Steroids 101 (2015) 78–89

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Synthesis of novel steroidal curcumin derivatives as anti-Alzheimer's disease candidates: Evidences-based on *in vivo* study

Gamal A. Elmegeed [a,*], Hanaa H. Ahmed [a], Maher A. Hashash [b], Mervat M. Abd-Elhalim [a], Dina S. El-kady [a]

[a] Hormones Department, National Research Centre, Dokki, Giza, Egypt[1]
[b] Chemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt

ARTICLE INFO

Article history:
Received 17 March 2015
Received in revised form 1 June 2015
Accepted 7 June 2015
Available online 13 June 2015

Keywords:
Alzheimer's disease
Curcumin analogues
Heterocycles
Rats

ABSTRACT

Alzheimer's disease (AD) is a complex disease in which a single monofunctional 'targeted' drug is ineffective for management. Hybrid drugs that impact multiple targets simultaneously are better at controlling such complex disease systems. Hybrid agents were synthesized through the combination of the steroid moiety with curcumin molecule. Also novel curcumin analogues containing promising heterocyclic nucleus fused to the essential pharmacophoric feature of the curcumin moiety, were synthesized. The aim of the present study was extended to elucidate the efficacy of these novel synthesized compounds in the regression of AD induced in adult female albino rats. The results revealed that treatment of AD groups with compounds **3**, **5**, **8c** or rivastigmin experienced significant increase in brain Ach, GSH, paraoxenase and BCL2 levels with respect to untreated group associated with significant decrease in brain AchE activity, urinary 8-OHG level, serum Caspase-3 level and brain P53 level relative to the untreated group. Immunohistochemical investigation revealed that the selected treatments caused marked increase in ChAT positive cells. These findings were documented by the histological investigation of the brain tissue. The activity of tested compounds showed gradual increase from compound b followed by compound **8c** then compound **5**. The anti-cholinesterase potential, anti-oxidant properties and anti-apoptotic activity are responsible for the anti-Alzheimer's disease potential of these compounds.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, responsible for over 50% of all cases of dementia, which affects up to 5% of people over 65 years, while its prevalence increases to more than 20% of those over 80 years [1]. AD is currently a major public health problem and will presumably be the most important disorder of this century in developed/developing countries. Sustained efforts have been made in the last decade to determine the etiopathogenesis of AD, and to carry out its early diagnosis and therapeutic control. Currently, there is no drug thereby that provides definite solution for curing Alzheimer's disease. The pharmacological treatment conventionally used to maintain cognitive functions of patients consist of two classes of drugs, the acetyl cholinesterase inhibitors (AchEI) and the glutamate modulators [2].

There is a growing body of evidence indicating that oxidative damage may contribute to AD pathogenesis before amyloid beta (Aβ) accumulation in the brain [3]. Several studies have consistently shown the presence of lipid, protein, and DNA oxidation products in the brain of AD patients [4]. Also, increases in brain levels of 8-hydroxyguanosine (8-OHG), an oxidized nucleoside, have been detected early in AD [5]. Furthermore, reduced antioxidant enzyme activity has been shown in AD brain [6]. Treatment with antioxidants has been suggested to be an alternative approach for slowing disease progression whereas oxidative damage may be responsible for the cognitive and functional decline observed in AD [7].

Tacrine and Trozamicol are heterocyclic drugs available for Alzheimer's disease based on the cholinergic approach. Many analogues of Tacrine and Trozamicol have been prepared which retain the pharmacologically rich heterocyclic moiety [8]. Also, many new heterocyclic compounds were synthesized as AchEI [9]. The investigation of new modified steroid derivatives condensed with

Abbreviations: Ach, acetylcholine; AchE, acetylcholinesterase; acetyl-CoA, acetyl coenzyme; AchEI, acetyl cholinesterase inhibitors; AD, Alzheimer's disease; Aβ, amyloid beta; AR, androgen receptor; BCL-2, 2B-cell lymphoma; b.wt., body weight; ChAT, choline acetyl transferase; DHEA, dehydroepiandrostane; DMSO, dimethylsulfoxide; GABA, γ-aminobutyric acid; GSH, glutathione reduced; 8-OHG, 8-hydroxy guanosine; MAP, mitogen activated protein; P53, tumor protein; ROS, reactive oxygen species; TLC, thin layer chromatography.

* Corresponding author at: Hormones Department, National Research Centre, 12622 Dokki, Giza, Egypt. Tel.: +20 2 33872607; fax: +20 2 33370931.
E-mail address: gamalae@hotmail.com (G.A. Elmegeed).
[1] Affiliation ID: 60014618.

http://dx.doi.org/10.1016/j.steroids.2015.06.003
0039-128X/

various heterocyclic rings has a great attention. The addition of heterocyclic rings to steroids often leads to a change of their physiological activity and the appearance of new interesting pharmacological and biological properties [10]. Curcumin is a major constituent of the plant rhizomes "*Curcuma longa Linn*" [11]. *C. longa* rhizome is used in traditional diet and as an herbal medicine in India. Curcumin has potent anti-inflammatory and antioxidant activities; it can decrease oxidative damage, inflammation and amyloid accumulation [12]. Curcumin is much stronger than vitamin E as a free-radical scavenger [13]; protects the brain from lipid peroxidation, and scavenges nitric oxide (NO)-based radicals. Curcumin inhibits amyloid aggregation (IC_{50} = 0.9 mm) *in vitro* and $A\beta_{40}$ (IC_{50} = 1 mm) [14]. The enhanced decrease in Aβ aggregation due to the metal chelation properties of curcumin as curcumin binds to redox-active metal ions such as iron and copper, and such complexes may cause a net protective effect through decreased Aβ aggregation [15]. Numerous analogues of curcumin have been developed for therapeutic purposes. It is well established that large quantities of curcumin can be consumed without toxicity. These distinctive properties make curcumin a valuable lead compound for drug development, and it remains the focus of several clinical trials [16].

In the scope of research program aimed at the development of new alternatives to treat neurological disorders [10,17,18]. We described in this study the synthesis of new candidates for regression of AD in experimental animal model. To achieve this goal, hybrid agents was synthesized through the combination of the steroid molecule with curcumin moiety, also, novel efficient curcumin analogues containing promising heterocyclic nucleus were synthesized. The potential anti-Alzheimer effect of these newly synthesized agents was investigated in experimental animal model.

2. Materials and methods

2.1. Synthetic methods, analytical and spectral data

Starting steroids and curcumin were purchased from Sigma Company, St. Louis, MO, 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 steroid derivatives showed the characteristic spectral data of cyclopentanoperhydrophenanthrene nuclei of androstane series were similar to those reported in literature [19]. Compounds **2**, **4** and **6** were prepared according to published procedures [20,21,22].

2.1.1. (17Z,17′Z)-17,17′-((2Z,2′Z)-((1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-diylidene)bis(hydrazine-2,1-diyilidine))bis(10,13-dimethylhexadecahydro-1H-cyclopenta]α[phenathren-3-ol) (3)

To a solution of curcumin **1** (0.36 g, 1 mmol) and 3β-hydroxy-17-hydrozone-5α-androstane **2** (0.60 g, 2 mmol) in absolute ethanol (20 mL), acetic acid (3 mL) was added and the reaction mixture was heated under reflux for 3 h until all the starting materials was disappeared as indicated by TLC. The reaction mixture was treated with ice/water mixture. The formed solid product was collected by filtration and crystallized from absolute ethanol to form dark orange crystals of compound **3**, yield 0.77 g (82%); mp 165–167 °C; IR (KBr, cm^{-1}): ν 3396 (OH), 2926 (CH_3), 2858 (CH_2), 1620 (C=N), 1592 (C=C). ^1H NMR ($CDCl_3$, ppm): δ = 0.78 (s, 6H, 2Me-19), 1.23 (s, 6H, 2Me-18), 2.22 (s, 2H, 2OH, D_2O-exchangeable), 3.82 (s, 6H, $2OCH_3$), 5.80 (s, 4H, olefinic proton), 6.58–7.30 (m, 6H, aromatic H), 9.86 (s, 2H, 2OH curcumin, D_2O-exchangeable). ^{13}C NMR (DMSO-d_6, ppm): δ = 27.30 (C-1), 30.60 (C-2), 68.10 (C-3), 34.70 (C-4), 37.00 (C-5), 25.50 (C-6), 25.80 (C-7), 42.01 (C-8), 36.25 (C-10), 29.04 (C-11), 30.72 (C-12), 45.10 (C-13), 50.34 (C-14), 29.40 (C-15), 111.00 (C-16), 164.62 (C-17), 20.60 (C-18), 27.52 (C-19), 136.12, 112.32 (C=C, curcumin moiety), 56.20 ($2OCH_3$), 112.00, 116.00, 120.00, 128.00 (aromatic carbon), 19.00 (CH_3), 20.70 (CH_3), 27.30, 29.90, 32.80, 35.40 (CH_2). MS (EI) *m*/*z* (%): 941 (M^+, 65%), 559 (46), 383 (100), 369 (22), 291 (42), 171 (47), 118 (90). Calc for $C_{59}H_{80}N_4O_6$ (940.600): C, 75.28; H, 8.57; N, 5.98%, found: C, 75.02; H, 8.32; N, 5.71%.

2.1.2. (S,S)-((1E,6E)-3,5-Dioxohepta-1,6-diene-1,7-diyl)bis(3-methoxy-4,1-phenylene)bis(2-((10S,13S)-10,13-dimethyl-3-oxohexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)amino)benzoate) (5)

To a mixture of 2-(3-oxoandrost-4-en-17-yl-amino)benzoic acid **4** (0.80 g, 2 mmol) and curcumin **1** (0.36 g, 1 mmol) in benzene (30 mL), H_2SO_4 (3 mL), was added. The reaction mixture was heated under reflux for 8 h until all the reactants had disappeared as indicated by TLC. The reaction mixture poured over crushed ice and then kept in refrigerator at 4 °C overnight. The solid product that formed was collected by filtration, crystallized from absolute ethanol to give brownish red powder of compound **5**, yield 0.91 g (79%); mp 85–87 °C; IR (KBr, cm^{-1}): ν 3415 (2NH), 2927 (CH_3), 2850 (CH_2), 1735, 1725, 1720, 1710, 1700, 1695 (6C=O), 1595 (C=C). ^1H NMR (DMSO-d_6, ppm): δ = 0.81 (s, 3H, Me-19), 1.10 (s, 3H, Me-18), 3.79 (s, 6H, $2OCH_3$), 4.45 (s, CH-C4-steroid), 6.74–7.52 (m, 18H, aromatic and 4 olefinic protons), 9.08 (s, 1H, NH), 9.20 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, ppm): δ = 26.10, 27.40, 33.90, 36.00, 37.40 ($5CH_2$ aliphatic), 55.9 ($2OCH_3$), 198.90 (2C=O), 126.30, 142.9 (2C=C), 113.60, 118.60,123.30, 129.50, 137.90,154.8 (aromatic carbons), 148.90 (C-N), 35.12 (C-1), 34.20 (C-2), 198.22 (C-3), 123.0 (C-4), 171.06 (C-5), 32.60 (C-6), 31.90 (C-7), 36.20 (C-8), 49.9 (C-9), 34.9 (C-10), 23.01 (C-11), 35.40 (C-12), 40.92 (C-13), 52.90 (C-14), 24.70 (C-15), 28.40 (C-16), 65.10 (C-17), 16.20 (C-18), 22.50 (C-19), 170.50 (C=O), 113.0, 116.10, 131.10, 134.82, 151.20, 169.40 (C-phenyl). MS (EI) *m*/*z* (%): 1150 (M^+, 22%), 523 (2), 288 (22), 203 (57), 124 (100), 105 (32), 91 (58), 77 (42), 63 (39). Calc for $C_{73}H_{86}N_2O_{10}$ (1150.470): C, 76.14; H, 7.53; N, 2.43% found: C, 75.84; H, 7.30; N, 2.19%.

2.1.3. General procedure for compounds 8a, 8b and 8c

To suspension of phenyl pyrazolocurcumin derivative **6** (0.44 g, 1 mmol) in glacial acetic acid (1.5 mL), sulfathiazole (**7a**) (0.44 g, 2 mmol), sulfamethoxazole (**7b**) (0.50 g, 2 mmol) or sulfadiazine (**7c**) (0.50 g, 2 mmol) was added. The reaction mixture was heated in an oil bath at 120 °C for 10 min and then left to cool at room temperature. The fused result was triturated with absolute ethanol (10 mL) and then heated again under reflux for 20 min. The solid product obtained upon cooling was collected by filtration and crystallized from appropriate solvent.

2.1.3.1. 4,4′-((((1E,1′E)-(1-Phenyl-1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl))bis(3-methoxy-4,1-phenylene))bis(azanediyl))bis(N-(thiazol-2-yl)benzenesulfonamide) (8a). Yellow crystals from absolute