Steroids 84 (2014) 64–69

Contents lists available at ScienceDirect

Steroids

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

Benzylidine pregnenolones and their oximes as potential anticancer agents: Synthesis and biological evaluation

Abid H. Banday [a,b,*], S.M.M. Akram [a,c], Shameem A. Shameem [a]

[a] Department of Chemistry, Islamia College of Science and Commerce, Srinagar 190009, India
[b] Department of Chemistry and Biochemistry, University of Arizona, Tucson 85721, USA
[c] Department of Chemistry, University of Kashmir, Srinagar 190002, India

ARTICLE INFO

Article history:
Received 8 November 2013
Received in revised form 5 March 2014
Accepted 15 March 2014
Available online 1 April 2014

Keywords:
Benzylidine pregnenolone
Aldol condensation
Nucleophillic addition
Cytoxicity
Dehydration

ABSTRACT

The present study reveals the anticancer activity of benzylidine pregnenolones and their oxime derivatives. The synthesis of the analogs of both series is very simple and involves aldol condensation in the first step followed by nucleophillic addition of hydroxylamine across carbonyl in the second step. Quantitative yields of more than 80% are obtained in both the steps. All the compounds were tested for their cytotoxic activities against a panel of six human cancer cell lines. Amongst all the compounds of both the series screened for their cytotoxic activity, compound **3e**, **3f** and **4e** are very potent especially against **HCT-15** and **MCF-7** cancer cell lines.

1. Introduction

Over the past twenty years, medicinal chemistry has gained enormous popularity for its role in drug discovery. A number of natural products, their semi synthetic analogs and small molecules have been discovered and evaluated for their role in the treatment and cure of various fatal diseases such as cancer, diabetes, microbial infections and cardiovascular diseases etc. [1,2]. However because of the drug resistance and drug tolerance problems, there is always scope for the design and development of new and modified analogs as more efficient drug candidates. This has been done through the rational design and synthesis of receptor based lead molecules which still remains an open area. Natural products have extensively been used as starting tools for the design and synthesis of lead therapeutic scaffolds. The use of natural products for curing human ailments dates back to 1550 BC, since natural products have been used in medicine especially for the treatment of cancer and related diseases. A number of plants have been used as folklore medicinal agents. The modern drug discovery program has made a great success as a number of lead compounds have been developed from the traditionally used medicinal plants. A number of plant based anticancer compounds such as vinblastine (Velban), vincristine (Oncovin), vinorelbine (Navelbine), etoposide (VP-16), teniposide (VM-26), Taxol (paclitaxel), and most recently Taxotere (docetaxel), topotecan (Hycamtin), and irinotecan (Camptosar) have been approved for use as anticancer drugs by the US FDA [3]. Steroids as natural products have been extensively studied as their biological and clinical importance is now well validated. Steroids as well as their derivatives have been found to have the potential to be developed as drugs for the treatment of a large number of diseases including cardiovascular [4], autoimmune diseases [5], brain tumours, breast cancer, prostate cancer, osteoarthritis, etc. [6]. The promise of using steroids for development of lead molecules lies in their regulation of a variety of biological processes and being a fundamental class of signaling molecules [7]. We have been interested in the area of steroid based medicinal chemistry for the past few years and we have successfully evaluated various steroid based congeners as potent pharmacological agents showing activities such as cytotoxic, antimicrobial, antioxidant etc. [8]. This is particularly true of pregnenolone which happens to be a precursor of most other steroids and has been extensively studied for various biological activities. It has been found that the molecule holds a great promise against brain disorders, memory loss, schizophrenia, insulin related disorders and cancer [9]. Thus in continuation of our program, we herein report the anticancer activities of two series of our compounds I,e benzylidine pregnenolones and their oxime derivatives. We are reporting

* Corresponding author at: Department of Chemistry, Islamia College of Science and Commerce, Srinagar 190009, India. Fax: +91 194 2429014.
E-mail address: abidrrl@gmail.com (A.H. Banday).

http://dx.doi.org/10.1016/j.steroids.2014.03.010
0039-128X/

the primary in vitro results as the mechanistic studies are in progress and will be communicated in due course of time.

2. Experimental

2.1. General methods

Melting points were recorded on Buchi Melting point apparatus D-545; IR spectra (KBr discs) were recorded on Bruker Vector 22 instrument. NMR spectra were recorded on Bruker DPX200 instrument in $CDCl_3$ with TMS as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values are mentioned in δ (ppm) and coupling constants are given in Hz. Mass spectra were recorded on EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. The progress of all reactions was monitored by TLC on 2 × 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck). The chromatograms were visualized under UV 254–366 nm and iodine.

2.2. Chemical synthesis

2.2.1. General procedure for the synthesis of Benzylidine pregnenolones (*3*)

To a solution of pregnenolone 1 (0.316 g, 1 mmol, 1 eq.) in ethanol (10 ml) was added a conc. aq. solution of KOH (2 eq.). Then aldehyde 2 (1.2 eq.) was charged into the reaction mixture to get the corresponding benzylidine derivative 3 (Scheme 1). After completion as revealed by thin layer chromatography (TLC) in an average span of around 1 h, the reaction mixture was precipitated using water because of the limited solubility. The precipitate was filtered, dried and monitored through TLC for the purity. Thin layer chromatography revealed just a single spot which proved the presence of a single product. For further purification, the product was recrystallized from EtOAc:Hexane to give product as solid white powder. It is to be mentioned that when non-aromatic aldehydes were used, the product was formed in a very minor quantity and that too not stable enough at ambient conditions. Thus the study was restricted to the use of aromatic aldehydes only (Tables 1 and 2).

2.2.2. General procedure for the synthesis of Benzylidine pregnenolone oximes (*4*)

To an ethanolic solution of compound 3d (0.418 g, 1 mmol) was added hydroxylamine hydrochloride (0.139 g, 2 mmol) and the reaction was stirred for 2 h at room temperature. A precipitate of the oxime was obtained as a single spot as revealed by the TLC. The reaction mixture was worked up by first evaporating the ethanol and then extracting the solid with EtOAc: H_2O (3 × 25 ml). Quantitative yields of 90–93% were obtained for all the compounds.The spectral data of various compounds from both the series is given as under.

*2.2.3.1. (2E)-1-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3(β)-hydroxy-10,13-dimethyl-1H-cyclopenta[α]phenanthren-17(β)-yl)-3-phenylprop-2-en-1-one (**3a**).* White powder (86%). M.p.: 128–131 °C; IR (KBr) cm^{-1}: 3425, 2938, 1804, 1637,1403, 1041, 689; 1H NMR (CDCl3): δ 0.63 (s, 3H), 1.00 (s, 3H),1.61–1.90 (m, 6H), 2.20–2.38 (m, 3H), 2.82 (t, J = 8.80, 1H); 3.51 (m, 1H); 6.78 (s, J = 16.00, 1H), 7.39 (m, 3H), 7.55 (m, 3H); 13C NMR (500 MHz, CDCl3): δ 13.33, 19.26, 21.07, 22.67, 24.62, 31.13, 31.78, 31.99, 37.22, 41.81, 45.11, 48.61, 48.78, 48.95, 49.12, 49.29, 50.04, 57.14, 61.97, 71.22, 121.24, 126.69, 128.29, 128.90, 130.41, 134.62, 140.85, 141.96, 201.32; ESI-MS: 405 (M+H); Anal. Calcd. for $C_{28}H_{36}O_2$: C, 83.12; H, 8.97; Found C, 83.37; H, 8.83.

Table 1
Nature of group "R" in the compounds **3a**–**3h**.

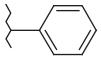
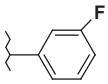
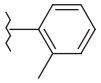
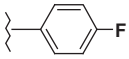
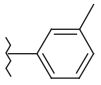
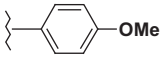
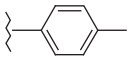
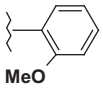
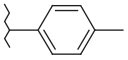
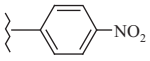
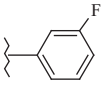
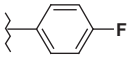
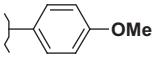
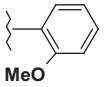
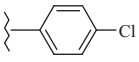
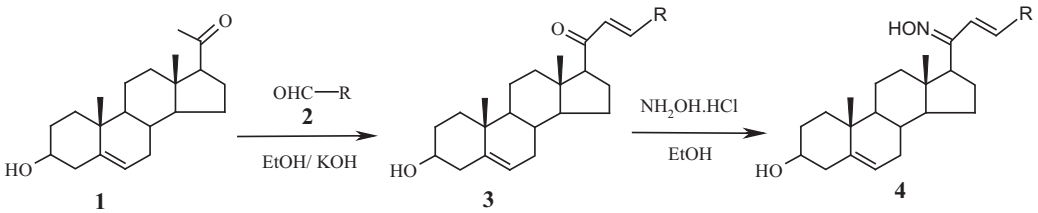
Entry	Nature of R	Entry	Nature of R
3a		**3e**	
3b		**3f**	
3c		**3g**	
3d		**3h**	

Table 2
Nature of group "R" in the compounds **4a**–**4g**.

Entry	Nature of R	Entry	Nature of R
4a		**4e**	
4b		**4f**	
4c		**4g**	
4d			

*2.2.3.2. (2E)-1-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3(β)-hydroxy-10,13-dimethyl-1H-cyclopenta[α]phenanthren-17(β)-yl)-3-o-tolylprop-2-en-1-one (**3b**).* Yellow powder (89%). M.p.: 208–210 °C; IR (KBr) cm^{-1}: 3405, 2941, 1774, 1674, 1403, 1041, 757; 1H NMR (CDCl3): δ 0.62 (s, 3H), 1.00 (s, 3H), 1.63–1.90 (m, 6H), 2.22–2.35 (m, 3H), 2.38 (s, 3H), 2.82 (t, J = 8.43, 1H); 3.52 (m, 1H), 5.32 (s, 1H), 6.69 (d, J = 15.78, 1H), 7.32 (m, 3H), 7.57 (d, J = 6.83, 1H), 7.82 (d, J = 15.78,1H); 13C NMR (500 MHz, CDCl3): δ 13.54, 19.46, 19.95, 21.21, 2.84, 24.77,



Scheme 1. Synthesis of D-ring substituted benzylidine pregnenolones and their oximes.