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ORIGINAL ARTICLE

*2nd Cancer Update*

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

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**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

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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 ($\delta$). 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, $CDCl_3$): $\delta$ 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, $CDCl_3$): $\delta$ 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, $CDCl_3$): $\delta$ 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, $CDCl_3$): $\delta$ 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, $CDCl_3$): $\delta$ 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

(Chiba et al., 2012) supplemented with 10% foetal bovine serum (FBS), 10U penicillin and 100 μg/mL streptomycin at 37 °C with 5% $CO_2$ in a humidified atmosphere. HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Shafi et al., 2009) supplanted with FCS and antibiotics as described above for RPMI 1640. MCF10A immortalized breast cells were maintained in mammary epithelial basal medium supplemented with an MEGM mammary epithelial singlequot kit (Cambrex). NL-20 (normal lung cells), HPC (normal pulp cells), and HPLF (periodontal ligament fibroblasts) were grown at 37 °C with 5% $CO_2$, 95% air under the humidified conditions. Fresh medium was given every second day and on the day before the experiments were done. Cells were passaged at preconfluent densities, using a solution containing 0.05% trypsin and 0.5 mM EDTA.

#### 2.3.2. Cell viability assay (MTT)

The anticancer activity in vitro was measured using the MTT assay. The assay was carried out according to known protocol (Berenyi et al., 2013; Wang et al., 2012; Das et al., 2012; Mosmann, 1983). Exponentially growing cells were harvested and plated in 96-well plates at a concentration of $1 \times 10^4$ cells/well. After 24 h incubation at 37 °C under a humidified 5% $CO_2$ to allow cell attachment, the cells in the wells were respectively treated with target compounds at various concentrations for 48 h. The concentration of DMSO was always kept below 1.25%, which was found to be non-toxic to the cells. A solution of 3-(4,5-dimethylthizao1–2-y1)-2,5-diphenyltetrazolium bromide (MTT), was prepared at 5 mg/mL in phosphate buffered saline (PBS; 1.5 mM $KH_2PO_4$, 6.5 mM $Na_2HPO_4$, 137 mM NaCl, 2.7 mM KCl; pH 7.4). 20 μL of this solution was added to each well. After incubation for 4 h at 37 °C in a humidified incubator with 5% $CO_2$, the medium/MTT mixtures were removed and the formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in 100 μL of DMSO per well. The absorbance of the wells was read with a microplate reader (Bio-Rad Instruments) at 570 nm. Effects of the drug cell viability were calculated using cells treated with DMSO as control.

#### 2.3.3. Data analysis

Cell survival was calculated using the formula: Survival (%) = [(absorbance of treated cells − absorbance of culture medium)/(absorbance of untreated cells − absorbance of culture medium)] × 100 (Hui et al., 2012; Liu et al., 2010; Saxena et al., 2007; Woerdenbag et al., 1993). The experiment was done in triplicate and the inhibitory concentration (IC) values were calculated from a dose response curve. $IC_{50}$ is the concentration in 'μM' required for 50% inhibition of cell growth as compared to that of control. $IC_{50}$ values were determined from the linear portion of the curve by calculating the concentration of agent that reduced absorbance in treated cells, compared to control cells, by 50%. Evaluation is based on mean values from three independent experiments, each comprising at least six microcultures per concentration level.

### 2.4. In vitro antibacterial activity

Steroidal benzothiazines **(4–6)** and the steroidal ketones **(1–3)** were screened for the in vitro antibacterial activity against the cultures of *S. pyogenes* (ATCC 19615), *S. aureus* (ATCC-25923), *P. aeruginosa* (ATCC-27853) and *E. coli* (ATCC-25922) by disc diffusion method (Cruickshank et al., 1975; Collins, 1976). Standard inoculums $1 \times 107 - 2 \times 10^7$ c.f.u. $mL^{-1}$ (0.5 McFarland standards) were introduced onto the surface of sterile agar plates and a sterile glass spreader was used for even distribution of the inoculums. The discs measuring 6 mm in diameter were prepared from Whatman No. 1 filter paper and sterilized by dry heat for 1 h at 140 °C. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were also kept. Chloramphenicol was used as a positive control while the disc poured in DMSO was used as a negative control. The plates were inverted and incubated at 37 °C for 24 h. The susceptibility was assessed on the basis of diameter of zone of inhibition against different strains of bacteria. Inhibition zones were measured and compared with standard drug.

Minimum inhibitory concentration (MIC) which is defined as the lowest concentration of an antibacterial drug that will inhibit the visible growth of a micro-organism after overnight incubation was determined by broth dilution technique. The nutrient broth which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately $5 \times 10^5$ c.f.u. $ml^{-1}$ of actively dividing bacterial cells. The cultures were incubated at 37 °C for 24 h and the growth was monitored visually and spectrophotometrically.

### 2.5. In vitro antifungal activity

Antifungal screening of steroidal benzothiazines **(4–6)** and the steroidal ketones **(1–3)** was done against the cultures of *Candida albicans* (ATCC 10231), *Aspergillus fumigatus* (ATCC 1022), *Trichophyton mentagrophytes* (ATCC 9533) and *Penicillium marneffei* (recultured) in DMSO by agar diffusion method (Khan, 1997; Verma et al., 1998). Sabouraud agar medium was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. 20 mL of agar media was poured into each petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. using an agar punch, wells were made and each well was labelled. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days.

Minimum inhibitory concentration (MIC) was determined by broth dilution technique as in antibacterial activity. The Inhibition zones of compounds **(1–6)** were compared with Nystatin used as standard drug. The nutrient broth which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately $1.6 \times 10^4$–$6 \times 10^4$ c.f.u. $mL^{-1}$. The cultures were incubated at 35 °C for 48 h and the growth was monitored.

## 3. Results and discussion

### 3.1. Chemistry

3β-Acetoxy-5α-cholestan-6-one **1**, 3β-chloro-5α-cholestan-6-one **2,** and 5α-cholestan-6-one **3** were prepared according to the literature procedure (Anagnostopoulos and Fieser, 1954;

Backer and Squire, 1948; Rajnikant et al., 2000). Steroidal benzothiazines **(4–6)** were conventionally synthesized by reacting 5α-cholest-6-one **(1–3)** with iodine and 2-aminothiophenol in absolute ethanol. The remarkable feature of the reaction is the formation of 5α-iodoketone *in situ* as an intermediate which might be obtained separately by the reaction of ketones with iodine (Khan et al., 2008). The advantages of this synthesis are to evade the cumbersome 5α-iodocholest-6-ones as starting materials and to obtain products in better yields. In spite of this modification, the method still remains cumbersome (19–21 h reflux), but still these types of multi component reactions have proven to be the practical way for achieving large functionalized molecules because these reactions generally are one-pot and afford better yields.

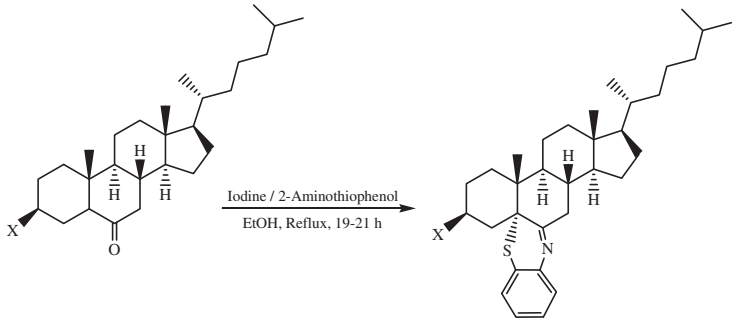
The tentative mechanism (Scheme 2) has been proposed for the formation of steroidal benzothiazines **(4–6)** from steroidal ketones **(1–3)** which involves the formation of 5α-iodo cholest-6-one *in situ* as an intermediate, which on further reaction with 2-aminothiophenol undergo $SN^1$ reaction at $C_5$ and condensation at $C_6$ resulting in cyclization that leads to the formation of corresponding product. (See Scheme 1).

The selected diagnostic bands of IR spectra of synthesized products provide useful information for determining structures of the benzothiazine derivatives. All compounds **(4–6)** exhibited absorption bands at 1626–1650 $cm^{-1}$ due to C=N stretching and 710–750 $cm^{-1}$ due to C–S. The absorption band at 3058–3062 $cm^{-1}$ and 1598–1603 $cm^{-1}$ as ascribed to the aromatic ring also confirmed the formation of benzothiazines. The formation of steroidal benzothiazines **(4–6)** was further confirmed with the NMR spectra. Assignments of the signals are based on the chemical shift and intensity pattern. In $^1H$ NMR spectra the multiplet at $\delta$ 6.43–6.24 shows the presence of aromatic protons. The singlets appeared at $\delta$ 1.18 and 0.70 are ascribed to three protons of the methyl group attached to '$C_{10}$' and three protons of the methyl group attached to '$C_{13}$' respectively. $^{13}C$ NMR signals are in good agreement with proposed structures of synthesized compounds. The compounds exhibited signals at $\delta$ 163–164 ($C_6$), 48 ($C_5$) while the signals obtained at $\delta$ 120–149 confirm the presence of aromatic ring. The distinctive signals were observed in the mass spectra of compounds **(4–6)** which followed the similar fragmentation pattern. The molecular ion peaks ($M^+$) for compounds **(4–6)** were observed at m/z 549, 525/527 and 491, respectively.

### 3.2. In vitro anticancer activity

Compounds **(1–6)** were screened for in vitro anticancer activity against different human cancer cells; **SW480**, **HeLa**, **A549** and **HepG2**. The anticancer activity in vitro was measured using the MTT assay during which the conversion of soluble yellowish MTT into the insoluble purple formazan by active mitochondrial lactate dehydrogenase of living cells has been used to develop an assay system for measurement of the cell proliferation. The anticancer screening data reported in Table 1 suggest that compounds **(4–6)** showed different levels of cytotoxicity during which compound **6** showed effective $IC_{50}$ = 13.73 μmol $L^{-1}$ against HeLa cell line. Compounds **4** and **6** also showed minimum $IC_{50}$ value in the range of 15.83 μmol $L^{-1}$ (HepG2) and 16.89 μmol $L^{-1}$ (A549), respectively. Next to these, compound **4** showed minimum $IC_{50}$ = 22.67 μmol $L^{-1}$ against SW480 cell line. The $IC_{50}$ shown by the starting compounds **(1–3)** were found either ⩾40 or much higher > 50 μmol $L^{-1}$. It is clear from the anticancer screening data that after the appending of benothiazine moiety to the steroidal ketone derivatives **(1–3)**, there occurs moderate to good increase in anticancer activity (Table 1). During anticancer screening, none of the compounds were found as effective as the standard anticancer drugs like Doxorubicin, 5-Fluorouracil or Cisplatin, except compound **6** which showed $IC_{50}$ = 13.73 μmol $L^{-1}$ which is more effective than 5-Fluorouracil ($IC_{50}$ = 16.32 μmol $L^{-1}$) against HeLa cell line. But further modifications and derivatization may lead to the development of more active cytotoxic agents.

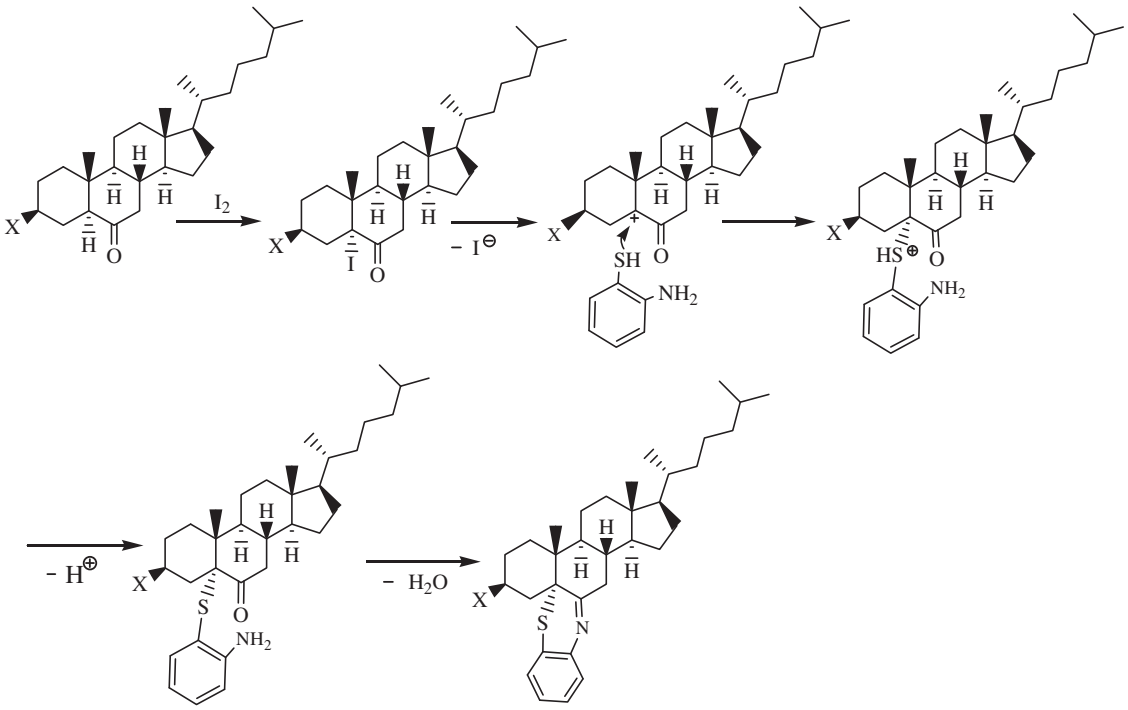
To confirm the cytotoxicity of steroidal benzothiazines, compounds **(4–6)** were tested with some non-cancer cell lines MCF10A (breast), NL-20 (lung), HPC (pulp) and HPLF (Periodontal) during which none of the synthesized compounds were found toxic, all the compounds showed $GI_{50}$ > 55 μmol $L^{-1}$. This also suggests that the steroidal benzothiazine derivatives can be used specifically for the treatment of cancer cells without showing toxicity to the non-cancer cells. The $GI_{50}$ which is the molar concentration causing 50% growth inhibition of non-cancerous cells by compounds **(4–6)** and Cisplatin are given in Table 2.




**X**
OAc (**1**)
Cl (**2**)
H (**3**)

**X**
OAc (**4**)
Cl (**5**)
H (**6**)

**Scheme 1** Showing the formation of steroidal benzothiazines **4–6**.



**Scheme 2** Mechanism for the formation of steroidal benzothiazines **4–6**.

**Table 1** Showing anticancer activity data of compounds 1–6 against human cancer cell lines.

| Compound | $IC_{50}$ (μmol $L^{-1}$)[a] | | | |
|---|---|---|---|---|
| | SW480 | A549 | HepG2 | HeLa |
| **1** | 39.77 | 46.13 | > 50 | > 50 |
| **2** | > 50 | > 50 | 49.22 | > 50 |
| **3** | 47.28 | 40.61 | > 50 | > 50 |
| **4** | 22.67 | 29.85 | 15.83 | 31.43 |
| **5** | > 50 | 41.52 | 37.65 | 49.18 |
| **6** | 27.73 | 16.89 | > 50 | 13.73 |
| **Doxorubicin** | 10.9 | 13.5 | 11.52 | 12.52 |
| **5-Fu** | 15.71 | 12.8 | 33.6 | 16.32 |
| **Cisplatin** | 3.52 | 12.1 | 9.63 | 9.43 |

Cell growth inhibition was analysed by the MTT assay.
**5-Fu** = 5-Fluorouracil.
[a] $IC_{50}$ = the concentration of compound that inhibits 50% of cell growth.

**Table 2** The $GI_{50}$ values shown by compounds **4–6**, Doxorubicin, Cisplatin and 5-Fluorouracil against noncancer cells.

| Compounds | **$GI_{50}$ (μ$ML^{-1}$)** | | | |
|---|---|---|---|---|
| | Breast MCF10A | Lung NL-20 | Pulp HPC | Periodontal HPLF |
| **4** | 68.41 | 90.21 | 63.31 | > 100 |
| **5** | 72.11 | > 100 | 59.16 | 66.33 |
| **6** | > 100 | 60.92 | 67.78 | > 100 |
| **Doxorubicin** | > 100 | 91.77 | > 100 | > 100 |
| **Cisplatin** | 26.17 | 51.25 | 63.35 | 61.17 |
| **5-FU** | > 100 | 83.54 | 73.62 | > 100 |

$GI_{50}$ is the molar concentration causing 50% growth inhibition of non-cancerous cells.
Values are the mean of triplicates of at least two independent experiments.
**5-FU** = 5-Fluorouracil.

### *3.3. In vitro antimicrobial activity*

The antimicrobial screening data obtained after treating different microbial strains with test doses of the steroidal derivatives **(1–6)** are given in Tables 3–6 and the values are reported in terms of the zone of inhibition in 'mm' and minimum inhibitory concentration in 'μg $mL^{-1}$'. It is clear from the antimicrobial screening data that compounds **(4–6)** showed moderate to good increase in antimicrobial activity than the starting compounds **(1–3)**. In antibacterial activity, compounds **4** and **6** showed potential inhibition zones against *E. coli* strain. Even compound **4** was found to be more potent than the reference drug, Chloramphenicol, in the case of *E. coli* strain. As shown in Table 3, compound **4** showed potential zones of inhibition *i.e.* 18.6, 19.6 and 21.5 mm against *S. aureus*, *S. pyogenes* and *P. aeruginosa*, respectively in comparison with Chloramphenicol. Compound **6** also showed influential zone of inhibition *i.e.* 19.4 mm against the *E. coli* strain which is almost equal to the inhibition zone of Chloramphenicol *i.e.* 20.0 mm against the same strain. The MIC's of compounds **1–6** with different bacterial strains are given in Table 4 and it is clear from the data that MIC's of steroidal ketones **(1–3)** are two to four fold higher than those of benothiazine derivatives **(4–6)** suggesting that compounds **(4–6)** are active at lower concentrations than those of compounds **(1–3)**. From Table 4 it is also clear that the lowest concentration at which compound **4** inhibited the visible growth of bacteria is 32 μg/mL and 64 μg/mL while compounds **5** and **6** inhibited the growth of bacteria at 32 μg/mL, 64 μg/mL and 128 μg/mL against the

**Table 3** Showing the zone of inhibition of compounds **1–6** with different bacterial strains.

| Compounds | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | *S. aureus* | *S. pyogenes* | *P. aeruginosa* | *E. coli* |
| **1** | 14.3 ± 0.4 | 12.2 ± 0.4 | 12.6 ± 0.2 | 14.2 ± 0.4 |
| **2** | 12.2 ± 0.2 | 10.3 ± 0.2 | 10.4 ± 0.4 | 11.2 ± 0.2 |
| **3** | 12.5 ± 0.4 | 11.6 ± 0.5 | 10.5 ± 0.5 | 10.2 ± 0.5 |
| **4** | 18.6 ± 0.2 | 19.6 ± 0.4 | 21.5 ± 0.5 | 23.2 ± 0.2 |
| **5** | 19.5 ± 0.4 | 15.3 ± 0.8 | 18.2 ± 0.4 | 15.6 ± 0.6 |
| **6** | 18.6 ± 0.1 | 15.1 ± 0.6 | 18.8 ± 0.4 | 19.4 ± 0.6 |
| Chloramphenicol | 21.6 ± 0.5 | 22.5 ± 0.4 | 25.2 ± 0.8 | 20.0 ± 0.2 |
| DMSO | – | – | – | – |

**Table 4** Showing the MIC's of compounds **1–6** with different bacterial strains.

| Strains | MIC (μg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | Chloramphenicol |
| *S. aureus* | 64 | 128 | 256 | 32 | 32 | 32 | 32 |
| *S. pyogenes* | 64 | 128 | 256 | 32 | 64 | 64 | 32 |
| *P. aeruginosa* | 128 | 128 | 128 | 64 | 64 | 128 | 32 |
| E. coli | 64 | 256 | 128 | 32 | 128 | 32 | 32 |

different bacterial strains. From Tables 3 and 4, it is clear that the compounds which show lower MIC are having larger zone of inhibition and are therefore active antibacterial agents.

It is clear also from the antifungal screening data that compounds **(4–6)** showed moderate to good increase in antifungal activity than the starting compounds **(1–3)**. In antifungal activity, compounds **4** and **5** showed potential inhibition zones against *P. marneffei* strain. But the inhibition zone of compound **5** is larger than compound **4** against *P. marneffei* in comparison with the standard drug, Nystatin. As shown in Table 5, compound **4** showed potential zones of inhibition *i.e.* 24.5, 21.4, 16.6 and 15.1 mm against *C. albicans*, *T. mentagrophytes*, *P. marneffei* and *A. fumigatus*, respectively in comparison with the reference drug, Nystatin. Compounds **5** and **6** also showed influential zone of inhibition *i.e.* 17.4 and 24.2 mm against the *P. marneffei* and *T. mentagrophytes* strains which are very close to that of Nystatin *i.e.* 19.5 and 29.0 mm, respectively. The MIC's of compounds **(1–6)** with different fungal strains are given in Table 6 and it is clear from the data that MIC's of steroidal ketones **(1–3)** are two to four fold higher than those of benothiazine derivatives **(4–6)** revealing that compounds **(4–6)** are active against different fungal strains at lower concentrations than those of compounds **(1–3)**. From Table 6 it is also clear that the lowest concentration at which compounds **4** and **5** inhibited the visible growth of fungi is 32 μg/mL and 64 μg/mL while compound **6** inhibited the growth of fungi at lower concentration *i.e.* 32 μg/mL against the different fungal strains. From the data given in Tables 5 and 6, it is quite clear that the compounds which are showing lower MIC are having efficacious zone of inhibition and are therefore active antifungal agent while the compounds which are not showing lower MIC did not show potential zone of

**Table 5** Showing the zone of inhibition of compounds **1–6** with different fungal strains.

| Compounds | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | *C. albicans* | *T. mentagrophytes* | *P. marneffei* | *A. fumigatus* |
| **1** | 13.1 ± 0.5 | 14.1 ± 0.5 | 11.3 ± 0.5 | 11.5 ± 0.2 |
| **2** | 12.2 ± 0.2 | 09.2 ± 0.2 | 10.1 ± 0.5 | 09.2 ± 0.4 |
| **3** | 14.2 ± 0.5 | 11.2 ± 0.4 | 12.1 ± 0.2 | 10.1 ± 0.3 |
| **4** | 24.5 ± 0.3 | 21.4 ± 0.6 | 16.6 ± 0.3 | 15.1 ± 0.4 |
| **5** | 19.1 ± 0.4 | 18.2 ± 0.4 | 17.4 ± 0.5 | 14.4 ± 0.2 |
| **6** | 23.5 ± 0.2 | 24.2 ± 0.5 | 15.8 ± 0.2 | 16.0 ± 0.5 |
| Nystatin | 29.0 ± 0.5 | 29.0 ± 0.5 | 19.5 ± 0.5 | 19.5 ± 0.5 |
| DMSO | – | – | – | – |

**Table 6** Showing the MIC's of compounds **1–6** with different fungal strains.

| Strains | MIC (μg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | Nystatin |
| *C. albicans* | 256 | 256 | 128 | 32 | 64 | 32 | 32 |
| T. mentagrophytes | 128 | 256 | 256 | 64 | 64 | 32 | 32 |
| *P. marneffei* | 256 | 128 | 128 | 32 | 32 | 32 | 32 |
| *A. fumigatus* | 128 | 256 | 256 | 32 | 32 | 32 | 32 |

inhibition at the fixed concentration against different fungal strains. This study also supports that benzothiazine moiety after being attached with steroid nucleus might be one of the factors responsible for enhanced antimicrobial behaviour.

## 4. Conclusions

In summary, the successfully developed, convenient and operationally simple reaction for an efficient synthesis of steroidal benzothiazines involved the reaction of steroidal ketone with iodine and 2-aminothiophenol in absolute ethanol. The reaction completed in 19–21 h and on completion, better yields (80–85%) were obtained. This strategy offered a very straight forward, one-pot and efficient method for access to steroidal benzothiazines. The anticancer screening data suggest that compounds **(4–6)** showed potential increase in anticancer activity than the starting compounds **(1–3)** and the possible reason may be the adjoining of the benzothiazine moiety to the steroidal nucleus because the presence of fold along the nitrogen and sulphur axis in benzothiazine derivatives is one of the features responsible for imparting the biological activity (Bandyopadhyay et al., 2012; Niewiadomy et al., 2011). Compounds **4** and **6** showed potential anticancer activity with $IC_{50}$ = 13.73 μmol $L^{-1}$ (HeLa) and 15.83 μmol $L^{-1}$ (HepG2), respectively. None of the compounds showed anticancer activity equivalent to the standard drugs except compound **4** which showed more effective anticancer activity than Doxorubicin against HeLa cells. Thus during anticancer screening compounds **4** and **6** were found to be potential anticancer agents among all the screened compounds **(1–6)**. All the newly synthesized compounds were found almost non-toxic to the normal cells which specify that these compounds can be used safely for the treatment of cancer without damaging the normal cells. During the antimicrobial screening, all the newly synthesized compounds **(4–6)** were found more active than the starting compounds **(1–3)**. Compound **4** showed potential behaviour against *E. coli* (being more active than the reference drug, Ciprofloxacin) in antibacterial activity while the compound **5** was found almost equally potent than the reference drug, Nystatin in the case of *P. marneffei* strain in antifungal activity. The reason for this increase in the antimicrobial activity in the synthesized compounds **(4–6)** may be due to the presence of aromatic functionality present in the product molecule because most of the heterocyclic compounds adjoined with the aromatic group, often show potential antimicrobial activity (Bandey et al., 2011; El-Nakkady et al., 2012). In conclusion, the present study showed that these one pot synthesized compounds can be used as a template for future development through modification and derivatization to design more potent and selective anticancer as well as antimicrobial agents.

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