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Synthesis and *in vitro* anti-breast cancer activity of some novel 1,5-benzothiazepine derivatives

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Abstract: The title compounds **3a**–**j**, substituted 1,5-benzothiazepines, were synthesized by the condensation of variously substituted chalcones **1** and 2-aminothiophenol **2** *via* conventional as well as non-conventional inorganic solid support microwave irradiation methods. The non-conventional protocol offers several advantages, such as simple procedure, fast reaction rate, mild reaction conditions and improved yields, compared to conventional methods. The structures of the products **3a**–**j** were established by elemental analysis, FTIR, ^{1}H-NMR, ^{13}C-NMR and mass spectroscopic studies. The synthesized compounds were also evaluated for their cytotoxicity against the human breast cancer cell line MDA-MB-435, with some exhibiting *in vitro* anti-breast cancer activity.

Keywords: 1,5-benzothiazepines; 2-aminothiophenol; chalcones; anti-breast cancer activity.

INTRODUCTION

Disease poses a major threat to human beings and scientists are fighting to find solutions in the form of various medications. When the era of synthetic drugs began, it opened thousand doors for the development of various synthetic molecules with potential action. Chalcones are the well known intermediates for the synthesis of various differently sized bioactive heterocycles, such as isoxazoles, pyrimidines, pyrazoles, *etc.*, which have been reported to possess various biological activities, such as antimicrobial,[1,2] anti-inflammatory,[3] antimalarial,[4] antioxidant[5] and antitubercular.[6]

Among these chalcone-derived heterocycles, benzothiazepines are well known important nitrogen and sulphur containing compounds that possess a broad spectrum of biological activities, such as antimicrobial,[7,8] anti-convulsant,[9] anti--HIV,[10] anti-cytotoxic,[11] anticancer,[12,13] DPPH free radical scavenger[14] and inhibition of cholinesterases, ureases and α-glycosidases. Due to biological acti-

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vities, various conventional[15–21] and non-conventional[22–24] approaches have been developed for the synthesis of 1,5-benzothiazepines derivatives.

Cancer is one of the most dangerous, fast propagating diseases of the present century with quite high mortality rates even in developed countries. The situation is even worse in undeveloped countries due to a lack of knowledge, poverty and the non-availability of quality drugs.[25]

As part of our continuing research in the development of new bioactive heterocycles containing nitrogen and sulphur atoms, simple and convenient methods for the transformation of chalcones into 1,5-benzothiazepines by conventional and non-conventional methods are described herein. The non-conventional approaches were based on the observation that organic solvents in classical procedures are used in much larger quantities than the solutes they carry. Furthermore, the employment of solid supports in conjugation with microwaves[26–30] leads to high yields, remarkable reaction rate enhancements, and high catalytic activities with the optimum utilisation of energy.

The anti-breast cancer activity of the synthesised compounds was also investigated.

EXPERIMENTAL

Materials, method and instrumentation

All the chemicals were of AR grade and were obtained from Sigma–Aldrich and Merck. Melting points (m.p.) were determined in open capillaries on a Veego (VMP-PM) melting point apparatus and are uncorrected. The microwave-assisted reactions were realised using a microwave synthesizer model CATA-R (Catalyst Systems, Pune, India), operating at 700 W, generating 2450 MHz frequency. The purity of the compounds was routinely checked by thin layer chromatography (TLC) with silica gel-G (Merck). The instruments used for obtaining the spectroscopic data were: IR – FTIR spectrophotometer Bruker Alpha-Zn-Se; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz), FT–NMR spectrometer Bruker AV III. The elemental analysis was realised on a Carlo Erba 1108 analyzer and the obtained values were within the ±0.5 % of the theoretical values. Column chromatography was performed on silica gel (Merck, 60–120 mesh).

*General procedure for the preparing of 1,5-benzothiazepines **3a–j***

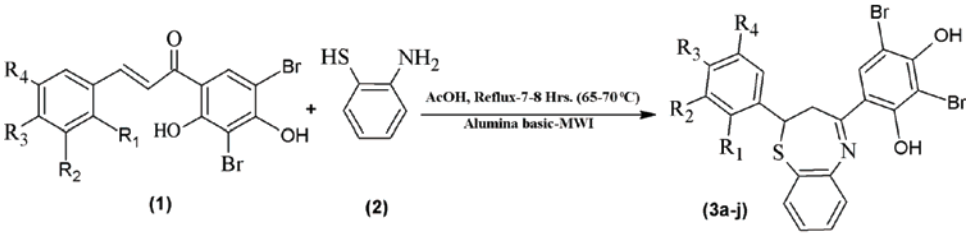
Condensation of the required chalcone (**1**) and 2-aminothiophenol (**2**) afforded the desired products **3a–j** in good yield, as shown in Scheme 1.

Conventional solution phase method. Compound **1** (0.010 mol) was dissolved in the minimum quantity of ethanol. To this, 2-aminothiophenol **2** (0.010 mol) was added and the resulting reaction mixture was refluxed for 3 h at 60–70 °C. Then, the mixture was acidified with 5–6 drops of glacial acetic acid and heating was continued for a further 4–5 h. After cooling, the content was poured onto crushed ice. The mixture was filtered and the solid was purified by recrystallization from methanol to afford compound **3a–j**.

Non-conventional solid phase method. Compound **1** (0.010 mol) was dissolved in the minimum quantity of DMF. To this, 2-aminothiophenol **2** (0.010 mol) and different inorganic solid supports (4.0 g) was added. The resulting mixture was uniformly mixed with a glass rod and air dried to remove the solvent. The absorbed material was irradiated inside the micro-

wave synthesizer for a specific time at medium power level (700 W). After completion of reaction (monitored by TLC), the reaction mixture was cooled to room temperature and the product was extracted with methanol (2×20 mL). Removal of the solvent and subsequent recrystallization with methanol resulted in analytical samples of **3a**–**j**. For the biological evaluation, the experimental drugs were solubilised in DMF solvent at 400-fold the desired final maximum test concentration and stored frozen prior to use.

Data of synthesized compounds are are given in Supplementary material.



Entry	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j
R_1	H	Cl	Cl	F	H	H	H	H	H	H
R_2	H	H	H	H	H	H	H	H	OCH_3	H
R_3	H	H	Cl	H	F	Cl	OCH_3	CH_3	OH	Br
R_4	H	H	H	H	H	H	H	H	H	H

Scheme 1. Preparation route of novel substituted 1,5-benzothiazepines (**3a**–**j**) from variously substituted chalcones (**1**) and 2-aminothiophenol (**2**) *via* conventional and non-conventional methods. The conventional method involved the 7–8 h reflux whereas the non-conventional method involved 5–7 min under microwave irradiation**.**

Biological evaluation

Protocol for in vitro *anti-cancer screening*. Human tumour breast cancer cell line (MDA-MB-435) was used in this study. The cytotoxic activity was measured *in vitro* for the newly synthesized compounds using the sulforhodamine B stain (SRB) assay method.[31,32] The cell lines were grown in RPMI 1640 medium containing 10 % foetal bovine serum and 2 mM L-glutamine. For the present screening experiment, cells were inoculated into 96-well microtiter plates. For the present screening experiment, cells were inoculated into 96 well microtiter plates in 90 µL at plating densities according to the SRB assay method. After cell inoculation, the microtiter plates were incubated at 37 °C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to the addition of the experimental drugs. After 24 h, one plate of the cell line was fixed *in situ* with trichloroacetic acid TCA, to represent a measurement of the cell population for the cell line at the time of drug addition (T_z). At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to 10 times the desired final maximum test concentration with complete medium containing test compound at a concentration of 10^{-3} M. Additional three 10-fold serial dilutions were made to provide a total of four drug concentrations plus control. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations. Different concentrations of the compound under test (10^{-7}, 10^{-6}, 10^{-5} and

10^{-4} M) were added to the cell monolayer. Triplicate wells were prepared for each individual concentration.

After compound addition, end point measurement was performed using the "Elisa" test under standard conditions for taking the absorbance at particular wavelength (690 nm reference wavelength). The percent growth was calculated on a plate-by-plate basis for the test wells relative to the control wells. The percent growth was expressed as the ratio of the average absorbance of the test well to the average absorbance of the control wells times 100. Using the six absorbance measurements (time zero (T_z), control growth (C), and test growth in the presence of drug at the four concentration levels (T_i)), the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as: $100((T_i–T_z)/(C–T_z))$ for concentrations for which $T_i \geq T_z$, *i.e.*, $(T_i–T_z)$ is positive or zero, or $100\ [(T_i–T_z)/T_z]$ for concentrations for which $T_i < T_z$, *i.e.*, $(T_i–T_z)$ is negative.

The dose response parameters were calculated for each test compound. The growth inhibition of 50 % (GI_{50}) was calculated from $100((T_i–T_z)/(C–T_z)) = 50$, which is the drug concentration resulting in a 50 % reduction in the net protein increase (as measured by SRB staining) compared to the control cells during the drug incubation.

RESULTS AND DISCUSSION

The aim of the present work was to design and synthesize some new 1,5--benzothiazepine derivatives carrying biologically active thiazepine moieties that were expected to have anti-breast cancer activity. Substituted chalcones **1** were reacted with 2-aminothiophenol **2** to give 1,5-benzothiazepine derivatives **3a**–**j**. The characterization data of the synthesized compounds are tabulated in Table I. The structures of the compounds **3a**–**j** were determined on the basis of analytical and spectroscopic data (given in the Supplementary material to this paper). Thus, the IR spectra showed bands at around 1606 (C=N), 665 (C–S) and 862 (C–Br). The ^{1}H-NMR spectra revealed the presence of a doublet at around δ 3.32 ppm, corresponding to the CH_2 group, and a triplet at around δ 4.98 ppm, corresponding to the CH group.

In view of the immense utility of eco-friendly synthetic approaches, improved syntheses of the 1,5-benzothiazepines were performed under microwave

TABLE I. Characterization data of the synthesized compounds

Entry	Molecular formula	M.p., °C	Reaction time		Yield, %	
			MW, min	Conventional, h	MW	Conventional
3a	$C_{21}H_{15}Br_2NO_2S$	80–81	5.0	6.0	78.0	64.0
3b	$C_{21}H_{14}Br_2ClNO_2S$	82–83	6.0	6.5	82.0	69.0
3c	$C_{21}H_{13}Br_2Cl_2NO_2S$	89–90	5.5	7.0	84.0	69.0
3d	$C_{21}H_{14}Br_2FNO_2S$	81–82	5.0	7.0	83.0	71.0
3e	$C_{21}H_{14}Br_2FNO_2S$	87–88	6.0	5.0	86.0	71.0
3f	$C_{21}H_{14}Br_2ClNO_2S$	90–91	6.0	6.0	80.0	69.0
3g	$C_{22}H_{17}Br_2NO_3S$	82–83	5.0	6.5	89.0	68.0
3h	$C_{22}H_{17}Br_2NO_2S$	85–86	7.0	7.5	81.0	68.0
3i	$C_{22}H_{17}Br_2NO_4S$	78–79	7.0	6.0	79.0	68.0
3j	$C_{21}H_{14}Br_3NO_2S$	93–94	6.0	7.5	90.0	72.0

irradiation. In this context, the suitability of different solid supports, *i.e.*, acidic alumina, basic alumina, neutral alumina, montmorillonite K 10 and K_2CO_3, were examined. From the results given in Table II, it is clear that basic alumina was the most adaptable and simplest catalyst and gave good yields in the synthesis of the 1,5-benzothiazepines **3a–j**. Finally in order to evaluate the use of microwave irradiation, the 1,5-benzothiazepines were also synthesised under conventional conditions. The yields and reaction times of the two preparation methods are compared in Table I.

TABLE II. Comparative study for the synthesis of **3a**–**j** by non-conventional MW using different solid supports

Exp. No.	Medium	MW Power, W	Time, min	Temp., °C	Yield, %
1	Alumina acidic	700	9–10	83	57
2	Alumina basic	700	5–7	92	80
3	Alumina neutral	700	11–12	81	64
4	Montmorillonite K 10	700	9–11	80	73
5	Potassium carbonate	700	12–13	72	69

The newly synthesized compounds were evaluated for their *in vitro* cytotoxicity against a human breast cancer cell line (MDA-MB-435) with adriamycin (ADR) as the reference drug. The relationship between control in % growth and molar drug concentration was plotted to obtain the control growth of the breast cancer cell line (MDA-MB-435). The response parameter calculated was the GI_{50} value, which corresponds to the concentration required for a 50 % inhibition of cell viability (Table III).

TABLE III. *In vitro* anticancer screening of the synthesised compounds against human breast cell line (MDA-MB-435); growth relative to the control (%), each value is the mean ± *SD* of three experiments

Compound	Molar drug concentration, mol dm^{-3}				GI_{50} / μM
	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
3a	100.00±0.00	100.00±0.00	37.54±4.95	–41.23±16.63	28.0
3b	99.66±0.39	97.44±2.60	68.84±6.13	–46.19±4.42	32.2
3c	100.00±0.00	96.72±3.18	33.62±3.67	–26.90±5.27	29.5
3d	90.95±8.00	99.99±0.01	88.24±8.34	80.39±7.35	>100
3e	97.64±2.68	95.62±4.60	77.61±7.35	–42.53±4.99	33.9
3f	99.88±0.20	98.75±2.01	79.60±4.71	–38.30±6.27	36.1
3g	99.43±1.00	98.97±1.96	66.40±3.14	–33.50±6.83	34.8
3h	98.58±2.45	96.74±3.26	84.74±7.44	–41.03±10.39	35.7
3i	95.26±4.56	91.80±7.10	78.39±3.18	–47.86±5.12	31.9
3j	100.00±0.00	100.00±0.00	95.98±3.56	–15.75±18.88	45.7
ADR	–22.63±8.93	–39.32±16.81	–48.07±19.26	–57.02±13.25	<0.1

CONCLUSIONS

By comparing different inorganic solid supports, it was concluded that basic alumina was the most suitable support for the synthesis of 1,5-benzothiazepines *via* a non-conventional method, giving the products in good yields. It was observed from the obtained results that most of the tested compounds showed some anti-breast cancer activity, as compared to the reference drug. These preliminary results of biologically screening of the tested compounds give an indication of the possible importance of the thiazepine moiety in anti-breast cancer compounds and give an encouraging framework in this field that may lead to the discovery of potent anticancer agents.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of synthesized compounds are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА НОВИХ ДЕРИВАТА 1,5-БЕНЗОТИАЗЕПИНА И ЊИХОВА *IN VITRO* АКТИВНОСТ ПРЕМА КАНЦЕРУ ДОЈКЕ

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Супституисани деривати 1,5-бензотиазепина (**3a**–**j**) синтетисани су реакцијом кондензације супституисаних халкона (**1**) и 2-аминофенола (**2**) класичним и новим поступцима под условима микроталасног озрачивања. Нова метода има неколико предности у поређењу са класичном, као што су једноставнији поступак, краће реакционо време, блажи реакциони услови, побољшан принос. Структуре производа **3a**–**j** утврђене су на основу елементалне анализе FT-ИЦ, ^{1}H-NMR и ^{13}C-NMR спектроскопије и масене спектрометрије. Испитана је цитотоксична активност синтетисаних једињења према ћелијској линији канцера дојке MDA-MB-435.

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