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One-pot three-component synthesis of novel heterocyclic steroids as a central antioxidant and anti-inflammatory agents

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ABSTRACT

Oxidative stress and inflammation have been implicated in several neurodegenerative and developmental brain disorders. The present work was devoted to the design and synthesis of novel steroid derivatives bearing promising heterocyclic moiety that would act to reduce neuro-inflammation and oxidative stress in brain. The novel heterocyclic steroids were synthesized and their chemical structures were confirmed by studying their analytical and spectral data. The tested compounds were assayed in the model of neuro-inflammation produced in rats by cerebral lipopolysacharide injection. The intracerebral administration of bacterial endotoxin resulted in cerebral inflammatory state evidenced by increased malondialdehyde (MDA), decreased reduced glutathione (GSH) level, increased nitric oxide as well as increased acetylcholinesterase (AChE) activity in the brain. Compounds **6**, **10**, **8b** and **13a** markedly increased reduced glutathione. Malondialadehyde and nitric oxide levels were reduced to normal values after treatment with all tested compounds. AChE activity was normalized by compound **8b** and reduced to below normal values by compounds **10** and **14a**. These results are exciting in that these agents might be useful candidates in treatment of cerebral inflammation.

1. Introduction

Multi component reactions (MCRs) have emerged an efficient method in synthetic organic chemistry, forcing the facile formation of several new bonds in one-pot reactions. In the past decade, research in academia and industry has increasingly emphasized the use of MCRs as well as domino reactions sequences for a broad range of products [1–3]. This is return to their convergence, productivity, ease of execution and generally high yields of products [4]. Pyridines generate a wide spread interest due to diverse pharmacological activities [5,6]. Pyrimidines owing to their natural occurrence in human in human genetic material are especially important as anti-inflammatory agents [7,8]. Thus fused pyridopyrimidines are associated with numerous biological activities [9,10]. This is responsible for the abiding interest in this class of compounds.

Oxidative stress and inflammation have been implicated in several neurodegenerative and developmental brain disorders. The pathogenesis of Alzheimer's disease, the most common form of dementia involves increased production and deposition of amyloid β-peptide in the brain [11]. Increased levels of lipid peroxidation products have been detected in brains of patients with Alzheimer's disease [12]. The main molecular mechanisms leading to neuronal cell death in Parkinson's disease are thought to be oxidative stress and mitochondrial dysfunction and the abnormal accumulation and processing of mutant or damaged proteins due to gene-environmental interactions [13]. Inflammation appears to be an important contributor to neuronal injury [14]. Multiple sclerosis the most common chronic inflammatory demyelinating disease is also associated with increased generation of reactive oxygen species [15]. Therefore, there is an immense need of findings new drugs that would decrease brain oxidative stress and alleviate neuroinflammation and/or neurodegenerative process. In light of the above discoveries and in continuation to our previous work [16–18], the present study investigate the use the MCRs technique for synthesizing pyridines and pyridopyrimidines on steroidal frame work. To our knowledge and according the literature survey, this is the first use of MCRs on steroids. The compounds under study were tested against oxidative stress and neuro-inflammation caused by cerebral injection of lipopolysacharide endotoxin. The

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administration of this bacterial wall endotoxin into the rat brain induced secretion of cytokines, causes brain inflammation and neuronal damage [19].

2. Experimental section

2.1. Synthetic methods, analytical and spectral data

Starting steroids, epi-androsterone, 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 ^1H NMR and ^{13}C NMR spectra were recorded with Jeol instrument (Japan), at 270 and 125 MHz, respectively, in DMSO-d_6 or $CDCl_3$ 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, q-quartet 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 described compounds showed the characteristic spectral data of cyclopentanoperhydrophenanthrene nuclei of androstane series were similar to those reported in literature [20,21]. For the nomenclature of steroid derivatives, we used the definitive rules for the nomenclature of steroids published by the Joint Commission on the Biochemical Nomenclature (JCBN) of IUPAC [22,23].

2.1.1. 2′-Amino-3β-hydroxy-4′H-4′-phenyl-5α-androstan[16,17:5′,6′]pyridin-3′-carbonitrile (**4**), 2′-amino-3β-hydroxy-4′H-4′-phenyl-5α-androstan[16,17:5′,6′]pyran-3′-carbonitrile (**6**)

2.1.1.1. General procedure. A solution of epi-androsterone **1** (1.45 g, 5 mmol), benzaldehyde **2** (0.53 g, 5 mmol) and malononitrile **3** (0.33 g, 5 mmol) in ethanol (10 ml) containing ammonium acetate (0.98 g, 2% excess) or piperidine (1 ml) was heated under reflux for 4–5 h until all starting materials had disappeared as indicated by TLC. The oil product that formed in each case on removal of the solvent in vacuum, was solidified by boiling in petroleum ether (60–80 °C), collected by filtration, dried and crystallized from the appropriate solvent.

Compound ***4***: Yellow crystals from absolute ethanol, mp 103–105 °C, yield 85%, IR (KBr, cm^{-1}): υ = 3654–3469 (OH, NH_2, NH), 3037 (CH-aromatic), 2930, 2859 (CH-aliphatic), 2224 (CN), 1583 (C═C). ^1H NMR (DMSO-d_6, ppm): δ = 0.81 (s, 3H, CH_3-19), 1.12 (s, 3H, CH_3-18), 1.53 (m, 1H, C_5-αH), 2.02 (s, 1H, OH, D_2O-exchangeable), 3.24 (m, 1H, C_3-αH), 4.50 (s, 1H, C-4 pyridine), 6.82 (s, 2H, NH_2), 7.24–7.49 (m, 5H, aromatic-H), 8.09 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (DMSO-d_6, ppm): δ = 31.29 (C-1), 30.32, (C-2), 72.85 (C-3), 34.43 (C-4), 38.50 (C-5), 27.11 (C-6), 27.86 (C-7), 35.31 (C-8), 50.56 (C-9), 44.00 (C-10), 21.34 (C-11), 34.3 (C-12), 38.80 (C-13), 47.61 (C-14), 120.18 (C-15), 111.64 (C-16), 134.30 (C-17), 19.32 (C-18), 20.02 (C-19), 44.67, 57.81, 162.31 (C-pyridine), 117.54 (CN), 129.19, 128.76, 142.31, 125.51 (C-phenyl). M.S (EI): *m/z* (%): 443 (M^+·, 33), 376 (87), 260 (16), 193 (46), 77 (100). Calc. for $C_{29}H_{37}N_3O$ (443.624): C, 78.51; H, 8.41; N, 9.47; found: C, 78.20; H, 7.24; N, 9.21%.

Compound ***6***: Pale brown crystals from absolute ethanol, mp 193–195 °C, yield 76%, IR (KBr, cm^{-1}): υ = 3563–3354 (OH, NH_2), 3032 (CH-aromatic), 2942, 2878 (CH-aliphatic), 2222 (CN), 1613 (C═C). ^1H NMR (DMSO-d_6, ppm): δ = 0.76 (s, 3H, CH_3-19), 1.15 (s, 3H, CH_3-18), 1.50 (m, 1H, C_5-αH), 2.52 (s, 1H, OH, D_2O-exchangeable), 3.19 (m, 1H, C_3-αH), 4.57 (s, 1H, C-4 pyrane), 6.65 (s, 2H, NH_2), 7.22–7.54 (m, 5H, aromatic-H). ^{13}C NMR ($CDCl_3$, ppm): δ = 31.18 (C-1), 30.32, (C-2), 70.95 (C-3), 34.53 (C-4), 39.25 (C-5), 27.14 (C-6), 27.80 (C-7), 34.71 (C-8), 50.36 (C-9), 44.00 (C-10), 22.34 (C-11), 35.78 (C-12), 38.80 (C-13), 48.41 (C-14), 27.04 (C-15), 110.92 (C-16), 149.30 (C-17), 19.52 (C-18), 20.22 (C-19), 43.07, 58.31, 159.34 (C-pyrane), 117.54 (CN), 129.32, 128.72, 142.41, 125.50 (C-phenyl). M.S (EI): *m/z* (%): 444 (M^+, 56), 248 (23), 196 (74), 156 (29), 77 (100). Calc. for $C_{29}H_{37}N_2O_2$ (444.608): C, 78.34; H, 8.16; N, 6.30; found: C, 78.56; H, 7.87; N, 6.17%.

2.1.2. 3β-Hydroxy-3′,5′,8′-trihydro-5′-phenyl-5α-androstan[16,17:6,7′]pyrido[2′,3′-d]pyrimidin-4′-one (**8a**), 3β-hydroxy-3′,5′,8′-trihydro-2′-methyl-5′-phenyl-5α-androstan[16,17:6,7′]pyrido[2′,3′-d]pyrimidin-4′-one (**8b**), 3β-hydroxy-3′,5′,8′-trihydro-2′-(chloromethyl)-5′-phenyl-5α-androstan[16,17:6,7′]pyrido[2′,3′-d]pyrimidin-4′-one (**8c**)

2.1.2.1. General procedure. A suspension of compound **4** (0.44 g, 1 mmol) in formic acid, acetic acid or chloroacetic acid (5 ml) was heated under reflux for 4–6 h until all starting materials had disappeared as indicated by TLC. The reaction mixture was evaporated under vacuum and the remaining residue was treated with ice/water mixture, neutralized with KOH (10%). The obtained solid product was collected by filtration, dried and crystallized from the appropriate solvent.

Compound ***8a***: Yellow crystals from absolute ethanol, mp 113–115 °C, yield 68%, IR (KBr, cm^{-1}): υ = 3560–3442 (OH, 2NH), 3028 (CH-aromatic), 2928, 2865 (CH-aliphatic), 1680 (CO), 1596 (C═C). ^1H NMR (DMSO-d_6, ppm): δ = 0.78 (s, 3H, CH_3-19), 1.14 (s, 3H, CH_3-18), 1.46 (m, IH, C_5-αH), 2.52 (s, 1H, OH, D_2O-exchangeable), 3.35 (m, 1H, C_3-α H), 4.53 (s, IH, C-5 pyridine), 7.06–7.18 (m, 5H, aromatic-H), 7.52 (s, IH, C-2 pyrimidine), 8.09, 8.85 (2s, 2H, 2NH, D_2O-exchangeable). ^{13}C NMR (DMSO-d_6, ppm): δ = 32.29 (C-1), 32.52 (C-2), 71.85 (C-3), 37.43 (C-4), 38.50 (C-5), 27.11 (C-6),27.60 (C-7), 38.31 (C-8),52.56 (C-9), 45.00 (C-10), 22.34 (C-11), 34.30 (C-12), 39.80 (C-13), 50.61 (C-14), 28.18 (C-15), 111.34 (C-16),134.30 (C-17), 20.32 (C-18), 20.02 (C-19), 33.70 (C-5 pyridine), 100.07, 163.21, 150.74, 157.28 (C-pyrimidine), 129.29, 128.70, 142.30, 125.25 (C-phenyl). M.S (EI): *m/z* (%): 470 (M^+-l, 67), 248 (12), 223 (29), 131 (67), 94 (100), 77 (58). Calc. for $C_{30}H_{37}N_3O_2$ (471.634): C, 76.40; H, 7.91; N, 8.91; found: C, 76.14; H, 8.16; N, 9.20%.

Compound ***8b***: Brown crystals from absolute ethanol, mp 178–180 °C, yield 73%, IR (KBr, cm^{-1}): υ = 3558–3440 (OH, 2NH), 3027 (CH-aromatic), 2930, 2864 (CH-aliphatic), 1687 (CO), 1595 (C = C). ^1H NMR (DMSO-d_6, ppm): δ = 0.78 (s, 3H, CH_3-19), 1.17 (s, 3H, CH_3-18), 1.39 (m, 1H, C_5-α H), 2.32 (s, 1H, OH, D_2O-exchangeable), 3.20 (m, 1 H, C_3-αH), 4.43 (s, 1 H, C-5 pyridine), 7.08–7.22 (m, 5H, aromatic-H), 8.29, 8.93 (2s, 2H, 2NH, D_2O-exchangeable). ^{13}C NMR (DMSO-d_6, ppm): δ = 32.12 (C-1), 32.42, (C-2), 71.25 (C-3), 37.40 (C-4), 38.65 (C-5), 27.00 (C-6), 27.16 (C-7), 38.30 (C-8), 52.46 (C-9), 44.28 (C-10), 22.34 (C-11), 34.30 (C-12), 40.28 (C-13), 50.76 (C-14), 27.28 (C-15), 111.04 (C-16), 133.70 (C-17), 20.52 (C-18), 21.02 (C-19), 33.75 (C-5 pyridine), 100.16, 163.20, 150.74, 157.34 (C-pyrimidine), 129.29, 128.70, 142.30, 125.53 (C-phenyl). M.S (EI): *m/z* (%): 485 (M^+, 34), 248 (84), 238 (34), 131 (100), 108 (24), 77 (65). Calc. for $C_{31}H_{39}N_3O_2$ (485.304): C, 76.67; H, 8.09; N, 8.65; found: C, 76.84; H, 8.36; N, 8.42%.

Compound ***8c***: Yellowish brown powder from 1,4-dioxane, m.p. 192–193 °C, yield 77%, IR (KBr, Cm^{-1}): υ = 3567–3446 (OH, 2NH), 3032 (CH-aromatic), 2945, 2860 (CH-aliphatic), 1693 (CO), 1607 (C = C). ^1H NMR (DMSO-d_6, ppm): δ = 0.83 (s, 3H, CH_3-19), 1.23 (s, 3H, CH_3-18), 1.40 (m, 1H, C_5-α H), 2.27 (s, 1 H, OH, D_2O-exchangeable), 3.18 (m, 1H, C_3-αH), 3.47 (s, 2H, CH_2Cl), 4.40 (s, 1H, C-5 pyridine), 7.06–7.25 (m, 5H, aromatic-H), 8.34, 8.98 (2s,

2H, 2NH, D_2O-exchangeable). ^{13}C NMR (DMSO-d_6, ppm): δ = 32.52 (C-1), 32.40, (C-2), 71.30 (C-3), 37.40 (C-4), 37.35 (C-5), 27.30 (C-6), 27.76 (C-7), 38.53 (C-8), 52.42 (C-9), 45.08 (C-10), 22.31 (C-11), 35.70 (C-12), 45.29 (C-13), 50.46 (C-14), 28.18 (C-15), 111.24 (C-16), 134.20 (C-17), 20.72 (C-18), 21.12 (C-19), 33.75 (C-5 pyridine), 100.12, 162.20, 164.34, 157.30 (C-pyrimidine), 49.74 (CH_2-Cl), 129.03, 128.74, 142.30, 125.43 (C-phenyl). M.S (El): *m/z* (%): 520 (M^+, 33), 272 (17), 247 (54), 141 (100), 91 (24). Calc. for $C_{31}H_{38}N_3O_2Cl$ (520.105): C, 71.59; H, 7.36; N, 8.08; found: C, 71.82; H, 7.13; N, 8.29%.

*2.1.3. 3β-Hydroxy-3′,5′,8′-trihydro-2′-methyl-5′-phenyl-5a-androstan [16,17:6′,7′]pyrido[2′,3′-d]pyrimidin-4′-one (**8b**), 3β-Hydroxy-3′,5′,8′-trihydro-2′,5′-phenyl-5a-androstan[16,17:6′,7′]pyrido[2′,3′-d]pyrimidin-4′-one (**9**)*

2.1.3.1. General procedure. To a solution of compound **4** (0.44 g, 1 mmol) in dimethyl formamide (DMF) (20 mL), equimolar amount of acetyl chloride (0.078 g, 1 mmol) or benzoyl chloride (0.14 g, 1 mmol) was added dropwise with stirring. After complete addition, the reaction mixture was refluxed for 6–8 h until all the starting materials had disappeared as indicated by TLC. The reaction mixture, then left to cool at room temperature, poured over crushed ice and left in a refrigerator at 4 °C overnight. The formed solid product, in each case, was collected by filtration and crystallized from the appropriate solvent.

Data of compound **8b** from this method is a fingerprint to that of the preceding method.

*Compound **9**:* Pale brown crystals from 1,4-dioxane, m.p. 213–214 °C, yield 74%, IR (KBr, cm^{-1}): υ = 3546–3475 (OH, 2NH), 3030 (CH-aromatic), 2986, 2845 (CH-aliphatic), 1697 (CO), 1587 (C=C). ^1H NMR ($CDCl_3$, ppm): δ = 0.87 (s, 3H, CH_3-19), 1.19 (s, 3H, CH_3-18), 1.42 (m, IH, C_5-αH), 2.07 (s, 1H, OH, D_2O-exchangeable), 3.27 (m, 1H, C_3-αH), 4.47 (s, 1H, C-5 pyridine), 7.14-7.35 (m, 10H, aromatic-H), 8.54, 9.20 (2s, 2H, 2NH, D_2O-exchangeable). ^{13}C NMR ($CDCl_3$, ppm): δ = 32.42 (C-l), 32.57, (C-2), 71.34 (C-3), 37.92 (C-4), 37.05 (C-5), 27.43 (C-6), 28.06 (C-7), 38.50 (C-8), 52.56 (C-9), 45.28 (C-10), 23.01 (C-11), 35.72 (C-12), 45.29 (C-13), 50.74 (C-14), 28.68 (C-15), 111.04 (C-16), 134.20 (C-17), 20.52 (C-18), 20.72 (C-19), 33.70 (C-5 pyridine), 100.12, 162.20, 161.30, 157.35 (C-pyrimidine), 129.03, 128.24, 142.30, 125.43, 126.43, 128.98, 128.71, 130.04 (C-phenyl). M.S (EI): *m/z* (%): 547 (M^+, 38), 299 (27), 248 (34), 131 (100), 170 (24), 77 (46). Calc. for $C_{36}H_{41}N_3O_2$ (547.730): C, 78.94; H, 7.54; N, 7.67; found: C, 78.72; H, 7.73; N, 7.39%.

*2.1.4. 3β-Hydroxy-1′,3′,5′,8′-tetrahydro-5′-phenyl-5α-androstan [16,17:6′,7′]pyrido[2′,3′d]pyrimidin-2′,4′-dithione (**10**)*

To a solution of compound **4** (0.44 g, 1 mmol) in 15 mL of ethanol/KOH (10%), excess of carbon disulphide was added. The reaction mixture was heated under reflux for 4 h until all the starting materials had disappeared as indicated by TLC. The reaction solvent was evaporated under vacuum and the remaining residue was treated with ice/water mixture, neutralized with dilute hydrochloric acid. The obtained solid product was collected by filtration, dried and crystallized from the absolute ethanol to afford dark yellow crystals of compound **10**. m.p. 220–222 °C, yield 72%, IR (KBr, cm^{-1}): υ = 3538–3454 (OH, 3NH), 3032 (CH-aromatic), 2986, 2845 (CH-aliphatic), 1589 (C=C), 1199, 1193 (2C=S). ^1H NMR (DMSO-d_6, ppm): δ = 0.98 (s, 3H, CH_3-19), 1.23 (s, 3H, CH_3-18), 1.40 (m, 1H, C_5-α H), 2.21 (s, 1H, OH, D_2O-exchangeable), 3.12 (m, 1H, C_3-α H), 4.43 (s, 1H, C-5 pyridine), 7.06–7.15 (m, 5H, aromatic-H), 8.63 (s, 1H, NH, D_2O-exchangeable), 9.34 (s, 2H, 2NH, D_2O-exchangeable). ^{13}C NMR (DMSO-d_6, ppm): δ = 32.54 (C-1), 32.70, (C-2), 70.74 (C-3), 38.02 (C-4), 37.15 (C-5), 27.40 (C-6), 28.06 (C-7), 38.50 (C-8), 52.40 (C-9), 44.85 (C-10), 22.43 (C-11), 35.70 (C-12), 46.20 (C-13), 50.72 (C-14), 28.38 (C-15), 111.24 (C-16), 134.20 (C-17), 20.70 (C-18), 21.32 (C-19), 38.37 (C-5 pyridine), 90.32, 152.24, 181.43, 196.05 (C-pyrimidine), 129.13, 128.27, 141.13, 125.43 (C-phenyl). M.S (EI): *m/z* (%): 519 (M^+, 32), 248 (64), 271 (26), 141 (100), 131 (76), 77 (45). Calc. for $C_{30}H_{37}N_{30}S_2$ (519.764): C, 69.32; H, 7.18; N, 8.08; S, 12.34; found: C, 69.50; H, 7.01; N, 7.79; S, 12.56%.

*2.1.5. N-(3β-hydroxy-3′-cyano-1′,4′-dihydro-4′-phenyl-5a-androstan [16,17:5′,6′]pyridine-2′-yl)-N,N-dimethylformamidine (**11**)*

To a solution of compound **4** (0.88 g, 2 mmol) in acetonitrile (10 mL), dimethylformamid-dimethylacetal (0.24 g, 2 mmol) was added dropwise with stirring. After complete addition, the reaction mixture was heated in a water bath at 70 °C for 2 h. After cooling at room temperature, the reaction mixture poured over ice/ water mixture and the resulted semisolid was subjected to extraction with chloroform (2 × 30 mL). The organic layer was dried over anhydrous magnesium sulfate and then filtered. The oil product that formed on removal of the solvent in vacuum, was solidified by boiling in petroleum ether (60–80 °C), collected by filtration and crystallized from absolute ethanol to afford pale yellow powder of compound **11**, m.p. 187–189 °C, yield 80%, IR (KBr, cm^{-1}): υ = 3487–3395 (OH, NH), 3028 (CH-aromatic), 2978, 2856 (CH-aliphatic), 2225 (CN), 1576 (C = C). ^1H NMR (DMSO-d_6, ppm): δ = 0.95 (s, 3H, CH_3-19), 1.19 (s, 3H, CH_3-18), 1.43 (m, IH, C_5-αH), 2.25 (s, 1H, OH, D_2O-exchangeable), 2.47 (s, 6H, NMe2), 3.24 (m, 1H, C_3-αH), 4.40 (s, 1H, C-5 pyridine), 7.07–7.18 (m, 5H, aromatic-H), 7.85 (s, 1H, enamine CH), 8.63 (s, 1H, NH, D_2O-exchangeable). M.S (EI): *m/z* (%): 497 (M^+-1, 43), 248 (14), 250 (36), 181 (100), 77 (65), 71 (28). Calc. for $C_{32}H_{42}N_4O$ (498.702): C, 77.07; H, 8.49; N, 11.23; found: C, 76.78; H, 8.71; N, 11.39%.

*2.1.6. N-(3β-hydroxy-3′-cyano-1′,4′-dihydro-4′-phenyl-5α-androstan [16,17:5′,6′]pyridin-2′-yl)ethoxymethanamine (**13a**), N-(3β-hydroxy-3′-cyano-4′H-4′-phenyl-5α-androstan [16,17:5′,6′]pyran-2′-yl) ethoxymethanamine (**13b**)*

A mixture of compound **4** (0.88 g, 2 mmol) or compound **6** (0.88 g, 2 mmol) and triethylorthoformate (0.30 g, 2 mmol) in acetic anhydride (10 mL) was heated under reflux for 5–7 h. The reaction was controlled by TLC, after cooling at room temperature, the reaction mixture poured over an ice/ water mixture and the resulted semisolid was subjected to extraction with chloroform (2 × 30 mL). The organic layer was dried over magnesium sulfate and then filtered. The oil product that formed on removal of the solvent in vacuum was solidified by boiling in petroleum ether (60–80 °C), collected and crystallized from the appropriate solvent.

*Compound **13a**:* Brown powder from 1,4-dioxane, m.p. 117–119 °C, yield 82%, lR (KBr, cm^{-1}): υ = 3482–3395 (OH, NH), 3030 (CH-aromatic), 2984, 2867 (CH-aliphatic), 2225 (CN), 1593 (C=C). ^1H NMR ($CDCl_3$, ppm): δ = 1.04 (s, 3H, CH_3-19), 1.15 (t, 3H, ester-CH_3), 1.25 (s, 3H, CH_3-18), 1.40 (m, 1H, C_5-α H), 2.08 (s, 1H, OH, D_2O-exchangeable), 3.28 (m, 1H, C_3-αH), 3.87 (s, 2H, ester-CH_2), 4.78 (s, 1H, C-5 pyridine), 7.23–7.37 (m, 5H, aromatic-H), 7.58 (s, 1H, N=CH), 8.73 (s, 1H, NH, D_2O-exchangeable). M.S (EI): *m/z* (%): 499 (M^+, 56), 248 (34), 179 (100), 77 (65), 72 (35). Calc. for $C_{32}H_{41}N_3O_2$ (499.687): C, 76.92; H, 8.27; N, 8.41; found: C, 77.12; H, 8.02; N, 8.29%.

*Compound **13b**:* Pale brown powder from methanol, m.p. 139–141 °C, yield 77%, IR (KBr, cm^{-1}): υ = 3432 (OH), 3042 (CH-aromatic), 2979, 2854 (CH-aliphatic), 2225 (CN), 1590 (C=C). ^1H NMR (DMSO-d_6, ppm): δ = 1.02 (s, 3H, CH_3-19), 1.17 (t, 3H, ester-CH_3), 1.20 (s, 3H, CH_3-18), 1.40 (m, 1H, C_5-αH), 2.27 (s, 1H, OH, D_2O-exchangeable), 3.35 (m, 1H, C_3-αH), 4.04 (s, 2H, ester-CH_2), 4.73 (s, 1H, C-5 pyrane), 7.20–7.27 (m, 5H, aromatic-H), 7.53 (s, 1H, N=CH), 8.73 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR ($CDCl_3$, ppm): δ = 37.02 (C-l), 31.07, (C-2), 71.13 (C-3), 37.92 (C-4), 43.25 (C-5), 27.63 (C-6), 31.16 (C-7), 34.50 (C-8), 51.06 (C-9), 38.28

(C-10), 21.21 (C-11), 34.72 (C-12), 44.59 (C-13), 50.04 (C-14), 27.88 (C-15), 110.34 (C-16), 149.87 (C-17), 18.02 (C-18), 19.70 (C-19), 44.12, 76.20, 170.30 (C-pyrane), 117.23 (CN), 156.09 (CHOEt), 63.00 (CH_2-ester), 15.78 (CH_3-ester), 127.03, 128.25, 142.13, 125.53 (C-phenyl). M.S (EI): *m/z* (%): 500 (M^+, 46), 248 (24), 180 (100), 77 (45), 72 (17). Calc. for $C_{32}H_{40}N_2O_3$ (500.672): C, 76.77; H, 8.05; N, 5.60; found: C, 76.89; H, 8.22; N, 5.39%.

*2.1.7. 3′-Amino-3β-hydroxy-4′-imino-5′-phenyl-5α-androstan [16,17:6′,7′]-5′H-pyrido[2′,3′-d]pyrimidine (**14a**), 3′-amino-3β-hydroxy-4′-imino-5′-phenyl-5α-androstan[16,17:6′,7′]-5′H-pyrano [2′,3′-d]pyrimidine (**14b**)*

To a mixture of compound **13a** (0.49 g, 1 mmol) or **13b** (0.50 g, 1 mmol) and hydrazine hydrate (0.05 g, 1 mmol) in 25 mL absolute ethanol, a catalytic amount of triethyl amine was added (0.5 mL). The reaction mixture was heated to 80 °C with stirring and maintained at this temperature for 4–6 h with continued stirring. The reaction was monitored by TLC, concentrated under vacuum and the remaining residue was treated with ice/water mixture (50 mL) and neutralized with dilute hydrochloric acid. The formed solid product, in each case, was filtered off, dried and crystallized from absolute ethanol.

*Compound **14a**:* Orange powder, m.p. 172–174 °C, yield 76%, IR (KBr, cm^{-1}): υ = 3480–3375 (OH, NH, NH_2), 3042 (CH-aromatic), 2988, 2863 (CH-aliphatic), 1603 (C = C). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.06 (s, 3H, CH_3-19), 1.17 (s, 3H, CH_3-18), 1.42 (m, 1H, C_5-αH), 2.87 (s, 1H, OH, D_2O-exchangeable), 3.28 (m, 1H, C_3-αH), 4.76 (s, 1H, C-5 pyridine), 6.52 (s, 2H, NH_2), 7.23-7.33 (m, 5H, aromatic-H), 7.56 (s, 1H, C-2 pyrimidine), 8.58, 8.73 (2s, 2H, 2NH, D_2Oexchangeable). ^{13}C NMR ($CDCl_3$, ppm): δ = 37.50 (C-l), 31.17, (C-2), 71.00 (C-3), 37.02 (C-4), 42.95 (C-5), 27.32 (C-6), 31.24 (C-7), 34.50 (C-8), 53.06 (C-9), 36.28 (C-10), 21.32 (C-11), 35.72 (C-12), 45.79 (C-13), 50.24 (C-14), 29.08 (C-15), 111.04 (C-16), 134.87 (C-17), 16.52 (C-18), 19.72 (C-19), 29.31 (C-5 pyridine), 151.12, 90.20, 158.09, 143.56 (C-pyrimidine), 142.45, 127.13, 128.25, 125.73 (C-phenyl). M.S (EI): *m/z* (%): 485 (M^+, 26), 248 (19), 128 (100), 77 (65). Calc. for $C_{30}H_{39}N_5O$ (485.664): C, 74.19; H, 8.09; N, 14.42; found: C, 73.95; H, 8.30; N, 14.23%.

*Compound **14b**:* Yellow powder, m.p. 186–188 CC, yield 78%, IR (KBr, cm^{-1}): υ = 3482–3373 (OH, NH, NH_2), 3038 (CH-aromatic), 2987, 2863 (CH-aliphatic), 1608 (C═C). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.02 (s, 3H, CH_3-19), 1.13 (s, 3H, CH_3-18), 1.42 (m, 1H, C_5-αH), 2.85 (s, 1H, OH, D_2O-exchangeable), 3.34 (m, 1H, C_3-αH), 4.75 (s, 1H, C-5 pyrane), 6.67 (s, 2H, NH_2), 7.26–7.35 (m, 5H, aromatic-H), 7.60 (s, 1H, C-2 pyrimidine), 8.96 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR ($CDCl_3$, ppm): δ = 37.25 (C-l), 31.07, (C-2), 71.08 (C-3), 37.12 (C-4), 42.46 (C-5), 27.13 (C-6), 31.25 (C-7), 34.62 (C-8), 53.06 (C-9), 36.25 (C-l0), 21.22 (C-11), 35.72 (C-12), 45.82 (C-13), 50.34 (C-14), 30.28 (C-15), 111.34 (C-16), 149.85 (C-17), 18.02 (C-18), 20.32 (C-19), 28.45 (C-5 pyrane), 159.30, 90.20, 158.04, 143.56 (C-pyrimidine), 142.35 127.23, 128.15, 125.70 (C-phenyl). M.S (EI): *m/z* (%): 486 (M^+, 54), 272 (39), 214 (65), 84 (100), 77 (43). Calc. for $C_{30}H_{38}N_4O_2$ (486.648): C, 74.04; H, 7.87; N, 11.51; found: C, 73.78; H, 8.12; N, 11.28%.

2.2. Biological assay

2.2.1. Experimental animals

The experimental animals used in the present study were adult Wistar albino rats weighing 130–150 g. The animals were obtained from the animal house of the National Research Center, Egypt. They were maintained on stock diet and kept under fixed appropriate conditions of housing and handling. All experiments were carried out in accordance with the research protocols established by the animal care committee of the National Research Center - Egypt.

2.2.2. Surgical procedures

At the beginning of the experiment, the number of rats was 50. Rats were anaesthetized with sodium pentobarbital (40 mg/kg, i.p.). After shaving the hair from the front-occipital area, antisepsis was performed with 2% iodine solution. A hole of 0.5 cm was made using orthodontic roof motor and number 2 drill to the right of the bregma until the dura matter was exposed. With the use of a Hamilton syringe fitted with a 30-gauge needle, lipopolysaccharide (5 μg in a volume of 2 μl PBS) was injected in the cisterna pontis (basal), an enlargement of the subarachnoid space on the ventral surface of the pons [24,25]. A group of rats (n = 6) was subjected to the same surgical procedure but injected with saline (0.9%) and served as negative control. The dura matter was left open and the skin together with the remainder of the subcutaneous tissue was sutured with a nylon thread 4.0.

2.2.3. Experimental design

Starting from the 2nd day of lipopolysaccharide injection, rats were randomly divided into six groups (n = 6 each). One of these groups was treated daily with saline orally and served as positive control. Each of the remaining lipopolysaccharide-injected groups was treated daily with one of compounds **6**, **10**, **8b**, **13a** or **14a** dissolved in 2% Tween 80 and injected subcutaneously in a dose of 50 mg kg^{-1} b.wt. for three consecutive days. Four hours after the last injection the rats were sacrificed and the cortex was dissected out on ice-cold Petri-dish. The samples were weighed and kept frozen at −20 °C until analysis.

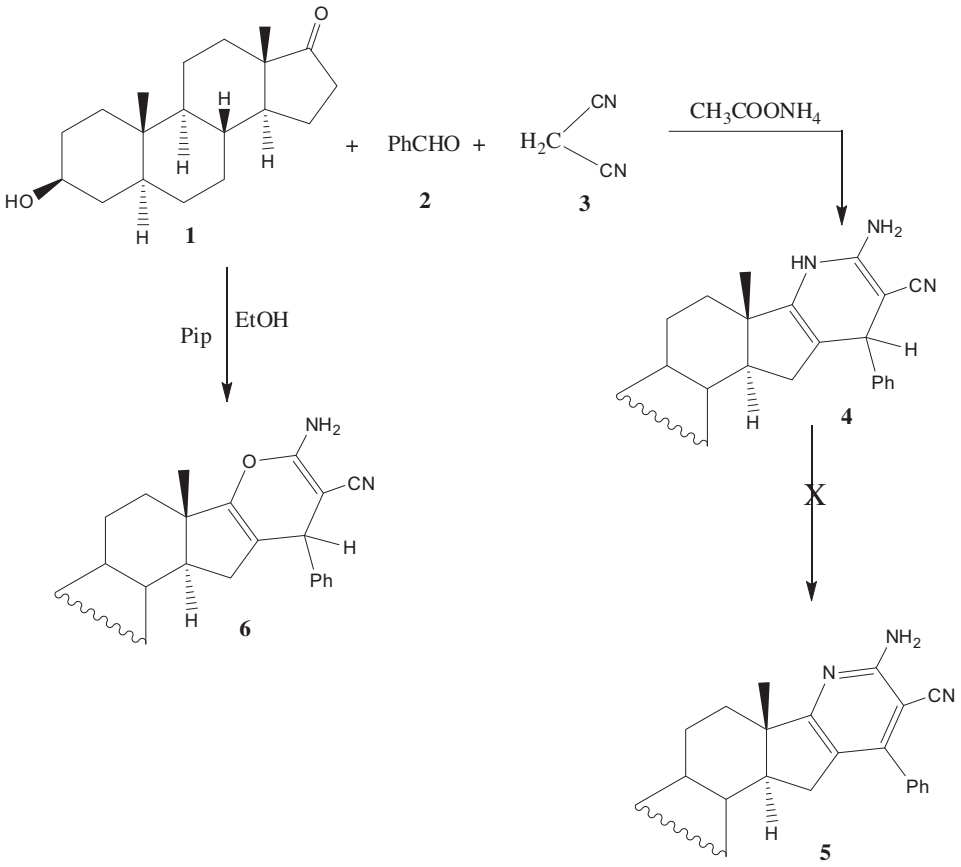
2.2.4. Neurochemical analysis

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA) in the cortex. MDA was determined by measuring thiobarbituric acid reactive species using the method of Ruiz-Larrea et al. [26] in which the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex having absorbance peak at 532 nm. The assay of reduced glutathione (GSH) levels was performed spectrophotometrically using the method of Ellman's [27]. It depends on the reduction of 5,5′-dithiobis-2-nitrobenzoic acid with glutathione to produce a yellow color whose absorbance is measured at 405 nm. The assay of nitric oxide was carried out using Biodiagnostic kit No. NO 25 33 (Biodiagnostic Co., Egypt). This method is based on the spectrophotometric method of Montgomery and Dymock [28] which depends on the measurement of endogenous nitrite concentration as an indicator of nitric oxide production. The procedure used for the determination of acetylcholinesterase activity (AChE) in the cortex was a modification of the method of Ellman et al. [29] as described by Gorun et al. [30]. The principle of the method is the measurement of the thiocholine produced as acetylthiocholine is hydrolyzed. The color was read immediately at 412 nm.

3. Results and discussion

3.1. Chemistry

Heterocyclic steroids serve as platform for developing pharmaceutical agents for various applications. We have attempted a straightforward synthesis of these compounds using MCRs. Thus, A mixture of epi-androsterone (3β-hydroxy-5α-androstan-3-one) **1**, benzaldhyde **2** and malononitrile **3** was heated in absolute ethanol containing ammonium acetate under reflux to afford the corresponding aminoandrostano-1,4-dihydropyridine derivative **4** (Scheme 1). The reaction proceeded via the formation of the benzylidene adduct at first followed by the addition of malononitrile and cyclization by loss of water. It was explained previously that the formed adduct may undergo aromatization via loss of



Scheme 1.

hydrogen to produce structure **5** [31]. The ^{1}H NMR spectrum of the synthesized adduct revealed the presence of hydrogen proton at C-4 of the 1,4-dihydropyridine ring at δ = 4.50 ppm and D_2O-exangeable NH proton at δ = 8.09 ppm. This excluded structure **5** and supported the formation of the adduct **4**. Carrying out the previous reaction in presence of piperidine instead of ammonium acetate led to the formation of the aminoandrostanodihydropyrane derivative **6** (Scheme 1). The ^{1}H NMR spectrum of product **6** showed the disappearance of the characteristic signal of NH group of the pyridine ring. All the microanalytical and spectroscopic data were in accordance with the suggested structure **6**.

Concerning the stereochemistry at position 4 of compounds **4** and **6**, the reaction conditions produced only one isomer [6]. Also, thin layer chromatography examination of the reaction mixture revealed one product. Identification of 1,4-dihydropyridine or dihydropyran isomers is beyond the scope of this study.

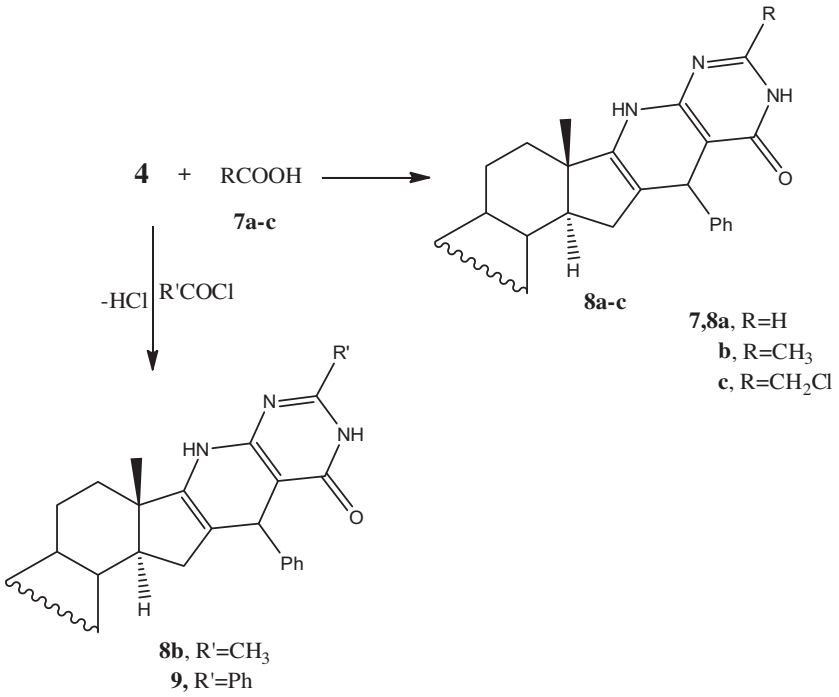
The androstanopyridopyrimidinone derivatives **8a–c** were formed via boiling compound **4** in different organic acids like formic, acetic and chloroacetic acids **7a–c** (Scheme 2). It is believed that the nitrile group was converted into amide via hydrolysis followed by cyclization. Dimorth rearrangement took place to furnish the final fused pyridopyrimidinone adduct [32,33]. On the other hand interaction of compound **4** with acetyl chloride or benzoyl chloride in the presence of triethylamine yielded the corresponding fused candidates androstanopyridopyrimidinone derivatives **8b** and **9**, respectively (Scheme 2).

Aminoandrostano-1,4-dihydropyridine **4** was allowed to react with excess of carbon disulfide in alcoholic-potassium hydroxide solution (10%) to form the androstanopyridopyrimidine-dithione adduct **10**. The microanalysis supported the presence of sulfur and the other spectroscopic data were in accordance with the structure of **10** (Scheme 3). Treatments of **4** with dimethylformamide-dimethylacetal in acetonitrile yielded the open enamine adduct, aminoandrostanopyridine-*N,N*-dimethylformamidine **11**. The ^{1}H NMR spectrum of compound **11** revealed, beside the disappearance of the characteristic signal of the amino group, the presence of singlet signal at δ = 2.47 (6H) for NMe_2 group and singlet at δ = 7.85 (1H) for the enamine CH *(c.f.* Experimental section).

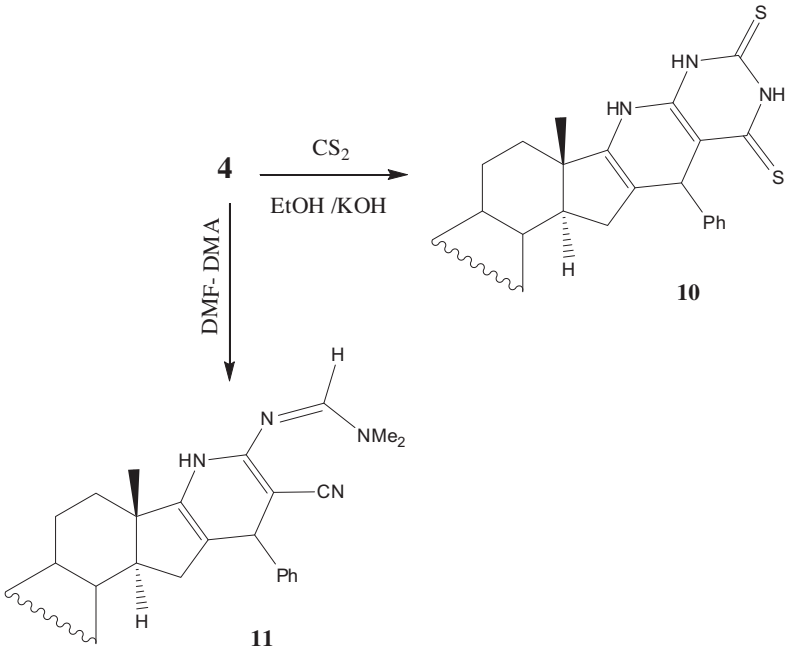
Furthermore, the interaction of either compound **4** or **6** with triethylorthoformate in acetic anhydride solution yielded the acyclic structures **13a** and **13b**, respectively. The expected cyclized adducts **12a**, **b** were excluded dependant on the spectroscopic data. The I.R spectrum of compound **13a** as example showed the presence of the nitrile group at υ = 2225 cm^{-1} and the absence of the carbonyl group of the pyrrole ring in **12a** structure. Treatment of compounds **13a**, **b** with hydrazine hydrate in ethanol absolute under reflux led to the formation of the fused aminoandrostanopyridopyrimidine adducts **14a**, **b** respectively (Scheme 4).

3.2. Bioassay

The in vivo potent anti-neuroinflammatory and anti-oxidative stress in brain of the novel synthesized heterocyclic steroids, compounds **6**, **10**, **8b**, **13a** and **14a** was investigated. The tested compounds were assayed in the model of neuro-inflammation produced in rats by cerebral lipopolysacharide injection. The intracerebral administration of bacterial endotoxin resulted in cerebral inflammatory state evidenced by increased lipid peroxidation (malondialdehyde; MDA), decreased reduced glutathione (GSH)



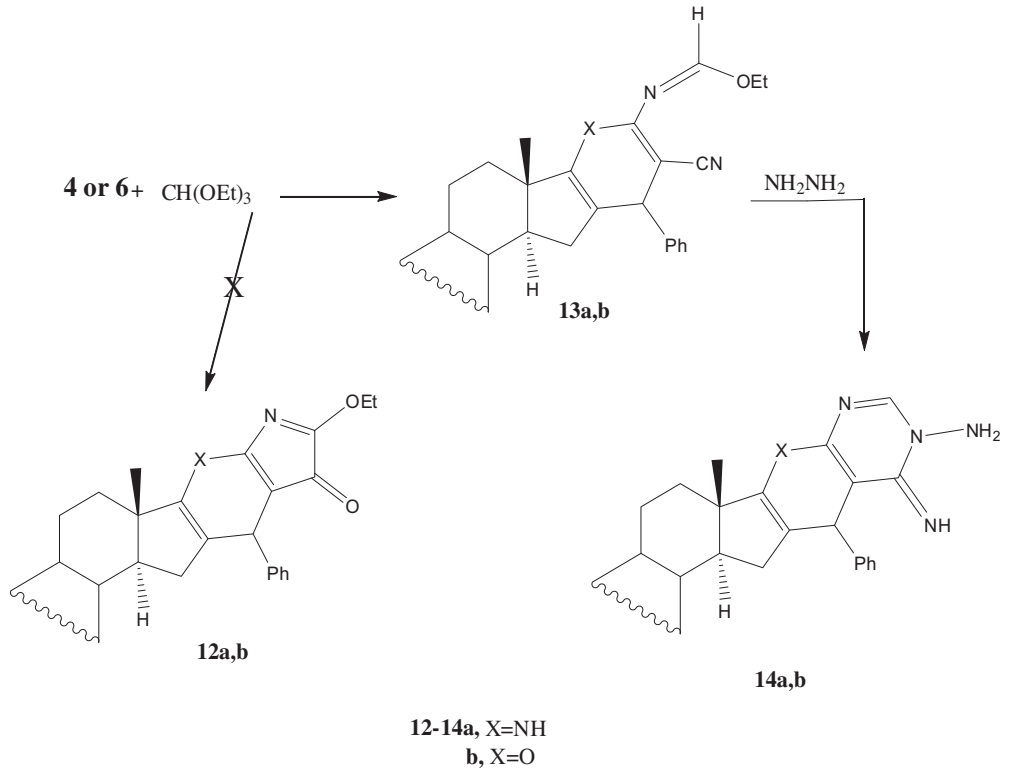
Scheme 2.



Scheme 3.

level, increased nitric oxide as well as increased acetylcholinesterase (AChE) activity in the brain (Table 1). The test compounds were administered subcutaneously to rats treated with intracerebral lipopolysaccahride. Compounds **6**, **10**, **8b** and **13a** markedly increased GSH that reached normal values with compounds **8b**, **10** and **13a** and was even raised above control value in rats treated with compound **6**. MDA was reduced to normal values after treatment with all tested compounds, almost normalized nitric oxide levels while marked inhibition of nitric oxide was seen after treatment with compound **6**. AChE activity, a marker of inflammation, was normalized by compound **8b** and reduced to below normal values by compounds **10** and **14a**. AChE activity was markedly reduced by compound **6**. These results are exciting in that, these compounds might be useful candidates in treatment of cerebral inflammation.

Directed by the structure of tested compounds it is probably that these compounds have a dual mechanism for their anti-neuroinflammatory and anti-oxidative activities. Further studies should be made to establish the mechanism of action with the possibility to formulate potent anti-cerebral inflammatory agents.



Scheme 4.

Table 1
Effect of test compounds on reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO) and acetylcholinesterase activity (AChE) in the brain of rats subjected to intracerebral lipopolysaccharide (LPS) injection.

	GSH (mmol/g fresh tissues)	MDA (nmol/g fresh tissue)	No (μ mol/g)	AChE (μ mol SH/g/min)
Vehicle	0.583 ± 0.082	18.476 ± 2.277	0.083 ± 0.007	4.729 ± 0.671
LPS	0.149 ± 0.033*	23.736 ± 1.468	0.141 ± 0.019*	6.830 ± 0.713
LPS + **8b**	0.606 ± 0.127	15.937 ± 1.22	0.073 ± 0.004	4.487 ± 0.561
LPS + **14a**	0.193 ± 0.045*	18.824 ± 0.895	0.102 ± 0.008	3.456 ± 0.288
LPS + **13a**	0.455 ± 0.051	18.427 ± 0.895	0.123 ± 0.014	6.145 ± 0.944
LPS + **10**	0.439 ± 0.053	21.194 ± 0.487	0.072 ± 0.007	2.176 ± 0.252*
LPS + **6**	0.818 ± 0.064	19.216 ± 1.742	0.077 ± 0.0 II	1.319 ± 0.401*

* Indicate significant difference compared vehicle-treated control values at $p < 0.05$.

3.3. Conclusion

In this study we have attempted a straightforward synthesis of novel pyrido- and pyrido-pyrimidine steroids that would act to reduce neuro-inflammation and oxidative stress in brain, using multi-component reactions. The tested compounds were assayed in the model of neuro-inflammation produced in rats by cerebral lipopolysacharide injection. We investigated also the pharmaceutical importance of incorporating heterocylic moiety to the steroid nucleus to form new effective hybrid molecules. Compounds **6**, **10**, **8b**, **13a** and **14a** showed anti-neuroinflammatory and anti-oxidant activities with various intensities. Results suggest tested compounds are useful candidates in treatment of cerebral inflammation.

References

[1] Yu L, Chen B, Huang X. Multicomponent reactions of allenes, diaryl diselenides, and nucleophiles in the presence of iodosobenzene diacetate: direct synthesis of 3-functionalized-2-arylselenyl substituted allyl derivatives. Tetrahedron Lett 2007;48(6):925–7.
[2] Heravi MM, Zakeri M, Pooremamy S, Oskooie HA. Clean and efficient synthesis of polyhydroquinoline derivatives under solvent-free conditions catalyzed by morpholine. Synth Commun 2011;41:113–20.
[3] Nemati F, Bigdeli MA, Mahdavinia GH, Kiani H. 2,4,6-Trichloro[1,3,5]triazine (TCT)-catalyzed one-pot Mannich-type reaction: three component synthesis of β-amino carbonyl compounds. Green Chem Lett Rev 2010;3(2):89–92.
[4] Quiroga J, Cruz S, Insuasty B, Abonía R, Nogueras M, Cobo J. Three-component synthesis of hexahydropyridopyrimidine–spirocyclohexanetriones induced by microwave. Tetrahedron Lett 2006;47(1):27–30.
[5] Kidwai M, Thakur R, Rastogi S. Ecofriendly synthesis of substituted pyridine and pyrido[2,3-d]pyrimidine derivatives. Russ Chem Bull 2005;54(6):1523–6.
[6] Mohamed NR, Khaireldin NY, Fahmy AF, El-Sayed AA. Facile synthesis of fused nitrogen containing heterocycles as anticancer agents. Der Pharma Chemica 2010;2(1):400–17.
[7] Amir M, Javed SA, Kumar H. Pyrimidine as antiinflammatory agent: a review. Indian J Pharm Sci 2007;69(3):337–43.
[8] Yejella RP, Atla SR. A study of anti-inflammatory and analgesic activity of new 2,4,6-trisubstituted pyrimidines. Chem Pharm Bull (Tokyo) 2011;59(9):1079–82.
[9] Gyires K, Fürst S, Miklya I, Budavari I, Knoll J. Analysis of the analgesic and anti-inflammatory effects of rimazolium, a pyrido-pyrimidine derivative, compared with that of prostaglandin synthesis inhibitors and morphine. Drugs Exp Clin Res 1985;11(8):493–500.
[10] Huron DR, Gorre ME, Kraker AJ, Sawyers CL, Rosen N, Moasser MM. A novel pyridopyrimidine inhibitor of abl kinase is a picomolar inhibitor of Bcr-abl-driven K562 cells and is effective against STI571-resistant Bcr-abl mutants. Clin Cancer Res 2003;9(4):1267–73.

[11] Lovell MA, Ehmann WD, Mattson MP, Markesbery WR. Elevated 4-hydroxynonenal in ventricular fluid in Alzheimer's disease. Neurobiol Aging 1997;18:457–61.
[12] Reitz C, Tang M, Luchsinger J, Mayeux R. Relation of plasma lipids to Alzheimer disease and vascular dementia. Arch Neurol 2004;61(5):705–14.
[13] Gandhi S, Wood NW. Molecular pathogenesis of Parkinson's disease. Hum Mol Genet 2005;14:2749–55.
[14] Nagatsu T, Sawada M. Inflammatory process in Parkinson's disease: role for cytokines. Curr Pharm Des 2005;11:999–1016.
[15] Carlson NG, Rose JW. Antioxidants in multiple sclerosis: do they have a role in therapy? CNS Drugs 2006;20(6):433–41.
[16] Mohareb RM, Elmegeed GA, Baiuomy AR, Eskander EF, William MG. Evaluation of anti-inflammatory, anti-nociceptive and anti-ulcerogenic, activities of novel synthesized thiazolyl and pyrrolyl steroids. Arch Pharm Chem Life Sci 2011;344:595–604.
[17] Mohareb RM, Elmegeed GA, Abdel-Salam OME, Doss SH, William MG. Synthesis of modified steroids as a novel class of non-ulcerogenic, anti-inflammatory and anti-nociceptive agents. Steroids 2011;76:1190–203.
[18] Mohareb RM, Ahmed HH, Elmegeed GA, Abd-Elhalim MM, Shafic RW. Development of new indole-derived neuroprotective agents. Bioorg Med Chem 2011;19:2966–74.
[19] Koprich JB, Reske-Nielsen C, Mithal P, et al. Neuroinflammation mediated by IL-lβ increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease. J Neuroinflammation 2008;5: 8–20.
[20] Gacs-Baitz E, Minuti L, Taticchi A. Synthesis and complete 1H and 13C NMR analysis of some 4-androsten-3- one derivatives. Synop 1996;27:324–5.
[21] Frenkel M, Marsh KN. TRC Data Series: Spectral Data for Steroids. Texas, TX: Thermodynamics Research Center; 1994. 386pp.
[22] IUPAC. Joint Commission on Biochemical Nomenclature (JCBN). Nomenclature of steroids. Pure Appl Chem, 1989;61:1783–1822.
[23] IUPAC. Joint Commission on Biochemical Nomenclature (JCBN). The nomenclature of steroids, Recommendations 1989. Eur J Biochem 1989;186:429–58.
[24] Kim W, Mohney RP, Wilson B, Jeohn G, Liu B, Hong J. Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: Role of microglia. J Neurosci 2000;20(16):6309–16.
[25] Cai Z, Fan LW, Lin S, Pang Y, Rhodes PG. Intranasal administration of insulin-like growth factor-1 protects against lipopolysaccharide-induced injury in the developing rat brain. Neuroscience 2011;194:195–207.
[26] Ruiz-Larrea MB, Leal AM, Liza M, Lacort M, de Groot H. Antioxidant effect of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsome. Steriods 1994;59:383–8.
[27] Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70–7.
[28] Montgomery HAC, Dymock JF. Determination of nitric oxide. Analyst 1961;86:414–7.
[29] Ellman GL, Courtney KD, Andres Jr V, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88–95.
[30] Gorun V, Proinov I, Baltescu V, Balaban G, Barzu O. Modified Ellman procedure for assay of cholinesterases in crude enzymatic preparation. Anal Biochem 1978;86:324–6.
[31] Hassan KM, El-Dean AMK, Youssef MSK, Atta FM, Abbady MS. Some reactions of 3-amino-2-carboethoxy-4,6-dimethylthieno[2,3-b]-pyridine: Synthesis of some new thienopyridopyrimidines. Phosphorus Sulfur Silicon Relat Elem 1990;47:283–9.
[32] Shishoo CJ, Devani MB, Ullas GV, Ananthan S, Bhadti VS. Studies on the synthesis of 2-(2-arylvinyl)thieno(2,3-d)pyrimidines and 5-(2-arylvinyl) triazolothieno(3,2-e)pyrimidines. J Heterocyc Chem 1985;22:825–30.
[33] Abdelrazek FM. Heterocyclic synthesis with nitriles: synthesis of some new thiophene and thieno[2,3-d]pyrimidin derivatives iv. Phosphorus Sulfur Silicon Relat Elem 1996;119:271–7.