Arabian Journal of Chemistry (2014) 7, 461–468

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

2nd Cancer Update

Anticancer and antimicrobial evaluation of newly synthesized steroidal 5,6 fused benzothiazines

Shamsuzzaman [a,*], **Ayaz Mahmood Dar** [a], **Hena Khanam** [a], **Manzoor Ahmad Gatoo** [b]

[a] *Department of Chemistry, Aligarh Muslim University, Aligarh 202 002, India*
[b] *Department of Biochemistry, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh 202 002, India*

Received 11 May 2012; accepted 23 June 2013
Available online 3 July 2013

KEYWORDS

Benzothiazine;
5α-Cholestan-6-one;
Antibacterial;
Antifungal;
Anticancer

Abstract A series of new 5α-cholestano [5,6-b] benzothiazines (**4–6**) has been synthesized by the reaction of 5α-cholestan-6-one (**1–3**) with 2-aminothiophenol in the presence of iodine. The structures of newly synthesized compounds have been established on the basis of spectral and analytical data. Compounds (**1–6**) were screened for in vitro anticancer activity against the human cancer cell lines; SW480 (colon adenocarcinoma cells), A549 (lung carcinoma cells), HepG2 (hepatic carcinoma cells) and HeLa (cervical cancer cells) using MTT assay during which the products (**4–6**) showed marked increase in anticancer activity and in particular, compound **6** showed IC_{50} = 13.73 μmol L^{-1} against HeLa cells, being more effective than Doxorubicin against the same cells. Compounds **4** and **6** also showed minimum IC_{50} of 15.83 μmol L^{-1} and 16.89 μmol L^{-1} against HepG2 and A549 cells, respectively. Compounds (**1–6**) were also tested for in vitro antimicrobial activity against different bacterial as well as fungal strains during which newly synthesized compounds (**4–6**) were found more potent than starting compounds (**1–3**). Compound **4** was found to be more potent than the reference drug, Chloramphenicol, in the case of *Escherichia coli* while compound **5** was found almost equally potential antifungal agent against P. marneffei in comparison with the reference drug, Nystatin.

1. Introduction

Steroids have been the important focus of research throughout the scientific history. But the recent past has seen an exhaustive focus of research being diverted towards these biologically important molecules. This is pertinently true of the rational semi-synthetic modifications of steroidal molecules. Probably, it is because of the various advantages associated with steroid based chemotherapeutics. These compounds turn out to be

* Corresponding author. Tel.: +91 9411003465.
E-mail address: shamsuzzaman9@gmail.com (Shamsuzzaman).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

1878-5352 © 2013 Production and hosting by Elsevier B.V. on behalf of King Saud University.
http://dx.doi.org/10.1016/j.arabjc.2013.06.027

non-toxic, less vulnerable to multi-drug resistance (MDR) and highly bioavailable because of being capable of penetrating the cell wall (Bandey et al., 2011). Although various modifications of steroids including derivatization cyclization, heterocyclization etc. have been tried but as far the literature precedents are concerned, little efforts have been made towards the efficient synthesis and simultaneous biological analysis of steroid based benzothiazines.

Steroid based antimicrobial agents continue to play a prominent role in those organisms which do not rely upon external supply of drugs to fight against pathogens (Savage, 2002) because the entire morbidity and mortality mostly in developing countries is due to these microbial infections (Qadri et al., 2005) among which *Escherichia coli* is responsible for the most common and serious infectious diseases like invasive dysentery and diarrhoea (Zhang et al., 2006). The different microbes such as *Staphylococcus aureus*, *Streptococcus pyogenes* and *Salmonella typhimurium* have important effect on the human's mucosal health. The infection with these microorganisms may have a significant impact on huge demolition of host tissue and severe diseases (Puerto et al., 2006; Nolan et al., 1979).

Nitrogen containing steroids have the ability to regulate a variety of biological processes and thus are potential drug candidates for the treatment of a large number of diseases including breast cancer (Visvanathan and Davidson, 2003), prostate cancer (Li et al., 1995), leukaemia (He and Jiang, 1999), autoimmune diseases (Latham et al., 2003) and osteoporosis (Hosking et al., 1998). So is the case with the nitrogen containing derivative, benzothiazines in which the presence of a fold along the nitrogen–sulphur axis is one of the features responsible to impart their biological activity (Gupta et al., 1993; Gautam et al., 2009; Khandelwal et al., 2013), hence they show broad spectrum of biological activities such as antagonists (Watanabe et al., 1996), anticancer (Niewiadomy et al., 2011; Srivastav et al., 2000; Gupta et al., 1993), vasorelaxant (Cecchetti et al., 2003), antidiabetic (Matsui et al., 1994), antihypertensive (Kajino et al., 1991) and antimicrobial (Rathore and Kumar, 2006). Intrigued by the above observations and in continuation of our previous work (Shamsuzzaman et al., 2013) herein we represent one-pot synthesis of steroidal benzothiazines and investigate their antimicrobial as well as anticancer behaviour.

2. Experimental

2.1. General remarks

Chemicals were purchased from Merck and Sigma–Aldrich as 'synthesis grade' and used without further purification. Melting points were determined on a Kofler apparatus and are uncorrected. The IR spectra were recorded on KBr pellets with Pye Unicam SP3–100 spectrophotometer and values are given in cm^{-1}. ^1H and ^{13}C NMR spectra were run in $CDCl_3$ on a JEOL Eclipse (400 MHz) instrument with TMS as internal standard and values are given in ppm (δ). Mass spectra were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer. Thin layer chromatography (TLC) plates were coated with silica gel G and exposed to iodine vapours to check the homogeneity as well as the progress of reaction. Sodium sulphate (anhydrous) was used as a drying agent.

2.2. Chemistry

2.2.1. General method for the synthesis of steroidal benzothiazines (4–6)

To a solution of steroidal ketones **(1–3)** (1 mmol) in absolute ethanol (10 mL) was added 2-aminothiophenol (1 mmol) and iodine (2 mmol) in the same solvent (25 mL) and the reaction mixture was refluxed for about 19–21 h. The progress of the reaction was monitored by TLC (Petroleum benzene: ether, 3:1). After completion of reaction the excess solvent was removed to three fourths of the original volume under reduced pressure. Then it was cooled to room temperature, diluted with $Na_2S_2O_7$ solution and subsequently with water. The mixture was extracted in ether, washed with water and finally dried over anhydrous Na_2SO_4. Evaporation of solvents and recrystallization from methanol afforded respective products **(4–6)**.

*2.2.1.1. 3β-Acetoxy 5α-cholestano [5,6-b] benzothiazine (**4**).* Yield: 80%; Solid; m p: 163–165 °C; Anal. Calcd. for $C_{35}H_{51}NO_2S$: C 76.17, H 9.08, N 2.32; Found C 76.45, H 9.35, N 2.55. IR (KBr, cm^{-1}): 1714 ($OCOCH_3$), 1650 (C═N), 3060, 1603 (aromatic), 750 (C–S), 1388 (C–N), 1206 (C–O). ^1H NMR (400 MHz, $CDCl_3$): δ 6.33–6.28 (*m*, 4H, aromatic), 4.7 (*m*, 1H, C_3 α-H, $W½ = 15$ Hz), 2.03 (*s*, 3H, $OCOCH_3$), 1.8 (*d*, 2H, C_4–H_2, $J = 8.0$ Hz), 1.9 (*d*, 2H, C_7–H_2, $J = 5.2$ Hz), 1.18 (*s*, 3H, C_{10}–CH_3), 0.70 (*s*, 3H, C_{13}–CH_3), 0.97 & 0.83 (other methyl protons). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174, 163, 148, 129, 128, 126, 124, 122, 73, 48, 46, 42, 39, 35, 26, 24, 22, 20, 17. ESI MS: 549 [M^+].

*2.2.1.2. 3β-Chloro 5α-cholestano [5,6-b] benzothiazine (**5**).* Yield: 85%; Solid; m p: 146–147 °C; Anal. Calcd. for $C_{33}H_{48}ClNS$: C 75.07, H 9.03, N 2.61; Found C 75.32, H 9.19, N 2.66. IR (KBr, cm^{-1}): 1626 (C═N), 3058, 1598 (aromatic), 710 (C–S), 1380 (C–N), 740 (C–Cl). ^1H NMR (400 MHz, $CDCl_3$): δ 6.43–6.24 (*m*, 4H, aromatic), 3.5 (*m*, 1H, C_3 α-H, $W½ = 17$ Hz), 2.07 (*d*, 2H, C_7–H_2, $J = 8.0$ Hz), 1.87 (*d*, 2H, C_4–H_2 $J = 4.8$ Hz), 1.18 (*s*, 2H, C_{10}–CH_3), 0.75 (*s*, 3H, C_{13}–CH_3), 0.97 & 0.80 (other methyl protons). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164, 146, 127, 126, 125, 123,122, 59, 48, 46, 42, 39, 35, 26, 24, 22, 20, 17. ESI MS: 525/527 [M^+].

*2.2.1.3. 5α-Cholestano [5,6-b] benzothiazine (**6**).* Yield: 83%; Solid; m p: 152–154 °C; Anal. Calcd. for $C_{33}H_{48}NS$: C 80.12, H 9.86 N 2.63 Found C 80.59, H 10.04, N 2.85. IR (KBr, cm^{-1}): 1628 (C═N), 3062, 1600 (aromatic), 711 (C–S), 1385 (C–N). ^1H NMR (400 MHz, $CDCl_3$): δ 6.43, 6.24 (*m*, 4H, aromatic), 2.05 (*d*, 2H, C_4–H_2, $J = 8.0$ Hz), 2.04 (*d*, 2H, C_7–H_2, $J = 4.4$ Hz), 1.17 (*s*, 2H, C_{10}–CH_3), 0.75 (*s*, 3H, C_{13}–CH_3), 0.97 & 0.80 (other methyl protons). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163, 149, 125, 124, 122.8, 122, 120, 48, 46, 42, 39, 35, 26, 24, 22, 20, 17. ESI MS: 491 [M^+].

2.3. Anticancer activity

2.3.1. Cell lines and culture conditions

Human cancer cell lines SW480 (colon adenocarcinoma cells), HeLa (cervical cancer cells), A549 (lung carcinoma cells), and HepG2 (hepatic carcinoma cells) were taken for the study. SW480, A549 and HepG2 cells were grown in RPMI 1640