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Synthesis of novel fluorinated 1,5-benzothiazepine derivatives and their biological evaluation as anticancer and antibacterial agents

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Abstract: A series of novel fluorinated 1,5-benzothiazepine derivatives were synthesized, characterized and evaluated for *in vitro* anticancer and antibacterial activity. The *in vitro* anticancer activity of the synthesized compounds **4a**–**h** was evaluated against four human cancer cell lines namely lung (A549), breast (MCF-7), liver (HEPG2) and prostate (PC-3). Compounds **4c**, **4d**, **4g** and **4h** exhibited good activity with GI_{50} <10 μg ml^{-1} against all four human cancer cell lines which was comparable to standard drug adriamycin. Additionally, antibacterial activity of synthesized compounds was estimated using Resazurin Microtiter Assay (REMA) and compared with standard drug ampicillin. Among the synthesized compounds, **4c**, **4d**, **4g** and **4h** showed good antibacterial activity and all the synthesized compounds were found to be more active towards gram negative than gram positive bacteria. These promising results obtained from *in vitro* anticancer and antibacterial activity, inferred that the synthesized compounds are capable of being anticancer as well as antibacterial agents.

Keywords: *in vitro*; lung cancer cell line; breast cancer cell line; liver cancer cell line; prostate cancer cell line.

INTRODUCTION

One of the major world problems is cancer; due to the increased cancer cases and deaths. In the year 2020, it was anticipated that there will be 19.3 million new cancer cases and 10.0 million deaths worldwide.[1] Despite the fact that chemotherapy is most commonly used to treat cancer, the failure of existing chemotherapeutics to treat cancer highlights the need for new chemical entities to be developed.[2] Additionally, chemotherapy in cancer treatment is usually associated with various side effects and appearance of resistance.[3] Moreover, despite

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of progressive development in cancer chemotherapy, there are still insufficient cytotoxic agents that act selectively to cancer cells.

Infections are one of the reasons for compromised immunity among cancer patients. They make patient vulnerable which leads to disturbance in treatment.[4] Infectious diseases put public life in jeopardy and are responsible for a large number of deaths globally. The major issue is the resistance of pathogenic microorganisms to the available antimicrobial agents, which makes it difficult to treat with conventional antibiotics and force clinicians to rely on restricted options.[5] Therefore, the development of both new anticancer and antimicrobial entities is a necessity.

In the last decades, owing to their structural properties and wide range of biological activities, benzothiazepines have piqued the interest of researchers.[6,7] 1,5-Benzothiazepine scaffold has been used as anticancer,[8–11] antimicrobial,[11–14] anti-inflammatory,[14] anticonvulsant[15] and anti-HIV[16,17]agent. Also, 1,5-benzothiazepine scaffold is reported as acetylcholinesterase inhibitor,[18] butyrylcholinesterase inhibitor,[19] VRV-PL-8a and H^+/K^+ ATPase inhibitor.[20] Clentiazem, diltiazem, thiazesim and quetiapine are among the commercially available drugs which contain the 1,5-benzothiazepine skeleton.

Heterocycles containing fluorine atoms have a wide range of applications in pharmaceutical industry.[21] The presence of a fluorine atom alters certain physicochemical properties such as basicity, lipophilicity, bioavailability as well as binding affinity of a drug molecule to the target protein.[22] Ciprofloxacin (antibiotic), fluconazole (antifungal), 5-fluorouracil (anticancer), paroxetine (antidepressant), linezolid (antibacterial), favipiravir (antiviral) and midazolam (sedative) are some of the marketed drugs that contain fluorine atom. Prasada Rao *et al.* reported compounds with fluorinated 1,5 Benzothiazepine skeleton as anti-cancer agents.[23] Similarly, Upreti *et al.* reported 8-fluoro-1,5-benzothiazepine as promising anti-AIDS agent.[24] A series of 1,5-benzothiazepines with fluorine and 4-fluorophenyl groups have also been reported for the treatment of cancer metastasis.[25]

Based on the wide spectrum of biological activities, 1,5-benzothiazepines are good candidate and could be taken in consideration as promising anticancer and antimicrobial agents. Herein we report the synthesis, anticancer and antibacterial activity of fluorinated 1,5-benzothiazepines. Anticancer activity was evaluated *in vitro* using four different cell lines namely human lung cancer cell line (A549), human breast cancer cell line (MCF-7), human liver cancer cell line (HEPG2) and human prostate cancer cell line (PC-3). Similarly, antibacterial activity was performed using two Gram-positive strains and two Gram-negative strains.

EXPERIMENTAL

Materials and method

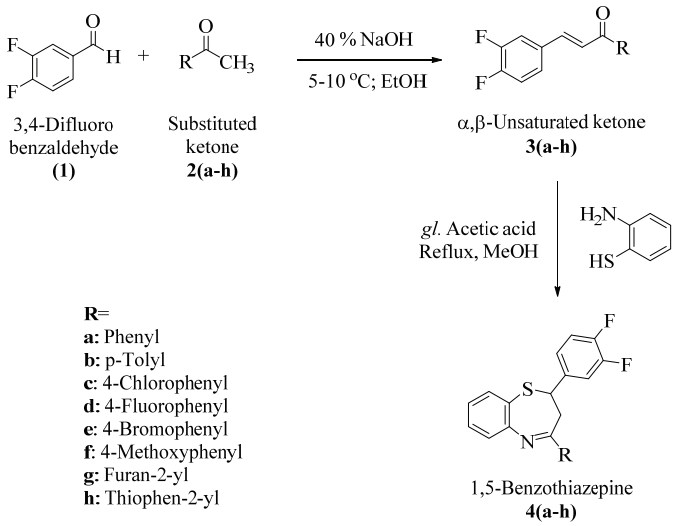
All reagents and chemicals were of analytical grade, procured from Sigma Aldrich (India), and used without further purification. Melting points are uncorrected and were recorded on Centrofix Syndicate MP apparatus. Merck silica gel 60 F254 TLC plates were used to monitor the reaction. FTIR spectra were recorded on Perkin Elmer, Frontier equipment with ATR. ^{1}H- (300 MHz) and ^{13}C-NMR (75 MHz) were recorded on Bruker Avance II using TMS as the internal standard in $CDCl_3$ and DMSO-d_6. ESI mass spectra were recorded on AB SCIEX 3200 QTRAP mass spectrometer. Elemental analysis (CHNS) was carried out on model EA300, Euro Vector, Italy. *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Salmonella typhi* (ATCC 23564) and *Escherichia coli* (ATCC 25922) cultures were procured from National Chemical Technology, Pune, Maharashtra and were used for antibacterial assay.

Analytical and spectral data are given in the Supplementary material to this paper.

Chemistry

Synthesis of α,β-*unsaturated ketone derivatives (**3a**–**h**).*[26] A mixture of 3,4-difluorobenzaldehyde derivative (5 mmol) and acetophenone derivative (6 mmol) were dissolved in 15 ml ethanol, then NaOH 40 % solution (5 ml) was added dropwise. The temperature of reaction was maintained below 10 °C and the reaction mixture was stirred until precipitation of solid. The precipitate was then filtered, washed several times with cold water and recrystallized from aqueous ethanol.

*Synthesis of 1,5-benzothiazepine derivatives (**4a**–**h**), Scheme 1.* A mixture of *α,β*-unsaturated ketone (5 mmol) and 2-aminothiophenol (5 mmol) were taken in methanol (10 ml) containing catalytic amount of glacial acetic acid. The reaction mixture was allowed to reflux until completion as indicated by TLC. After completion, the reaction was allowed to cool to room temperature and the solid obtained was filtered, washed several times with methanol and recrystallized using ethanol to afford yellow solid.



Scheme 1. Synthetic pathway of fluorinated 1,5-benzothiazepine derivatives.

Biology

In vitro *anticancer activity*. The sulforhodamine B (SRB) assay was used to test the anticancer activity of the synthesized derivatives against four human cancer cell lines namely lung (A549), breast (MCF-7), liver (HEPG2) and prostate (PC-3) cancer.[27] The cell lines were grown in RPMI 1640 medium for 24 h before being inoculated into 96-well plates and incubated at 37 °C. After that, the cells were fixed with 10 % trichloroacetic acid (TCA) and tested with drugs at four dose levels (10, 20, 40, and 80 μg ml^{-1}) with doxorubicin serving as a control. After adding the compounds, the plates were incubated for 48 h until the assay was terminated with the addition of cold TCA. TCA (30 %) was used to fix the cells, which were then incubated at 4 °C for 1 h before being stained with SRB solution for 20 min. Further, the excess dye was discarded by washing with 1 % acetic acid and air dried. The protein-bound dye was then eluted with a 10mM Tris base, and the absorbance was measured at 540 nm using a plate reader. Growth was calculated and expressed as the ratio of average absorbance of the test well (T_i) to the average absorbance of the control wells (C):

$$\text{Growth inhibition} = 100(T_i/C) \quad (1)$$

where C = control growth and T_i = test growth in the presence of drug at the four concentration levels. The experiment was done in triplicate and the average values were plotted as control growth versus drug concentrations.

Antibacterial assay

The antimicrobial activity of synthesized compounds was investigated using the resazurin microtiter assay (REMA) in aseptic conditions using a 96 well microtitre plate as described previously.[28] 50 μl solution of test material dissolved in 2 % DMSO was added after filling all the wells of the microtiter plate with 50 μl of nutrient broth. Two-fold serial dilution was achieved by transferring 50 μl test material from the top well of the first row to the following wells in the next row of the same column and resulted in a graded sequence of concentrations (500, 250, 125, 62.5, 31.25, 15.63, 7.81 and 3.90 μg ml^{-1}). After that, 50 μl of bacterial suspension were added to each well, resulting in a final concentration of 0.5 Mcfarland standard cfu ml^{-1}. Later, all plates containing test materials were incubated for 24 h at 37 °C. After 24 h, each well was given 0.2 % resazurin, and a visual change in colour was observed in the wells. The transition from purple to pink/colorless was taken as a positive. The *MIC* value for that particular sample was recorded, as the lowest concentration in the column containing the sample at which no color shift occurred, and compared to the standard drug ampicillin.

RESULTS AND DISCUSSION

Chemistry

Synthesis of fluorinated 1,5-benzothiazepines **4a**–**h** followed the path shown in Scheme 1. The compounds were synthesized by reaction of *α*,*β*-unsaturated ketone with 2-aminothiophenol in presence of catalytic amount of glacial acetic acid. The synthesized novel compounds **4a**–**h** were verified by various techniques like IR, NMR (^{1}H and ^{13}C), MS and elemental analyses (CHN).

FTIR spectra of the synthesized compounds **4a**–**h** showed appearance of C–H in the range of 2844–3105 cm^{-1}, a characteristic band in the range of 1595––1608 cm^{-1} confirms presence of C=N bond in the synthesized compound, also presence of band in range of 1311–1322 cm^{-1} indicates presence of C–N.

Presence of band in the range of 681–688 cm^{-1} indicates formation of C–S–C bond. ^{1}H-NMR spectra of synthesized compounds shows presence of three characteristics peaks apart from aromatic protons (δ 8.12–6.50 ppm). The three peaks in the aliphatic region are due to CH_2 and CH. Two protons of CH_2 are diastereotopic in nature (H_a and H_b), H_a shows triplet at δ 2.88–2.99 ppm with J_{ab} value 12.4–12.7 Hz and the other H_b along with C<u>H</u> (H_x) shows doublet of doublet at δ 3.19–3.28 ppm with J_{bx} in range12.9–13.0 Hz; J_{ab} in range 4.8–5.1 Hz and δ 4.88–4.99 ppm with J_{ax} in range 11.8–12.5 Hz; J_{ab} in range 4.6–5.1 Hz, respectively, due to abx system. The ^{13}C-NMR showed characteristics peak at δ 37–38 ppm denoting <u>C</u>H and at δ = 59 ppm confirming presence of <u>C</u>H_2. Hence, the interpreted data obtained from the spectra corroborated with the structure of synthesized compound. For further verification, ESI-MS was performed, *m*/*z* values obtained were in good agreement with measured mass. Further elemental analysis confirmed the purity of the synthesized compounds as the experimental composition was found to be similar with the theoretical composition.

Biology

In vitro *anticancer activity*. The *in vitro* anticancer activity of the synthesized compounds **4a**–**h** was assessed using sulforhodamine B (SRB) assay with adriamycin as standard drug. The results were described in terms of GI_{50} (concentration that reduces total cell growth by 50 values) and are delineated in Table I. The results concluded that among the synthesized compounds, compound **4c**, **4d**, **4g** and **4h** exhibited good activity with $GI_{50} < 10$ μg ml^{-1} against all four cell lines, while rest of the compounds exhibited moderate to poor activity with $GI_{50} > 10$ μg ml^{-1}.

TABLE I. *In vitro* anticancer activity (GI_{50} / μg ml^{-1}); GI_{50} = concentration of drug causing 50 % inhibition of cell grow. For pure compounds, GI_{50} value ≤ 10 μg ml^{-1} is considered to demonstrate activity

Compound	Cell line			
	A549	MCF-7	HEP G2	PC-3
4a	68.2	68.6	57.6	>80
4b	27.8	48.2	32.6	28.1
4c	<10	<10	<10	<10
4d	<10	<10	<10	<10
4e	22.1	<10	16.9	<10
4f	32.3	35.1	33.4	11.6
4g	<10	<10	<10	<10
4h	<10	<10	<10	<10
Adriamycin	<10	<10	<10	<10

From the structure activity relationship, the activity of heterocyclic and halogenated compounds was outstanding when compared to other substituents. The

compounds were found to be in the order: –F group > –Cl group > –Br group > > –OMe group > –Me group > –H denoting that the compounds having electronegative groups showed excellent activity.

Antibacterial assay. The antibacterial activity of the synthesized compounds **4a**–**h** were evaluated against two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative (*Escherichia coli* and *Salmonella typhi*) bacteria using REMA. Ampicillin was taken as positive control. The antibacterial potential of the synthesized compounds were assessed by minimum inhibitory concentration (*MIC*) values and are displayed in Table II. Result obtained indicates that all the synthesized compounds are potent antibacterial agents. Among which, compound **4c**, **4d**, **4g** and **4h** exhibited excellent activity w.r.t the standard (ampicillin) against all the bacterial strains inferring that hetero and halogenated substances show excellent results. The results outlined suggested that the synthesized compounds are more active towards Gram-negative strain than Gram-positive strain.

TABLE II. *In vitro* bacterial activity using REMA method (*MIC* / μg ml^{-1})

Compound	Strain			
	Gram-positive		Gram-negative	
	S. aureus	*B. subtilis*	*E. coli*	*S. typhi*
4a	62.5	62.5	62.5	62.5
4b	62.5	125	62.5	62.5
4c	31.25	31.25	15.625	31.25
4d	15.625	15.625	15.625	15.625
4e	62.5	125	62.5	62.5
4f	62.5	125	62.5	62.5
4g	15.625	31.25	15.625	15.625
4h	15.625	15.625	31.25	15.625
Ampicillin	31.25	31.25	15.625	31.25

CONCLUSION

To conclude, we synthesized series of fluorinated 1,5-benzothiazepine derivatives **4a**–**h**. The synthesized compounds were evaluated for *in vitro* anticancer and antibacterial activity. The *in vitro* anticancer activity was performed using four human cancer cell lines namely A549, MCF-7, HEPG2 and PC-3. Compounds **4c**, **4d**, **4g** and **4h** exhibited excellent activity with GI_{50} <10 μg ml^{-1} against all four cell lines which is comparable to standard drug adriamycin. Further, the synthesized compounds were subjected to antibacterial activity using resazurin microtiter assay (REMA) with ampicillin as standard drug. The compounds **4c**, **4d**, **4g** and **4h** exhibited elegant antibacterial activity and the synthesized compounds were found to be more active towards Gram-negative than

Gram-positive bacteria. Hence, the synthesized fluorinated 1,5-benzothiazepine derivatives have potential to be an anticancer as well as antibacterial agents.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/10712, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА НОВИХ ФЛУОРИСАНИХ ДЕРИВАТА 1,5-БЕНЗОТИАЗЕПИНА И ЊИХОВА БИОЛОШКА ПРОЦЕНА КАО АНТИКАНЦЕРОГЕНИХ И АНТИБАКТЕРИЈСКИХ СРЕДСТАВА

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Синтетисана је серија флуорованих деривата 1,5-бензотиазепина, једињења су окарактерисана и испитана је њихова *in vitro* антиканцерска и антибактеријска активност. *In vitro* антиканцерска активност једињења **4a**–**h** испитана је према ћелијским линијама хуманог канцера плућа (A549), груди (MCF-7), јетре (HEPG2) и простате (PC-3). Једињења **4c**, **4d**, **4g** и **4h** имају добру активност, која износи GI_{50} < 10 μg ml^{-1} према свим испитиваним ћелијским линијама, и блиска је активности стандардног лека адриамицина. Такође, антибактеријска активност једињења је испитана употребом ресазурин микротитар есеја (resazurin microtiter assay, REMA) и добијене вредности су упоређене са активношћу стандардног лека ампицилина. Од синтетисаних једињења, једињења **4c**, **4d**, **4g** и **4h** показују добру антибактеријску активност и утврђено је да су сва синтетисана једињења активнија према грам-негативним него према грам-позитивним бактеријама. Резултати добијени из *in vitro* антиканцерске и антибактеријске активности су охрабрујући и указују да би синтетисана једињења могла да буду добри антиканцерски и антибактеријски агенси.

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