ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 10 | October - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

IN SILICO DESIGN, SYNTHESIS, CHARACTERISATION, AND BIOLOGICAL EVALUATION OF 1, 3-BENZOTHIAZOLE DERIVATIVES

Medical Biochemistry

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ABSTRACT

Benzothiazole nucleus serves as unique and versatile scaffolds in heterocyclic chemistry due to its increasing importance in drug discovery. 2 amino benzothiazole have been synthesized by using aniline and ammonium thiocyanate in presence of an acid. Further they undergo Schotten-Baumenn reaction to form the derivatives. For the synthesis of derivatives different acid chlorides such as nitrobenzoyl chloride, 4-flurobenzoyl chloride, 4-chlorobenzoyl chloride, benzoyl chloride and propionyl chloride are used. The formed derivatives were named as ABT1, ABT2, ABT3, ABT4 and ABT5. The identities of synthesized compounds were confirmed on the basis of their IR, NMR, and MASS spectra. Among the synthesized compounds, ABT4 and ABT2 show significant anticancer activity based in-vitro cytotoxicity study on DLA cell lines. From this, we know that 2 aminobenzothiazole derivatives are an interesting lead molecule for synthetic and biological evaluation.

KEYWORDS

benzothiazole, synthesis, derivatives, characterization, in-vitro cytotoxicity study

INTRODUCTION

The origin and advancements of medicinal chemistry and drug discovery are interwoven in nature.[1] Medicinal chemistry is a specialized science which deals with a broad range of disciplines concerned with the discovery, design and development of drug-like compounds for therapeutic use, based on molecular interactions in terms of molecular structures or its physicochemical properties involved. Moreover, it includes isolation, characterization and chemical synthesis of novel compounds that can be used in medicine for the prevention, treatment and cure of disease. [2] Drug discovery process have been focusing on highly potent leads that interact with single targets, producing measurable outcomes.[3]

In silico methods have been developed and contain quantitative structure activity relationships, databases, pharmacophores, similarity searching, homology models and other molecular modeling, which uses a computer. These methods have been frequently used in the drug discovery process for identifying drug targets via bioinformatics tools and for the illumination of absorption, distribution, metabolism, excretion and toxicity properties as well as physicochemical characterisation.[4]

Benzothiazole is an important group of aromatic heterocyclic compound that has a significant role in medicinal chemistry.[5] Benzothiazole is a bicyclic ring system having molecular formula C_7H_5NS. It contains a benzene ring fused to a thiazole ring and occupies an important position in medicinal chemistry. These two rings together constitute the basic nucleus 1, 3- benzothiazole.[6] The numbering in thiazole starts from sulphur. It is a weak base, having a variety of biological activities and still having greater attention in the scientific area.[7]

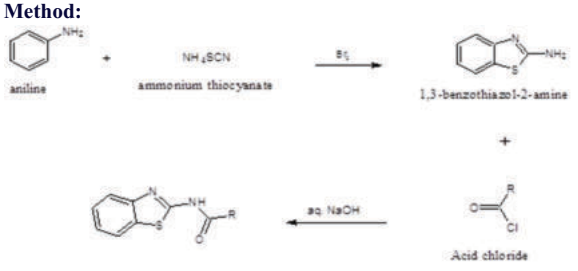
Benzothiazole plays a key role in heterocyclic compound due to the wide range of biological activities such as antimicrobial, anti-tubercular, anti-tumour, antimalarial, anti-convulsant, anthelmintic, anti-oxidant, analgesic and anti-inflammatory activity.They are found in the field of bioorganic and medicinal chemistry with application in new drug development. The study of these privileged structures in drug discovery is a rapidly emerging in the field of medicinal chemistry.[8] For instance, benzothiazole derivatives have demonstrated a wide spectrum of biological properties in both biochemical and pharmaceutical fields.[9] In view of these valid observations and as a combination of our work, provoked us to synthesize new 2 amino benzothiazole derivatives and synthesized compounds were screened for their anticancer studies.

MATERIALS AND METHOD

Materials:

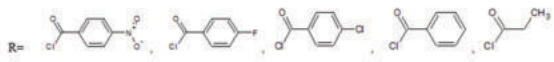
All the chemicals and reagents used in this research work were of analytical or synthetic grade. Compounds obtained were properly purified and dried using standard conditions before use, where ever necessary.

Method:



Scheme of substituted 2- amino benzothiazole

Fig. 1



Synthetic Procedure

General method for the synthesis of 2 amino benzothiazole

0.05 mol of aniline and 0.05 mol of ammonium thiocyanate were dissolved in absolute ethanol containing 4 ml of conc. Hydrochloric acid. To this mixture bromine water (0.125mol) was added and the reaction mixture was refluxed for 1:30 hr. After the completion of the reaction, the reaction mixture was mixed with crushed ice. The precipitate obtained was filtered, washed with cold water and dried. The crude product was recrystallized from ethanol. [10,11]

General method for the synthesis of 2 amino benzothiazole derivatives

In a 250 ml iodine flask, 0.25 g of 2 amino benzothiazole, 2.5 ml of 10% sodium hydroxide solution and added 0.4 ml of acid chloride derivatives (nitro benzoyl chloride, 4-chlorobenzoyl chloride, 4-flurobenzoyl chloride, benzoyl chloride, propionyl chloride). Corked the flask and shook it vigorously for about 15 minutes until the components were completely dissolved. 5 ml of cold water was added to the flask. The crude product formed was filtered in a Buchner funnel with suction and washed with cold water. Drained and dried in hot air oven at 100 ^{0}C. The product was recrystallized from ethanol.[12]

Characterization

The structural characterisation of the synthesised derivatives was done by IR, NMR and MASS spectroscopy.

Biological evaluation in-vitro cytotoxicity study

Trypan blue exclusion method: The tumor cells taken from the peritoneal cavity of tumour bearing mice were washed thrice with PBS

or normal saline. Viable cell suspension (1x 10^6 cells in 0.1 ml) was then combined with tubes containing various concentrations of test compounds and the volume was made up to1ml using phosphate buffered saline (PBS). Set control tube with cell suspension. The assay mixture was then incubated for 3 hrs at 37^0C. Further it was mixed with 0.1ml of 1% trypan blue and kept for 2-3 min and loaded on haemocytometer. Dead cells taken up the blue colour of trypan blue but live cells not take. The number of stained and unstained cells was counted separately[13].

$$\% \text{ cytotoxicity} = \frac{\text{No. of dead cell}}{\text{No.of dead cell} + \text{No.of live cell}} \times 100$$

RESULT:

ABT1: N-(1, 3-benzothiazol-2-yl)-4-nitrobenzamide

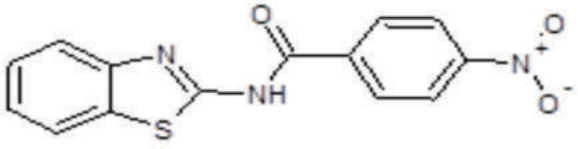


Fig. 2

Pale yellow powder; (Yield: 58%); IR Vmax/cm, (KBr): 2926 cm^{-1}(Ar-CH str), 1518 cm^{-1} (Ar-C=C str), 3174 cm^{-1} (NH str), 1693 cm^{-1} (C=O), 686 cm^{-1} (C-S str), 1286 cm^{-1} (C-N str), 1605 cm^{-1} (C=N str), 1346 cm^{-1} (Ar-NO2), 748 cm^{-1} (Ar-H); ^{1}HNMR ppm (400 MHz in DMSO-d6) data; this synthesized compound complies with standard protocol of ABT1. Mass: molecular ion peak at m/z: 300.043Da $[M+H]^+$, Molecular formula: $C_{14}H_9N_3O_3S$; Molecular weight (g/mol): 299.31

ABT 2: N-(1, 3-benzothiazol-2-yl)-4-fluorobenzamide

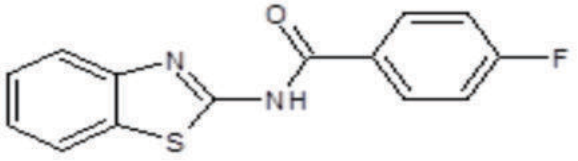


Fig. 3

Yellowish white powder; (Yield: 68%); IR Vmax/cm, (KBr): 2941 cm^{-1}(Ar-CH str), 1534 cm^{-1} (Ar-C=C str), 3519 cm^{-1} (NH str), 1672 cm^{-1} (C=O), 603 cm^{-1} (C-S str), 1288 cm^{-1} (C-N str), 1672 cm^{-1} (C=N str), 1098 cm^{-1} (C-F), 761 cm^{-1} (Ar-H); ^{1}HNMR ppm (400 MHz in DMSO-d6) data; this synthesized compound complies with standard protocol of ABT2. Mass: molecular ion peak at m/z: 273.049Da $[M+H]^+$, Molecular formula: $C_{14}H_9FN_2OS$; Molecular weight (g/mol): 272.30

ABT 3: N-(1, 3-benzothiazol-2-yl)-4-chlorobenzamide

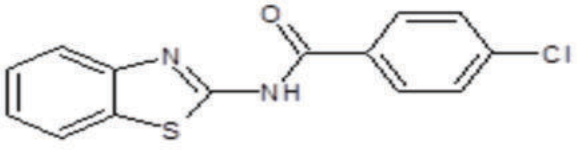


Fig. 4

White color powder; (Yield: 55%); IR Vmax/cm, (KBr): 1528 cm^{-1} (Ar-C=C str), 3344 cm^{-1} (NH str), 1722 cm^{-1} (C=O), 642 cm^{-1} (C-S str), 1322 cm^{-1} (C-N str), 1653 cm^{-1} (C=N str), 792 cm^{-1}(C-Cl), 690 cm^{-1}(Ar-H); ^{1}HNMR ppm (400 MHz in DMSO-d6) data; this synthesized compound complies with standard protocol of ABT3. Mass: molecular ion peak at m/z: 289.019Da $[M+H]^+$, Molecular formula: $C_{14}H_9ClN_2OS$; Molecular weight (g/mol): 288.76

ABT 4: N-(1, 3-benzothiazol-2-yl) benzamide

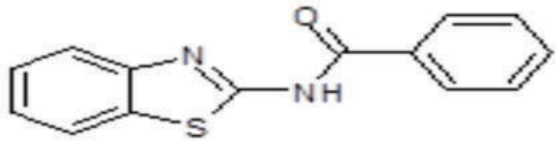


Fig. 5

Whitish yellow powder (Yield: 72%); IR Vmax/cm, (KBr): 2978 cm^{-1}(Ar-CH str), 1526 cm^{-1} (Ar-C=C str), 3233 cm^{-1} (NH str), 1600 cm^{-1} (C=O), 604 cm^{-1} (C-S str), 1249 cm^{-1} (C-N str), 1560 cm^{-1} (C=N str), 746 cm^{-1} (Ar-H); ^{1}HNMR ppm (400 MHz in DMSO-d6) data; this synthesized compound complies with standard protocol of ABT4. Mass: molecular ion peak at m/z: 255.058Da $[M+H]^+$, Molecular formula: $C_{14}H_9ClN_2OS$; Molecular weight (g/mol): 254.31

ABT 5: N-(1, 3-benzothiazol-2-yl) propanamide

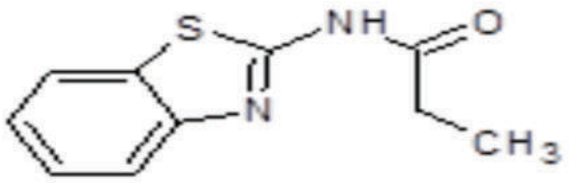


Fig. 6

Light greyish powder; (Yield: 65%); IR Vmax/cm, (KBr):3165 cm^{-1}(Ar-CH str), 1510 cm^{-1} (Ar-C=C str), 3272 cm^{-1} (NH str), 1607 cm^{-1} (C=O), 632 cm^{-1} (C-S str), 1254cm^{-1} (C-N str), 2995 cm^{-1} (CH_3 str), 1441 cm^{-1} (CH_2 str), 743 cm^{-1} (Ar-H); ^{1}HNMR ppm (400 MHz in DMSO-d6) data; this synthesized compound complies with standard protocol of ABT5. Mass: molecular ion peak at m/z: 207.058Da $[M+H]^+$, Molecular formula: $C_{10}H_{10}N_2OS$; Molecular weight (g/mol): 206.27

in-vitro cytotoxicity study

Table 1: In-vitro cytotoxicity of synthesized compounds against DLA cell lines

Compound Code	Percentage cell death (Concentration in µg/ml)					Ic50
	10 µg/ml	20 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	
ABT1	3	7	12	20	32	315.4632
ABT2	6	14	24	30	42	232.7134
ABT3	5	12	22	28	38	259.5253
ABT4	4	10	25	58	78	113.7253
ABT5	1	3	6	17	27	356.7765

DISCUSSION

The present study involves synthesis of 2 amino benzothiazole derivatives and evaluation of invitro cytotoxicity. The preliminary in silico design of different analogues of 2 amino benzothiazole was performed. The synthetic method includes two step processes. In the first step, aniline reacts with ammonium thiocyanate in presence of an acidic environment. Bromine is used as a catalyst in this reaction to yield 2 amino benzothiazole. In second step, 2 amino benzothiazole reacts with different acyl chloride in presence of a base such as sodium hydroxide to yield N-(1, 3-benzothiazol-2-yl)-4-nitrobenzamide, N-(1, 3-benzothiazol-2-yl)-4-fluorobenzamide, N-(1, 3-benzothiazol-2-yl)-4-chlorobenzamide, N-(1, 3-benzothiazol-2-yl) benzamide, N-(1, 3-benzothiazol-2-yl) propenamide The structure of ABT1, ABT2, ABT3, ABT4, and ABT5, series of compounds were established by IR, H1NMR, and mass spectra. In- vitro cytotoxicity studies of synthesized compounds were carried out by Tryphan blue dye exclusion method on DLA cell lines and the result revealed that all the test compounds had anticancer activity against DLA cell lines. It seems that ABT4 containing benzoyl group and ABT2 containing fluro compound on 2 amino benzothiazole shows significant anticancer activity.

CONCLUSION

The present study was carried out for the synthesis of some effective therapeutic derivatives of 2 amino benzothiazole derivatives with the aim of anticancer activity. The new scaffolds were prepared according to the standard protocol. From the above scaffolds, a series of new benzothiazole derivatives were synthesized in moderate to good yield. The spectral studies were performed for all the synthesized compounds for their confirmation. The compounds were further subjected to IR, NMR, and MASS study for confirmation of functional group, presence of total number of proton of compounds and molecular weight. Five synthesized compounds namely ABT1, ABT2, ABT3, ABT4, ABT5 show prominent anticancer, the present study showed that ABT4 containing benzoyl group and ABT2 containing fluro group shows enhanced cytotoxic activity. ABT1, ABT3, ABT5 shows moderate cytotoxic activity. From the whole data, we can conclude that 2 -amino

benzothiazole can be an important building block for the development of new drug applicants.

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