to react with ethylcyanoacetate **6a** or malononitrile **6b** in presence of catalytic amount of piperidine, it yielded 2-imino-4-amino-1-(((1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-diene-4-ylidene)amino)-1,2,5,6,7,8-hexahydrobenzo[2,3b]pyridine-3-carbonitrile **7a** and ethyl-2-imino-4-amino-1-((1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dien-4-ylidene)amino)-1,2,5,6,7,8-hexahydrobenzo[4,5]thieno[2,3b]pyridine-3-carboxylate **7b** respectively (scheme 3).



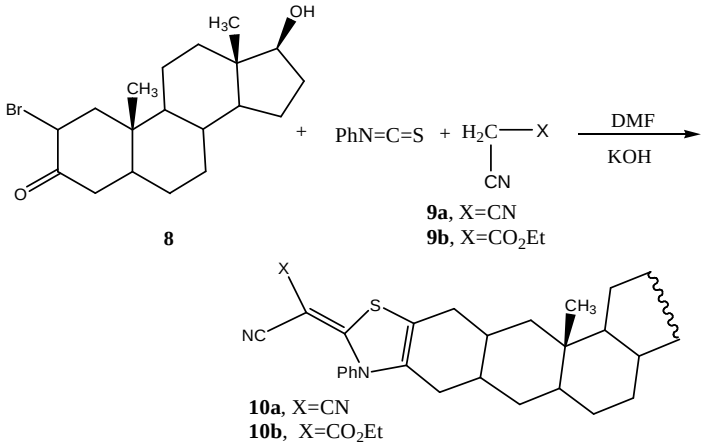
Scheme 3

The IR spectra of compound **7a** exhibited strong absorption bands at 1700, 1720, 2210, 2875, 2930 and 3400-3440 cm^{-1} attributable to ν 2C=O, ν C≡N, ν CH_2, ν CH_3 and ν NH and ν OH respectively. ^{1}HNMR, ^{13}CNMR and EIMS are agreed well with the proposed structure of (cf. Materials and methods).

The IR spectra of compound 7b was revealed strong absorption bands at 1720, 1710, 1700, 2875, 2930, 3320- 3440 cm^{-1} due to ν max of three carbonyl group, ν CH_2, ν CH_3 and ν NH, ν NH2 and ν OH respectively and devoid any band for ν CN ^{1}HNMR, ^{13}CNMR and EIMS are agreed well with the proposed structure of (*cf*. Materials and methods).

In this context, utilization of multicomponent reactions (MCRs) with at least three different simple substrates reacting in a well defined manner to form a single compound has emerged as a powerful strategy [32].

When α–bromoandrostanalone **8** [17] was submitted to react with phenylisothiocyanate and malononitrile **9a** or ethylcyanoacetate **9b** in DMF in the presence of of potassium hydroxide, it yielded the thiazoloandorstane derivative **10a** or **10b** respectively (scheme 4).

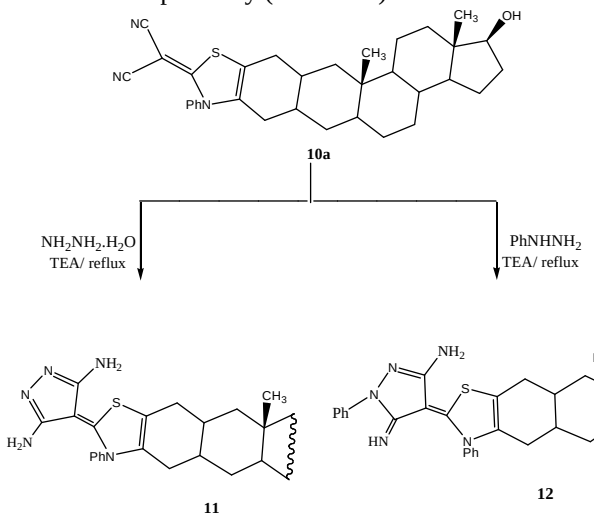


Scheme 4

The IR spectrum of compound **10a** was exhibited absorption bands 2205, 2860, 2933and 3429 cm^{-1} attributable to ν C≡ N, ν CH and ν OH.^{1}HNMR, ^{13}CNMR and EIMS are agreed well with the proposed structure of (*cf*. Materials and methods).

The structure of compound **10b** was established from IR spectrum that revealed strong absorption bands at 1740, 2210, 2860, 2930, and 3415 cm^{-1} due to ν C=O ν CN, ν CH_2, ν CH_3 and ν OH respectively. ^{1}HNMR, ^{13} CNMR and EIMS are agreed well with the proposed structure of (*cf*. Materials and methods).

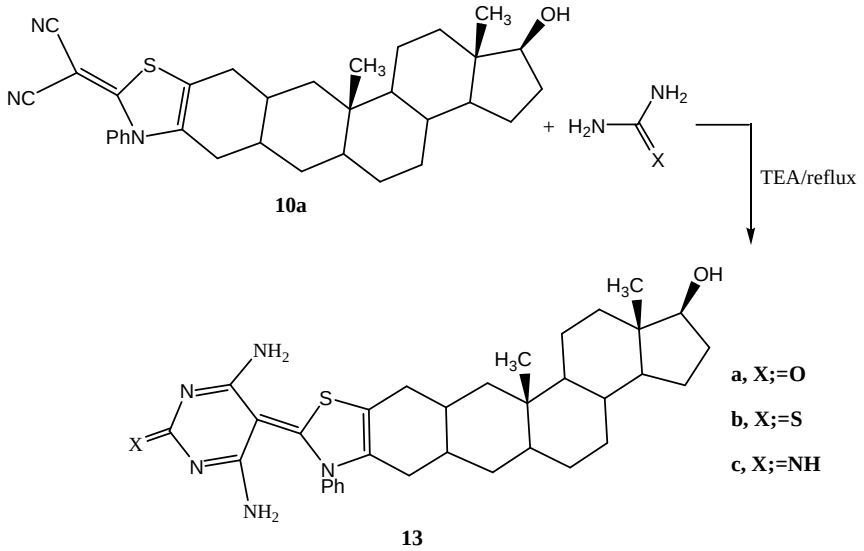
When compound **10a** was allowed to react with hydrazines namely, hydrazine hydrate or phenyl hydrazine in boiling ethanol in presence of a catalytic amount of triethylamine, it yielded 3,5-diaminopyrazolothiazoloandorstane and 3-amino-5-iminophenylpyrazoly thiazoloanorstane **11** and **12** respectively (Scheme 5). The structure of compound **19** was inferred chemically *via* its reaction with hydrazines, urea, and thiourea and guanidine hydrochloride in boiling ethanol and in presence of triethylamine as a catalyst it yielded pyrimidinyl thaiazoloandorstane derivative s **13 a-c** respectively (Scheme 6).



Scheme 5

IR spectrum of compound **11** and **12** showed absorption attributable to ν NH and ν OH respectively. ^{1}HNMR, ^{13}CNMR and EIMS agreed well with the proposed structure of experimental part. The mechanism of the reaction involving nucleophilic addition of hydrazines to nitrile groups.

When thiazoloandrostane derivatives **10a** allowed to react with nitrogen nucleophiles namely, urea, thiourea and guanidine hydrochloride in boiling ethanol and in presence of tri ethyl amine as a catalyst it yielded pyrimidino thaiazoloandorstane derivative s **13 a-c** respectively (Scheme 6).

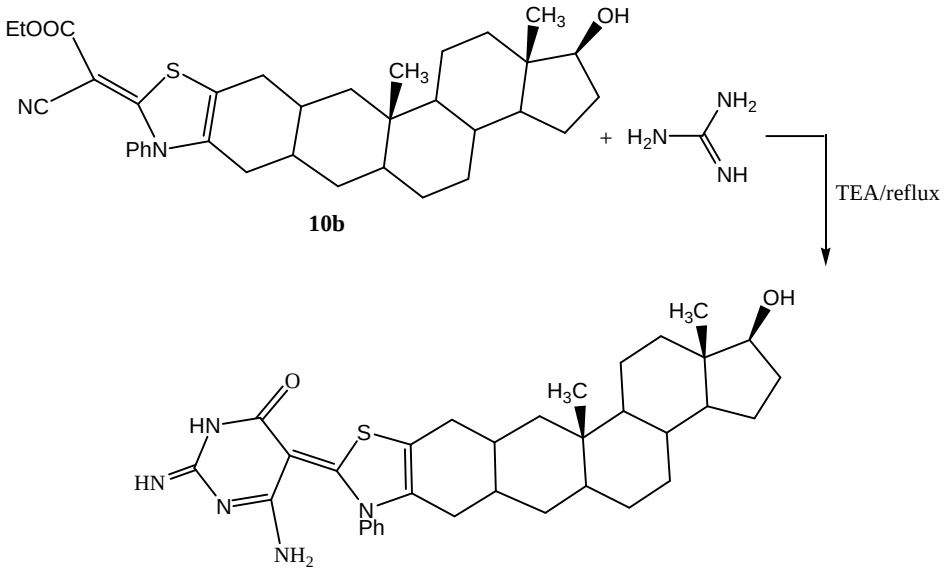


13

Scheme 6

The structure of compounds **13** showed absorption at 2845, 2976 and 3415-3429 cm^{-1} attributable to ν CH_2, ν CH_3, ν NH ν NH_2 and ν OH respectively. ^{1}HNMR, ^{13}CNMR and EIMS agreed well with the proposed structure of experimental part. The reaction possibly takes place *via* nucleophilic attack of nitrogen nucleophiles on nitrile groups.

When the thiazoloandrostane derivative **10b** was allowed to react with guanidine hydrochloride in boiling ethanol in the presence of triethylamine as a catalyst, it yielded pyrimidine derivative **14** (Scheme 7).



14

Scheme 7

IR, ^{1}HNMR, ^{13} CNMR and EIMS are consistent with the proposed structure of experimental part.

Biological assays

In vivo anti-Alzheimer's disease effect of the newly synthesized compounds **7a** and **13c** in rat model was investigated. The cholinergic activity in AD model in this study was detected by measuring brain acetylcholinesterase (AchE) activity, acetylcholine (Ach) level and cholineacetyltransferase (ChAT) activity. Moreover, brain oxidant/antioxidant status of AD-challenged rats was estimated. As well, levels of brain caspase-3, tumor protein (P 53) and 2B-cell lymphoma BCL2 were quantified in AD- bearing rats.

The data in Table (1) illustrated the effect of treatment with rivastigmine and the tested compounds on cholinergic markers represented by brain AchE activity and brain Ach level in AD-bearing rats. In comparison with the control group, $AlCl_3$ administration produced significant elevation ($P<0.05$) in brain AchE activity (26.64%) associated with significant reduction ($P<0.05$) in brain Ach level (-26%). The underlying mechanism for the stimulation of AChE activity in the brain by $AlCl_3$ is related to the neurotoxicity afford by this heavy metal due to its effect in promoting the accumulation of insoluble Aβ protein [33]. The pro-oxidant activity of Aβ is responsible for the elevation in AchE activity *via* stimulation of lipid peroxidation in neuronal membranes as a consequence of generation of H_2O_2 [34]. Moreover, Al could reduce Ach levels in the brain due to the interaction of Al with cholinergic system, by altering cholinergic projection functioning and also by intensifying its inflammation. This represents the way by which Al contributes to the pathological process in AD [35].

Administration of healthy rats with the tested compounds (**7a** or **13c**) resulted in insignificant increase ($P>0.05$) in brain AchE activity (6.53%for compound **7a** and 4.53%for compound **13c**) with respect to the control counterparts.
Treatment of AD- bearing rats with rivastigmine or compound **13c** resulted in significant decrease ($P<0.05$) in brain AchE activity (-18.12% for rivastigmine and-15.61%for compound **13c** respectively) as compared to untreated AD group. Meanwhile, treatment of AD- bearing rats with compound **7a** showed insignificant decrease ($P>0.05$) in brain AchE activity (-10.93%) versus the untreated AD group.

Treatment of AD-bearing rats with the tested compounds elicited insignificant increase ($P>0.05$) in brain AchE activity (8.77%for compound **7a**; 3.56%for compound **13c**) with respect to rivastigmine-treated group.

Administration of healthy rats with the tested compounds (**7a** or **13c**) resulted in insignificant decrease ($P>0.05$) of brain Ach level (-5.3%for compound **7a**, -4%for compound **13c**) relative to the control counterparts.

Treatment of AD- bearing rats with rivastigmine or compound **13c** experienced in significant increase ($P<0.05$) in brain Ach level (24.3% for rivastigmine and 13.5%for compound **13c** respectively) as compared to the untreated AD group. However, treatment of AD- bearing rats with compound **7a** showed insignificant increase ($P>0.05$) in brain Ach level (4.5%) as compared with untreated AD group.

Treatment of AD bearing rats with compound **7a** resulted in significant decrease ($P<0.05$) in brain Ach level (-15.9%) relative to rivastigmine- treated group. Meanwhile, treatment with compound **13c** resulted in insignificant decrease ($P>0.05$) in brain Ach level (-8.6%) with respect to rivastigmine-treated group.

Concerning the inhibitory effect of compound **7a** on AchE activity and in turn Ach preservation, this could be attributed to the presence of curcumin, 4-aminopyridine (4-AP) and tetrahydrobenzothiophene moieties in this compound. It was reported that, curcumin could adopt many different conformations suitable for maximizing hydrophobic contacts with the protein to which it is bound [36]. Hence, curcumin could offer significant inhibitory activity against AchE [37]. Moreover, it has been found that administration of 4-AP could ameliorate the sympyoms of Alzheimer's disease, *via* enhancement of neurotransmitter release by blocking of presynaptic K channels [38]. Additionally, the structural optimization of 4-AP derivatives, has been studied to enhance their potency against cholinesterase in order to be useful in the treatment of AD [39]. It has been reported that 4-AP potentiated Ach release in two different ways: 1) Promoting a sustained quantal release of transmitter during several hundreds of milliseconds without any significant change in the maximal synchronous release and 2) Interacting with Mg^{2+} in a manner that the sensitivity of the nerve terminals to Ca^{2+} is increased [40]. All together with, curcumin, that possesses AchE inhibitory activity in the hippocampus and frontal cortex, provided longer time for Ach to stimulate postsynaptic muscarinic receptors [41]. Besides, it was belived that benzothiophenes possessed anticholinesterases activity [42].

Compound **23c** in the present study contains steroid, thiazole and pyrimidine moieties. Steroids significantly increased Ach release in the hippocampus *via* antagonizing AchE activity [43]. In addition, thiazole ring has been found to process a remarkably anticholinesterase potential [44]. Furthermore, a central pyrimidine ring serves as a suitable template to develop dual inhibitors of cholinesterase and AchE-induced Aβ aggregation thereby targeting multiple pathological routes in AD [45]. It was reported that pyrimidine derivatives represented lead compounds to

generate AchE inhibitors as novel therapeutical entities for severe neurodegenerative diseases [46]. Additionally, compounds bearing thiazole moiety have been reported to exhibit a wide spectrum of biological effects including anticholinesterase activity [47].Therefore, this compound (**13c**) proved its promising role in maintaining brain Ach level in AD- bearing rats. Rivastigmine appears to inhibit cholinesterases (chEs) in plaques and tangles with the same potency as those in neurons and axons by interacting with the esteratic site in chE molecules [48]. It prevents the hydrolysis of Ach released from surviving nerve terminals and correlates best with the increasing of the steady-state levels of Ach in the brain [49]. In general, cholinesterase inhibitors increased the availability of Ach, hence they enhanced the cholinergic transmission in the brain and improved the symptoms of AD [50].

Table (1): Effect of treatment of with rivastigmine and newly synthesized compounds on brain acetylcholineesterase (AChE) activity and acetylcholine (Ach) level in AD- bearing rats.

Groups	AchE (U/mg protein)	Ach (u/mg protein)
Control	570.9±11.68	7.50 ± 0.27
AD	722±11.3[a] (26.64%)	5.55 ± 0.14[a] (-26%)
Compound **7a**	608.2±20.31 (6.53%)	7.10 ± 0.31 (-5.3%)
Compound **13c**	595.79±44.9 (4.35%)	7.20 ± 0.29 (-4%)
AD+ rivastigmin.	591.17±52.6[b] (-18.12%)*	6.90 ± 0.17[b] (24.3%)*
AD+ Compound **7a**	643.07±37.7 (-10.93%)* (8.77%)**	5.80 ± 0.12[c] (4.5%)* (-15.9%) **
AD+Compound **13c**	612.26±62.7[b] (-15.19%)* (3.56%)**	6.30 ± 0.16[b] (13.5%)* (-8.6%)* *

Data were expressed as means ±standard Error for 8 animals /group.
a: P<0.05 vs. the control group.
b: P<0.05 vs. AD group.
c: P <0.05 vs. AD+rivastigmine-treated group.
% percent of change with respect to the corresponding control value.
*% *percent of change with respect to AD group.*
*% **percent of change with respect to rivastigmine-treated group.*

The data in Table (2) demonstrated the effect of treatment with rivastigmine and compounds **7a** and **13c** on brain glutathione reduced (GSH) level and paraoxenase activity and urinary 8-hydroxy guanosine (8-OHG) level in AD-bearing rats. The present findings revealed that $AlCl_3$ administration produced significant reduction (P<0.05) in brain GSH level (-50.42%) paralled with insignificant (P>0.05) decline in brain paraoxenase (-14.55%) activity while, it induced significant increase (P<0.05) in urinary 8-OHG level (176.59%) when compared with the control group. Earlier *in vitro* and *in vivo* studies demonstrated the increased reactive oxygen species (ROS) including H_2O_2 production in different brain areas as a consequence of Al administration [51]. It can be speculated that Al-induced ROS production may be one of the underlying mechanisms for the onset of Al-induced neurotoxicity [52]. It has been found that Al could induce depletion of glutathione (GSH) due to its consumption to overcome the excessive oxidative stresss produced by Al [53]. High accumulation of aluminum in the brain areas decreases the activities of the antioxidant enzymes and increases the level of lipid peroxidation, leading to neurotoxicity [54].

Administration of compound **7a** in healthy rats caused significant decrease (P<0.05) in brain GSH level (-37.60%) while administration of compound **13c** led to insignificant decrease (P>0.05) in brain GSH level (-11.96%) relative to the control counterparts.

Treatment of AD-bearing rats with rivastigmine or either one of the tested compounds (**7a** or **13c**) resulted in significant increase (P<0.05) in brain GSH level (86.20% for rivastigmine; 46.55% for compound **7a** and 67.24% for compound **13c**) as compared to untreated AD group. Meanwhile, treatment of AD- bearing rats with compound **7a** resulted in significant decline (P<0.05) in brain GSH level (-21.29%) when compared with rivastigmine-treated group. Interestingly, the treatment of AD- bearing rats with compound **13c** elicited insignificant reduction (P>0.05) in brain GSH level (-10.18%) with respect to rivastigmine-treated group.

Healthy rats administered compound **7a** showed significant decrease (P<0.05) in brain paraoxenase activity (-28.61%) while, those administered with compound **13c** revealed insignificant decrease(P>0.05) in brain paraoxenase activity (-21.48%) with respect to the control counterparts.

Treatment of AD- bearing rats with rivastigmine or either one of the tested compounds (**7a** or **13c**) recorded insignificant increase (P>0.05) in brain paraoxenase activity (14.85% for rivastigmine; 5.71% for compound **7a** and

8.91% for compound **13c**) when compared with untreated AD group. In comparison with rivastigmin –treated group, the treatment of AD-bearing rats with any one of the tested compounds produced insignificant decrease (P>0.05) in brain paraoxenase activity (-7.96%for compound **7a** and -5.17%for compound **13c**) .

The data in Table (2) also indicated that the administration of healthy rats with compound **7a** caused significant increase (P<0.05) in urinary8-OHG level (38.68%) while insignificant increase (P>0.05) in urinary 8-OHG level was recorded upon administration of healthy rats with compound **13c** (20.50%) verses the control counterparts.

Treatment of AD-bearing rats with rivastigmine or compound **13c** resulted in significant reduction (P<0.05) in urinary 8-OHG level (-55.52% for rivastigmine, -24.33% for compound **13c**) when compared with untreated AD group. Meanwhile, treatment of AD-bearing rats with compound **7a** led to insignificant reduction (P>0.05) in urinary 8-OHG level (-7.69%) as compared to untreated AD group.

In comparison with rivastigmin-treated group, treatment of AD- bearing rats with either one of the tested compounds induced significant increase (P<0.05) in urinary 8-OHG level (107.54% for compound **7a** and 70.12% for compound **13c**).

Concerning these effect of compound **7a**, it could be attributed to curcumin which is a well known antioxidant and it possesses a strong free radical scavenging capacity, that is implicated in its various biological activity [55]. Moreover, benzothiophene in compound **7a** has been extremely exploited for its antioxidants activity [56].

Regarding the activity of compound **13c**, steroid moiety is in this compound considered as antioxidant candidate as it has capability to upregulate the expression of the antioxidant enzymes *via* intracellular signalling pathways [57].In addition the synthesized thiazole derivatives have been reported to have high affinity to scavenge free radicals preventing damage of proteins and lipids [58]. Pyrimidine derivatives as well showed their power to accept free radicals due to the presence of NH group [59].Taken together, the above mentioned activity for the tested synthesized compounds accounts for their ability to enhance brain antioxidant capacity and reduce oxidative stress in the brain of AD- bearing rats.

The observed effect of rivastigmin on the oxidant/antioxidant homeostasis of the brain in AD-bearing rats might be due to that rivastigmin could reverse the biochemical impairments particularly oxidative stress parameters caused by Al as reported by Bihaqi et al [60]. It has been found that the alterations in the antioxidant defence system like GSH levels were brought to normal by rivastigmine treatment [61]. Moreover, it has been reported that paraoxonase activity showed better clinical response to acetylcholinesterase inhibitors [62]. This means that rivastigmine possesses powerful antioxidant and free radical scavenging properties.

Table (2): Effect of treatment with rivastigmine and the newly synthesized compounds on oxidant/antioxidant parameters in AD-bearing rats

Groups	GSH (mg / g brain tissue)	Paraxosnase (ku/mg protein)	8-OHG (ng/mg creatinine)
Cont	1.17±0.08	10.24±0.49	5.17±0.16
AD	0.58±0.039[a] (-50.42%)	8.75±0.67 (-14.55%)	14.3±0.51[a] (176.59%)
Compound **7a**	0.73±0.047[a] (-37.60%)	7.31±0.66[a] (-28.61%)	7.17±0.006[a] (38.68%)
Compound **13c**	1.03±0.081 (-11.96%)	8.04±0.73a (-21.48%)	6.23±0.62 (20.50%)
AD+ rivast.	1.08±0.043 [b] (86.20%)*	10.05±0.82 (14.85%)*	6.36±0.82[b] (-55.52%)*
AD+ Compound **7a**	0.85±0.028 [bc] (46.55%)* (-21.29%) **	9.25±0.39 (5.71%)* (-7.96%) **	13.2±0.32[c] (-7.69%)* (107.54%) **
AD+Compound **13c**	0.97±0.042 [b] (67.24%)* (-10.18%) **	9.53±0.41 (8.91%)* (-5.17%) **	10.82±0.51[bc] (-24.33%)* (70.12%) **

Data were expressed as means ±Standard Error for 8 animals /group.
a: P<0.05 vs. the control group.
b: P<0.05 vs. AD group.
c: P <0.05 vs. AD+rivastigmine-treated groups.
% percent of change with respect to the corresponding control value.
*% *percent of change with respect to AD group.*
*% **percent of change with respect to rivastigmine-treated group.*

The results in Table (3) spoke for the influence of treatment with rivastigmine and the tested compounds (**7a** and **13c**) on brain apoptotic/antiapoptotic markers in AD- bearing rats.

The current results revealed that $AlCl_3$ administration produced significant (P<0.05) increase in brain caspase-3 (26.92%) and P53 (47.33%) levels paralleled by significant decrease (P<0.05) in brain BCL2 level (-49.36%) when compared with the control group. Al has been found to have capability to induce apoptosis and produce neurodegenerative impact on the brain *in vivo* [63] and *in vitro* [64]. Hence, Al could enhance the proapoptotic markers (caspase-3 and P53) and reduce the anti-apoptotic mediator (BCL2) levels in the brain of rats as shown in the present work.

Administration of healthy rats with either one of the tested compounds (**7a** or **13c**) caused insignificant increase (P>0.05) in brain caspase-3 level (5.12%for compound **7a** and 3.84% for compound **13c**) versus the control counterparts.

Treatment of AD- bearing rats with rivastigmin elicited significant reduction (P<0.05) in brain caspase-3 level (-20.20%) relative to untreated AD group. Meanwhile, the treatment of AD- bearing rats with either one of the selected compounds (**7a** or **13c**) produced insignificant reduction (P>0.05) in brain caspase-3 level (-6.06%for compound **7a** and -14.14%for compound **13c**) as compared to untreated AD group.

In comparison with rivastigmine-treated group, the treatment of AD- bearing rats with either one of the tested compounds (**7a** or **13c**) caused insignificant increase (P>0.05) in brain caspase-3 level (17.72% for compound **7a** and 7.59%for compound **13c**).

The results in Table (3) also revealed that the administration of healthy rats with either one the tested compounds (**7a** or **13c**) caused significant increase (P<0.05) in P53 level (43.29%for compound **7a** and 37.67% for compound **13c**) with respect to the control counterparts.

Treatment of AD- bearing rats with rivastigmine produced significant reduction (P<0.05) in brain P 53 level (-22.14%) versus the untreated AD group. Meanwhile, treatment of AD- bearing rats with either one of the tested compounds (**7a** or **13c**) led to insignificant decrease (P>0.05) in P 53 level (-0.54% for compound **7a** and -12.06 for compound **13c**) with respect to the untreated AD group.

On the other hand, treatment of AD- bearing rats with compound **7a** elicited significant increase (P<0.05) in P 53 level (27.74%) relative to rivastigmine-treated group. Meanwhile, the treatment of AD- bearing rats with compound **13c** induced insignificant increase (P>0.05) in P 53 level (12.95%) versus rivastigmine-treated group.

The data in Table (3) also revealed that administration of healthy rats with compound **7a** caused significant decrease (P<0.05) in brain BCL2 level (-35.44%) with respect to the control counterparts. Meanwhile, the administration of healthy rats with compound **13c** recorded insignificant decrease (P>0.05) in brain BCL2 level (-12.11%) with respect to the control ones.

Table (3): Effect of treatment with rivastigmine and the newly synthesized compounds on brain pro-apoptotic and anti-apoptotic markers in AD- bearing rats

Groups	Caspase-3 (ng/mg protein)	P 53 (ng/mg protein)	BCL2 (ng/mg protein)
Control	0.78±0.03	61.9±0.38	5.53±0.34
AD	0.99±0.004[a] (26.92%)	91.2±2.6[a] (47.33%)	2.80±0.34[a] (-49.36%)
Compound **7a**	0.82±0.075 (5.12%)	88.7±6.64[a] (43.29%)	3.57±0.32[a] (-35.44%)
Compound **13c**	0.81±0.045 (3.84%)	84.6±6.9[a] (37.67%)	4.86±0.19 (-12.11%)
AD+ rivastigmin.	0.79±0.031[b] (-20.20%)*	71.0±1.97[b] (-22.14%)*	5.39±0.2[b] (92.5%)
AD+ Compound **7a**	0.93±0.052 (-6.06%)* (17.72%) **	90.7±3.18[c] (-0.54%)* (27.74%) **	3.63±0.069[bc] (29.64%)* (-32.65%) *
AD+Compound **13c**	0.85±0.095 (-14.14%)* (7.59%) **	80.2±6.64 (-12.06%)* (12.95%) **	4.76±0.19[b] (70%)* (-11.68%) *

Data were expressed as means ±standard Error for 8 animals /group.
a: P<0.05 vs. the control group.
b: P<0.05 vs. AD group.
c: P <0.05 vs. AD+rivastigmine -treated group.
% percent of change with respect to the corresponding control value.
*% *percent of change with respect to AD group.*
*% **percent of change with respect to rivastigmine-treated group.*

Treatment of AD- bearing rats with rivastigmine or either one of the tested compounds (**7a** or **13c**) resulted in significant increase (P<0.05) in brain BCL2 level (92.5% for rivastigmine, 29.64%for compound **7a**, and 70% for compound **13c**) as compared to the untreated AD group. However, in comparison with rivastigmine- treated group, the treatment of AD- bearing rats with compound **7a** recorded significant reduction (P<0.05) in brain BCL2 level (-32.65%) while, the treatment of AD-bearing rats with compound **13c** caused insignificant reduction (P>0.05) in brain BCL2 level (-11.68%) relative to rivastigmine -treated group.

Curcumin (as present in compound **7a)** has been reported to have apoptotic inhibitory activity *via* antiapoptotic effect by downregulating P 53 level [65]. Additionally, K channel blockers like 4-AP are likely to be very important for protecting neurons from apoptosis by enhancement of K effluxes from intracellular sources [66]. Similarly, steroid moiety (as in compound **13c**) is known to be neuroprotective agent protecting neurons against cell death due to different neurotoxic substances[67].Regarding the antiapoptotic effect of rivastigmin as shown in the present work, it has been reported that the expression level of BCL 2 increased with ACHEIs treatment [68]. The blockade of voltage-activated K currents by rivastigmine may lead to the suppression of apoptosis and substantial increase in cell survival [69].

Immunohistochemical investigation of ChAT in the brain

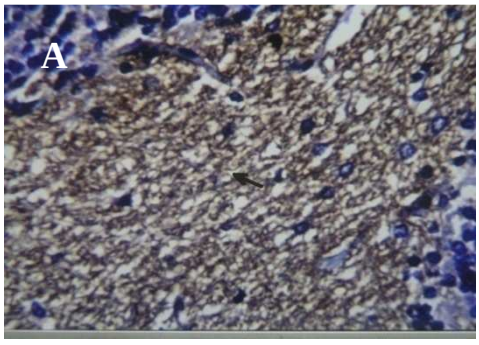
Photomicrograph of brain tissue section of rat in the control group showed positive reaction for cholineacetyltransferase (ChAT) (Fig.1A). Photomicrograph of brain tissue section of rat in AD group showed negative reaction for ChAT (Fig.1B). Al has been known to be cholinotoxic acts by blocking the provision of acetyl CoA which is required for ACh synthesis [70]. Also, Al displayed neurotoxicity by inhibiting the activity of the biosynthetic enzyme ChAT [71].

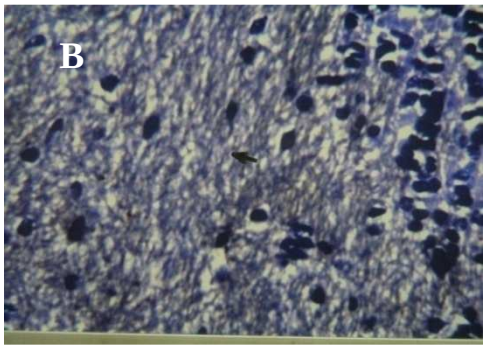
Photomicrograph of brain tissue section of healthy rat administered compound **7a** showed positive reaction for ChAT (Fig.1C). Similarly, photomicrograph of brain tissue section of healthy rat administered compound **13c** revealed positive reaction for ChAT (Fig.1D).

Photomicrograph of brain tissue section of AD- bearing rat treated with compound **7a** showed positive reaction for ChAT (Fig.1E). It was reported that benzothiophene derivatives (Raloxifene) increased ChAT activity in the hippocampus [72]. In addition curcumin (present in compound **7a**) could increased cholineacetyltransferase (ChAT) activity in the hippocampus [73].

Photomicrograph of brain tissue section of AD- bearing rat treated with compound **13c** showed positive reaction for ChAT (Fig.1F). Research studies have suggested that steroid moiety (found in compound **13c**) might improve cognitive functions *via* stimulating cholinergic neurons to produce Ach *via* activiation of ChAT [74].

Photomicrograph of brain tissue section of AD- bearing rat treated with rivastigmine showed positive reaction for ChAT (Fig.1G). The underlying mechanisms for rivastigmine to compensate cholinergic deficits include the downregulation of the destruction of acetylcholine and the upregulation of the expression of ChAT enzyme [75].





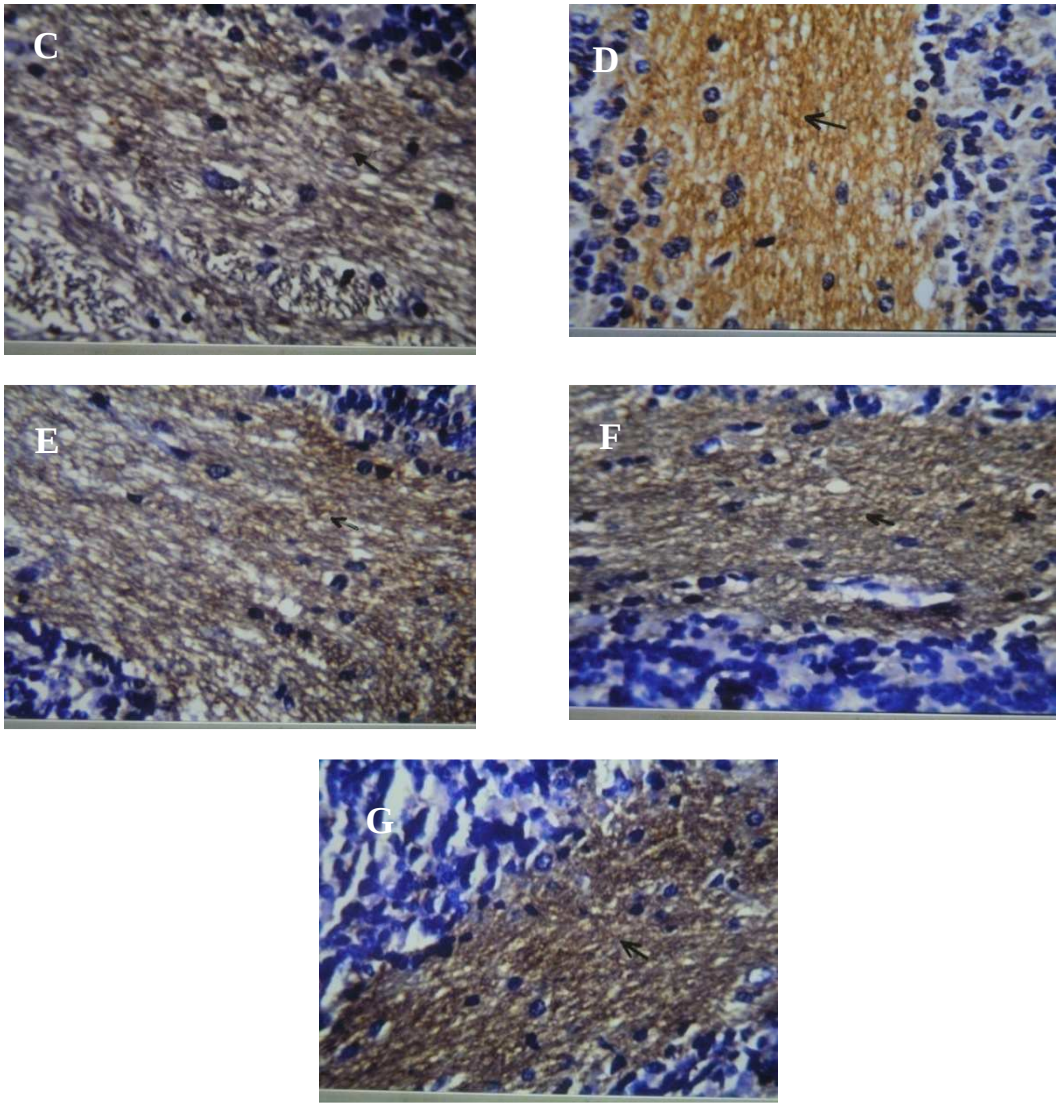


Fig 1:Immunohistochemical examination of ChAT expression in rat brain: (A) control group, (B) AD group, (C) group administered compound 7a, (D) group administered compound 13c, (E) AD + compound 7a group, (F) AD + compound 13c group, (G) AD + rivastigmine group

Histological examination of the brain

Microscopic examination of brain tissue section of rat in the control group showed no histopathological alteration and the normal histological feature of the meninges, cerebral cortex (cc), cerebrum striatum (c) (Fig.2A), hippocampus (hp) (Fig., 2B) and cerebellum (cr) (Fig. 2C) has been observed.

Microscopic investigation of brain tissue section of rat in AD group showed multiple numbers of eosinophilic plaque formation (p) and the diffused glia cells proliferation in between (arrow) is detected in the striatum of cerebrum (Fig.2D). These findings are in agreement with those of Salem et al who found that Al administration caused the formation of neuratic plaques that appeared with dark center and neuronal damage as well as degeneration in the cerebral cortex and the hippocampus [76].

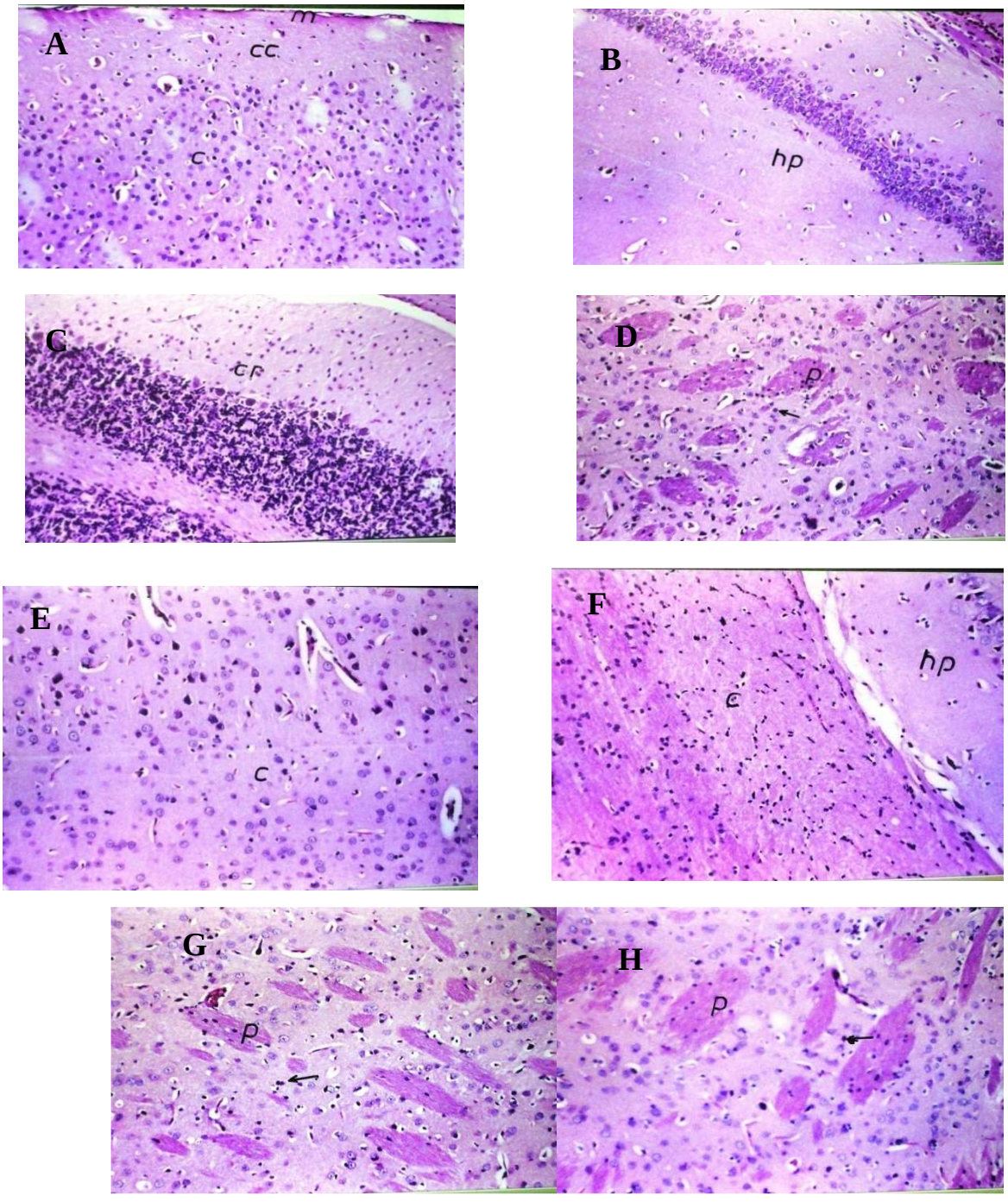
Microscopic examination of brain tissue section of healthy rat administered compound **7a** showed no histopathological alteration in the striatum of cerebrum (c) (Fig.1E). Microscopic investigation of brain tissue section of healthy rat administered compound **13c** showed no histopathological alteration and the normal structural organization of the cerebrum striatum (c) and hippocampus (hp) has been observed (Fig.1F).

Microscopic examination of brain tissue section of rat in AD group treated with compound **7a** revealed plaques (p) with diffuse gliosis in the cerebrum striatum (Fig.1G). Franciosi et al reported that 4-AP can block Aβ1–42 induced neurotoxicity *in vivo*. They demonstrated that peptide injection -induced significant loss of dentate granule neurons is mainly prevented in the presence of 4- AP. Importantly, their data also showed a reduced microgliosis in peptide

injected brain as aconsequence of 4- AP [77]. In addition curcumin has been shown to inhibit Aβ (1–40) fibril formation and lower its toxicity [78].

Microscopic investigation of brain tissue section of rat in AD group treated with compound **13c** showed few plaques (p) with diffuse gliosis in the cerebrum striatum (Fig.1H). Functionalization of pyrimidine ring is necessary for strong interaction with β and γ secretases receptor. Furthermore, such chemical transformations could facilitate the formation of hydrogen bonding, thus reduced β-amyloid (Aβ) formation [79]. Moreover, it has been reported that, steroid moiety in compound **13c** possesses antiamyloidogenic activity [80].

Microscopic investigation of brain tissue section of rat in AD group treated with rivistagmin showed that there was focal gliosis (g) in the cerebrum (Fig.1I). Rivastigmine has been known to possess considered neuroprotective and neurorestorative activity in the brain [48].



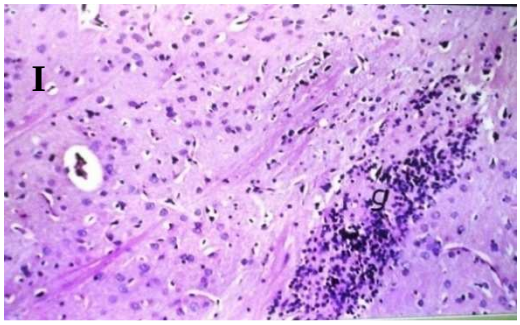


Fig (2): Photomicrograph of brain tissue section of rat in control group (A, B and C), AD group (D), compound 7a administered group (E), compound 13c administered group (F), AD + compound 7a group(G), AD + compound 13c group (H), AD + rivastigmine group (I).

CONCLUSION

In conclusion, the modification of curcumin moiety by incorporating heterocyclic rings enhances its activity against neurodegeneration in AD model .Also fusion of heterocyclic rings in steroid molecule improves its potential towards neurodeterioration as manifested by our biological investigation. The present bioassay findings offer a multi-mechanistic explanation of the novel synthesized compounds-induced regression of neurodegeneration characterized AD in the experimental animal. Our results clearly identify that the modulation of the neurodeterioration by the newly synthesized compounds is contributed to acetylcholinesterase inhibitor activity, anti-oxidative property and anti-apoptotic potential. Finally, the anti-Alzheimer's disease activity displayed by these compounds may be of interest for further derivatization, further studies in the hope of finding more active and selective anti-Alzheimer's disease agents.

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