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Original Article

IN-SILICO DESIGN, SYNTHESIS AND *IN VITRO* ANTICANCER AND ANTITUBERCULAR ACTIVITY OF NOVEL AZETIDINONE CONTAINING ISATIN DERIVATIVES

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ABSTRACT

Objective: To design, synthesize and *in vitro* anticancer and antitubercular evaluation of some new isatin derivatives containing azetidinone.

Methods: Novel azetidinone containing isatin derivatives were designed by using various softwares such as ACD Lab ChemSketch 12.0, Marvin Sketch, Molinspiration, PASS and Schrodinger. The designed molecules having required physico-chemical properties, drug likeness and obeying Lipinski's rule of five were selected for the synthesis. These compounds were synthesized by conventional and microwave assisted synthetic methods through a series of three steps. The synthesized compounds were subjected to TLC, melting point determination, IR, ^{1}HNMR and Mass spectroscopic studies. The *in vitro* anticancer activity of selected compounds was evaluated against MCF7 and L929 by MTT assay method. The antitubercular activity of selected compounds was evaluated by REMA method.

Results: Ten derivatives (AZ-2, AZ-3, AZ-5, AZ-8, AZ-9, AZ-10, AZ-12, AZ-13, AZ-14 and AZ-15) were selected for synthesis with the help of *in-silico* modeling. In the synthesis, the microwave method took minimum reaction time and gave maximum yield comparing with conventional method. All the synthesized compounds showed characteristic peak in IR, ^{1}HNMR and Mass spectroscopic studies. Based on the Schrodinger Glide XP score, the compounds AZ-13, AZ-5 and AZ-3 were selected for *in vitro* anticancer and AZ-9, AZ-14 and AZ-3 were selected for antitubercular evaluation. The compound AZ-13 showed significant anticancer activity particularly against L929 cell line and the compound AZ-9 showed significant antitubercular activity comparing with other selected compounds.

Conclusion: These results are useful for further investigation in the future.

Keywords: Azetidinone containing isatin derivatives, Microwave assisted synthesis, Spectral study, *In vitro* anticancer activity, Antitubercular activity.

INTRODUCTION

In nature, isatin is found in plants of the genus *Isatis*, in *Calanthe discolor* LINDL., and in *Couroupita guianensis* Aubl., and has also been found as a component of the secretion from the parotid gland of *Bufo* frogs and in humans as it is a metabolic derivative of adrenaline. Substituted isatins are also found in plants, for example the melosatin alkaloids (methoxy phenylpentyl isatins) obtained from the Caribbean tumorigenic plant *Melochia tomentosa* as well as from fungi, 6-(3'-methylbuten-2'-yl) isatin was isolated from *Streptomyces albus* and 5- (3'-methylbuten-2'-yl)isatin from *Chaetomium globosum*. Isatin has also been found to be a component of coal tar [1]. The biological and pharmacological properties of isatin and its derivatives led to extensive use of these compounds as key intermediates in organic synthesis [2]. Literature surveys reveal that various derivatives of isatin possess diverse activities such as antibacterial [2, 3], antifungal [2, 4], antiviral [2, 5], anti-HIV [2, 6] antimycobacterial [2, 7] anticancer [2, 8] anti-inflammatory and anticonvulsant activities [2].

Azetidinones, commonly known as β-lactams, are well-known heterocyclic compounds among the organic and medicinal chemists. The activity of the famous antibiotics such as penicillins, cephalosporins and carbapenems are attributed to the presence of 2-azetidinone ring in them. Recently, some other types of biological activity besides the antibacterial activity have been reported in compounds containing 2-azetidinone ring. Such biological activities include antifungal, antitubercular, antitumor, inhibition of cholesterol absorption and enzyme inhibition activity. The β-lactams also serve as synthons for many biologically important classes of organic compounds [9].

Our ongoing investigations have been directed toward the *in-silico* design, synthesis and pharmacological evaluation of some novel isatin derivatives containing azetidinone.

MATERIALS AND METHODS

In-silico molecular modification

In-silico molecular modification was the most important preliminary step in the rational drug designing of novel drugs. In the present study different proposed derivatives are screened for different physico-chemical properties by using different softwares. ACD Lab Chemsketch 12.0 and Marvin Sketch were used for 3-D drawing, optimizing and calculating various molecular descriptors such as hydrophobicity, lipophilicity, steric and electronic parameters of the proposed molecules. The Molinspiration software was used for calculating LogP values, Lipinski's rule of five and drug likeness.

The proposed molecules were screened for whether they obey the rule of five or not. The general biological activities of proposed molecules were predicted by using PASS (Prediction of activity spectra for substances) software and the ADME (absorption, distribution, metabolism and excretion) profiles were predicted by Qik Prop program provided by Schrodinger Maestro. Schrodinger XP Glide (Extra precision Grid based ligand docking with energetics) program was used for the molecular docking of proposed molecules. Ten azetidinone containing isatin derivatives were selected for synthesis with the help of these selection parameters. They are

3-{[3-chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl] imino}-2, 3-dihydro-indol-2-one. (AZ-2)

3-{[3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl] imino}-2, 3-dihydro-indol-2-one. (AZ-3)

3-{[3-chloro-2-(4-hydroxy-3-methoxyphenyl)-4-oxoazetidin-1-yl] imino}-2, 3-dihydro-indol-2-one. (AZ-5)

3-{[3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl] imino}-2, 3-dihydro-indol-2-one. (AZ-8)

3-{[3-chloro-2-(4-fluorophenyl)-4-oxoazetidin-1-yl] imino}-2, 3-dihydro-indol-2-one. (AZ-9)

3-{[2-(5-bromo-2-hydroxyphenyl)-3-chloro-4-oxoazetidin-1-yl] imino}-2, 3dihydro-indol-2-one. (AZ-10)

3-{[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl] imino}-2, 3-dihydro--indol-2-one. (AZ-12)

3-{[3-chloro-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl] imino}-2, 3-dihydro-indol-2-one. (AZ-13)

3-{[3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl] imino}-2, 3-dihydro-indol-2-one. (AZ-14)

3-{[3-chloro-2-(3-nitrophenyl)-4-oxoazetidin-1-yl] imino}-2, 3-dihydro-indol-2-one. (AZ-15)

Synthesis of selected derivatives

The selected compounds were synthesized by conventional and microwave assisted synthetic methods through a series of three steps.

Conventional method

Step-1: Synthesis of Isatin-3-hydrazone

Isatin (0.01 mole) was dissolved in 20 ml of alcohol. Hydrazine hydrate (99%, 0.015 mole) was added to this mixture with stirring and the reaction mixture was warmed on the water bath for 10 minutes and allowed to cool for 3 h in the refrigerator. The resultant yellow coloured crystalline solid was filtered, washed repeatedly with cold water and finally with cold alcohol. The product obtained was dried and recrystallized from alcohol. A single spot on the TLC plate established the purity of the compound. The solvent system used was toluene: ethyl acetate (7:5).

Step-2: Synthesis of different Schiff bases

A mixture of Isatin-3-hydrazone (0.01 mole, 1.61g) in absolute ethanol, a few drops of glacial acetic acid and an aromatic aldehyde (0.01 mole) were refluxed for 6-8 h. The excess solvent was distilled off and the residue was poured on to ice. The separated solid was filtered and recrystallized from appropriate solvent. A single spot on the TLC plate established the purity of the compound. The solvent system used was toluene: ethyl acetate (7:5).

Yield and melting point of the product were recorded. Ten different Schiff bases were synthesized by using different aldehydes. They are

2-chloro benzaldehyde (0.01 mole, 1.12 ml) was used for the synthesis 3-[(2-chlorobenzylidene) hydrazinylidene]-2, 3-dihydro-indol-2-one. (IIa)

Para hydroxy benzaldehyde (0.01 mole, 1.22g) was used for the synthesis of 3-[(4-hydroxybenzylidene) hydrazinylidene]-2, 3-dihydro-indol-2-one. (IIb)

Vanillin (0.01 mole, 3.72g) was used for the synthesis of 3-[(4-hydroxy-3-methoxybenzylidene) hydrazinylidene]-2, 3-dihydro-indol-2-one. (IIc)

Para nitro benzaldehyde (0.01 mole, 1.51g) was used for the synthesis of 3-[(4-nitrobenzylidene) hydrazinylidene]-2, 3-dihydro-indol-2-one. (IId)

Para fluoro benzaldehyde (0.01 mole, 1.24g) was used for the synthesis of 3-[(4- fluoro benzylidene) hydrazinylidene]-2, 3-dihydro-indol-2-one. (IIe)

5-bromo salicylaldehyde (0.01 mole, 1.51g) was used for the synthesis of 3-[(5- bromo-2-hydroxy benzylidene) hydrazinylidene]-2, 3-dihydro-indol-2-one. (IIf)

Para chloro benzaldehyde (0.01 mole, 1.41g) was used for the synthesis of 3-[(4- chloro benzylidene) hydrazinylidene]-2, 3-dihydro-indol-2-one. (IIg)

Salicylaldehyde (0.01 mole, 0.73 ml) was used for the synthesis of 3-[(2-hydroxy benzylidene) hydrazinylidene]-2, 3-dihydro-indol-2-one. (IIh)

Para methoxy benzaldehyde (0.01 mole, 1.36 ml) was used for the synthesis of 3-[(4- methoxy benzylidene) hydrazinylidene]-2, 3-dihydro-indol-2-one. (IIi)

Meta nitro benzaldehyde (0.01 mole, 1.51g) was used for the synthesis of 3-[(3- nitrobenzylidene) hydrazinylidene]-2, 3-dihydro-indol-2-one. (IIj)

Step-3: Synthesis of azetidinone containing isatin derivatives

A mixture of Schiff's base (0.01 mole) and triethyl amine (5-6 drops) was dissolved in 1, 4-dioxan (50 ml) and cooled with stirring. To this mixture, chloroacetyl chloride (0.015 mole, 1.68 ml) was added drop wisely within a period of 30 min. The reaction mixture was then stirred for an additional 3 hours at room temperature and refluxed for 7 h. The reaction mixture was filtered to remove tri ethylamine hydrogen chloride and the resultant solution was concentrated, cooled and poured into ice-cold water with stirring. The solid thus obtained was recrystallized from ethanol. A single spot on the TLC plate established the purity of the compound. The solvent system used was Methanol: Petroleum ether (1:1). Yield and melting point of the product were recorded.

Schiff bases IIa, IIb, IIc, IId, IIe, IIf, IIg, IIh, IIi, IIj were used for the synthesis of compounds AZ-2, AZ-3, AZ-5, AZ-8, AZ-9, AZ-10, AZ-12, AZ-13, AZ-14, AZ-15 respectively.

Microwave assisted synthesis

Step-1: Synthesis of Isatin-3-hydrazone

Isatin (0.25g, 1.7 mmole), 55% hydrazine (0.30g, 0.425 mmole) and ethylene glycol (1 ml) were added to a 50 ml beaker. The mixture was shaken gently to ensure proper mixing. The beaker was then covered with a watch glass and irradiated in the microwave oven at medium power for 30 seconds. Then the beaker was removed from the oven and cooled at room temperature, the mixture was further cooled in an ice bath for 5 min. The yellow powders were collected in a suction flask, washed with cold ethanol (2.5 ml), and air dried. Yield, melting point and R_f value of the product were recorded.

Step-2: Synthesis of different Schiff bases

Isatin-3-hydrazone (0.01 mole), substituted aldehyde (0.001 mole) and 1-2 drops of glacial acetic acid were subjected to microwave radiation at 360W at an interval for specified time (1-3 minutes). The completion of the reaction was checked by TLC. The reaction mixture was cooled and poured into cold water. Solid product obtained was filtered, dried and recrystallised from ethanol to form red crystalline solid. Yield, melting point and R_f value of the product were recorded. 2-chloro benzaldehyde (0.001 mole, 0.112 ml), Para hydroxy benzaldehyde (0.001 mole, 0.122g), Vanillin (0.001 mole, 0.372g), Para nitro benzaldehyde (0.001 mole, 0.151g), Para fluoro benzaldehyde (0.001 mole, 0.124g), 5-bromo salicylaldehyde (0.001 mole, 0.151g), Para chloro benzaldehyde (0.001 mole, 0.141g), Salicylaldehyde (0.001 mole, 0.073 ml), Para methoxy benzaldehyde (0.001 mole, 0.136 ml), Meta nitro benzaldehyde (0.001 mole, 0.151g) were used for the synthesis of Schiff bases IIa, IIb, IIc, IId, IIe, IIf, IIg, IIh, IIi, IIj respectively.

Step-3: Synthesis of azetidinone containing isatin derivatives

A mixture of Schiff's base (0.01 mole) and triethyl amine (5-6 drops) was added to chloroacetyl chloride (0.015 mole, 1.68 ml). Then it was irradiated for 3.5 min. in microwave oven and diluted with ice cold water. The solid obtained was filtered, washed with water and recrystallized from ethanol. Yield, melting point and R_f value of the product were recorded. Schiff bases IIa, IIb, IIc, IId, IIe, IIf, IIg, IIh, IIi, IIj were used for the synthesis of compounds AZ-2, AZ-3, AZ-5, AZ-8, AZ-9, AZ-10, AZ-12, AZ-13, AZ-14, AZ-15 respectively.

Characterization of synthesized compounds by spectral study

IR Spectrum

IR spectra were recorded by using KBr pellets in the range of 4000 – 500 cm-1 on Jasco FTIR Model 4100 Type A to elucidate the structure of the compounds.

^{1}HNMR Spectrum

Proton NMR (400 MHz) spectra were recorded by using Bruker ultra shield model 400 MHz spectrometer using Di methyl sulphoxide as the solvent and Tetra Methyl Silane (TMS) as internal standard.

Mass spectrum

Mass spectra of the synthesized compounds were recorded by FAB+ ionization mode on JEOL JMS 600 instrument.

***In vitro* anticancer activity**

Anticancer activities of selected compounds were evaluated against MCF7 (Breast cancer cell line) and L929 (Fibro sarcoma T cells) by MTT (3-[4,5-dimethyl thiazol-2yl]-2,5- diphenyl tetrazolium bromide) assay method. The cell lines were procured from NCCS, (National centre for cell science) Pune, India.

Sub culturing and maintenance of cell line

The cell lines were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% heat inactivated foetal bovine serum (FBS) and incubated at 37^{0}C in a humidified atmosphere of 5% CO_2

Assessment of anticancer activity by MTT assay

Cells were transferred in to 96- well flat bottom plates at the concentration of 1×10^4 cells/ml and incubated at 37^{0}C in a humidified incubator (5% CO_2) for 24 h followed by exposure to various concentrations of tested compounds for 48 h. Then 20 µl of MTT (3-[4,5-dimethyl thiazol-2yl]-2,5- diphenyl tetrazolium bromide) reagent dissolved in PBS (phosphate buffered saline, pH 7.4) was added to each well and mixed and incubated for an additional 4 h. Subsequently, the supernatant was removed, 150 µl DMSO (dimethyl sulphoxide) was added to each well for dissolving the MTT- formazan crystals. Finally absorbance was recorded at 570 nm using a micro plate reader with DMSO as a blank and the proliferation rate (PR) was calculated by using the following equation

$$PR = \frac{\text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Cytotoxicity was calculated as cell growth inhibition rate (IR).

$$IR = 100 - PR.$$

Antitubercular activity

Anti-tubercular activity of selected compounds was evaluated by Resazurin micro titre assay (REMA) method. Mycobacterium tuberculosis $H_{37}Rv$ used for the evaluation was procured from MTCC, Chandighar, India. Black view, flat bottom 96-well micro plates were used for the experiment. The initial drug dilutions were prepared by using dimethyl sulphoxide and subsequent two fold dilutions were prepared in the micro plates by using 0.1 ml of 7H9GC broth. 100 µl of 2000CFU/ml of test organism in 7H9GC broth was added to each well of 96 well micro titre plate containing test compounds. Three controls- medium only, drug and medium, test organism and medium were prepared and all are incubated at 37°C for seven days. On 7th day alamar blue dye solution (20 µl alamar blue solution and 12.5 ml of 20% Tween 80) was added to all the wells and the plates were re-incubated at 37 °C for 24 h. Results were recorded at 365 nm using a micro plate reader.

RESULTS

In the present study, *in-silico* molecular modifications of proposed derivatives were done by using different softwares. 3-D drawing, optimizing and calculating various descriptors of proposed derivatives were done by using ACD Lab Chemsketch 12.0 and Marvin Sketch software. The results are shown in table 1.

Table 1: Molecular descriptors of proposed derivatives

Compound	Molecular formula	Molar Volume (cm^3)	Parachor (cm^3)	Surface Tension (dyne/cm)	Polarisability (10-[24] cm^3)	Molar Refractivity (cm^3)
AZ-1	$C_{17}H_{12}ClN_3O_2$	216.2 ± 7.0	605.3 ± 8.0	61.3± 7.0	34.51 ± 0.5	87.06 ± 0.5
AZ-2	$C_{17}H_{11}Cl_2N_3O_2$	225.5 ± 7.0	634.2 ± 8.0	62.4 ± 7.0	36.33 ± 0.5	91.66 ± 0.5
AZ-3	$C_{17}H_{12}ClN_3O_3$	213.5 ± 7.0	611.0 ± 8.0	67.0 ± 7.0	34.85 ± 0.5	87.91 ± 0.5
AZ-4	$C_{17}H_{10}Cl_3N_3O_2$	234.8± 7.0	663.0 ± 8.0	63.5 ± 7.0	38.16 ± 0.5	96.26 ± 0.5
AZ-5	$C_{18}H_{14}ClN_3O_4$	235.2 ± 7.0	661.2 ± 8.0	62.4 ± 7.0	37.15 ± 0.5	93.72 ± 0.5
AZ-6	$C_{18}H_{14}ClN_3O_3$	237.9 ± 7.0	655.6 ± 8.0	57.6 ± 7.0	36.81 ± 0.5	92.87 ± 0.5
AZ-7	$C_{18}H_{14}ClN_3O_3$	237.9± 7.0	655.6 ± 8.0	57.6 ± 7.0	36.81 ± 0.5	92.87 ± 0.5
AZ-8	$C_{17}H_{11}ClN_4O_4$	221.5 ± 7.0	650.8 ± 8.0	74.4 ± 7.0	36.75 ± 0.5	92.72 ± 0.5
AZ-9	$C_{17}H_{11}ClFN_3O_2$	219.1 ± 7.0	605.5 ± 8.0	58.2 ± 7.0	34.46 ± 0.5	86.93 ± 0.5
AZ-10	$C_{17}H_{11}BrClN_3O_3$	226.0 ± 7.0	654.5 ± 8.0	70.2 ± 7.0	37.84 ± 0.5	95.47 ± 0.5
AZ-11	$C_{17}H_{11}ClN_4O_4$	221.5 ± 7.0	650.8 ± 8.0	74.4 ± 7.0	36.75 ± 0.5	92.72 ± 0.5
AZ-12	$C_{17}H_{11}Cl_2N_3O_2$	225.5 ± 7.0	634.2 ± 8.0	62.4 ± 7.0	36.33 ± 0.5	91.66 ± 0.5
AZ-13	$C_{17}H_{12}ClN_3O_3$	213.5 ± 7.0	611.0 ± 8.0	67.0 ± 7.0	34.85 ± 0.5	87.91 ± 0.5
AZ-14	$C_{18}H_{14}ClN_3O_3$	237.9 ± 7.0	655.6 ± 8.0	57.6 ± 7.0	36.81 ± 0.5	92.87 ± 0.5
AZ-15	$C_{17}H_{11}ClN_4O_4$	221.5 ± 7.0	650.8 ± 8.0	74.4 ± 7.0	36.75 ± 0.5	92.72 ± 0.5
AZ-16	$C_{17}H_{10}ClN_5O_6$	226.8 ± 7.0	696.2 ± 8.0	88.7 ± 7.0	39.00 ± 0.5	98.38 ± 0.5
AZ-17	$C_{19}H_{16}ClN_3O_5$	256.8 ± 7.0	711.5 ±8.0	58.8 ± 7.0	39.46 ± 0.5	99.54 ± 0.5
AZ-18	$C_{20}H_{18}ClN_3O_5$	281.2 ± 7.0	756.1 ± 8.0	52.2 ± 7.0	41.42 ± 0.5	104.5 ± 0.5
AZ-19	$C_{19}H_{16}ClN_3O_4$	259.6 ± 7.0	705.8 ±8.0	54.6 ± 7.0	39.12 ± 0.5	98.68 ± 0.5
AZ-20	$C_{18}H_{14}ClN_3O_4$	235.2 ± 7.0	661.2 ± 8.0	62.4 ± 7.0	37.15 ± 0.5	93.72 ± 0.5
AZ-21	$C_{18}H_{14}ClN_3O_2$	231.4 ± 7.0	636.4 ± 8.0	57.1 ± 7.0	36.26 ± 0.5	91.48± 0.5
AZ-22	$C_{19}H_{16}ClN_3O_2$	246.6 ± 7.0	667.5± 8.0	53.6 ± 7.0	38.02± 0.5	95.91 ± 0.5
AZ-23	$C_{17}H_{13}ClN_4O_2$	213.0 ± 7.0	612.5 ±8.0	68.3 ± 7.0	35.27 ± 0.5	88.97 ± 0.5
AZ-24	$C_{19}H_{17}ClN_4O_2$	257.4 ± 7.0	701.6 ± 8.0	55.1 ± 7.0	39.59 ± 0.5	99.86 ± 0.5
AZ-25	$C_{21}H_{22}ClN_5O_2$	298.6 ± 7.0	797.9 ±8.0	100.9 ± 3.0	44.66 ± 0.5	112.67 ± 0.5
AZ-26	$C_{15}H_{10}ClN_3O_3$	190.6 ± 7.0	547.3 ± 8.0	110.1 ± 5.0	31.41 ± 0.5	96.78 ± 0.5

The molinspiration software was used to study the LogP values, violation of Lipinski's rule of five and drug likeness by comparing with already existing standard drugs. The results are shown in tables 2, 3 and figures 1, 2.

The ADME profiles of the designed molecules were predicted by QikProp program provided by Schrodinger Maestro. The PASS software was used to predict the general biological activities of proposed molecules. Schrodinger Glide XP software was used for predicting the protein-ligand binding modes. In this study, the compound having high (-) value is considered as the best one. Of the

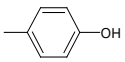
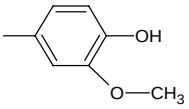
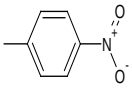
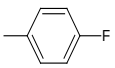
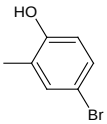
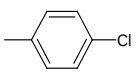
26 proposed derivatives, 10 derivatives were selected for synthesis with the help of these selection parameters. They were named as AZ-2, AZ-3, AZ-5, AZ-8, AZ-9, AZ-10, AZ-12, AZ-13, AZ-14 and AZ-15. The selected compounds were synthesized by conventional and microwave assisted synthetic methods through a series of three steps. The general scheme for the synthesis is presented in figure 3.

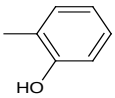
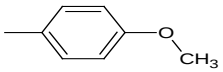
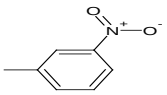
Table 2: Lipinski's rule analysis of standard drugs and proposed derivatives

Compound	MiLog P	M. W	n. HDO	n. HAC	n. rotb	n. violation
Isoniazid	-0.89	137.14	3	4	1	0
Ethambutol	0.35	204.314	4	4	9	0
Mechlorethamine	1.554	156.056	1	0	4	0
Cyclophosphamide	0.755	261.081	4	1	5	0
AZ-1	2.903	322.755	5	1	2	0
AZ- 2	3.533	360.2	5	1	2	0
AZ-3	2.423	341.754	6	2	2	0
AZ-4	4.187	394.645	5	1	2	0
AZ-5	2.242	370.78	7	2	3	0
AZ-6	2.911	355.781	6	1	3	0
AZ-7	2.935	355.781	6	1	3	0
AZ-8	2.862	370.752	8	1	3	0
AZ-9	3.066	343.745	5	1	2	0
AZ-10	3.628	420.65	6	2	2	0
AZ-11	2.814	370.752	8	1	3	0
AZ-12	3.581	360.2	5	1	2	0
AZ-13	2.843	341.754	6	2	2	0
AZ-14	2.959	355.781	6	1	3	0
AZ-15	2.838	370.752	8	1	3	0
AZ-16	2.748	415.749	11	1	4	1
AZ-17	2.258	401.806	8	2	4	0
AZ-18	2.929	415.833	8	1	5	0
AZ-19	2.549	385.807	7	1	4	0
AZ-20	2.447	371.78	7	2	3	0
AZ-21	3.303	339.782	5	1	2	0
AZ-22	3.728	353.809	5	1	2	0
AZ-23	1.979	340.77	6	3	2	0
AZ-24	3.005	368.824	6	1	3	0
AZ-25	3.035	411.893	7	1	4	0
AZ-26	2.16	315.716	6	1	2	0

M. W- molecular weight; nHDO- number of hydrogen bond donar; nHAC- number of hydrogen bond acceptor; n. rotb- number of rotatable bonds.

Table 3: SMILES and Log P values of standard drugs and the derivatives selected for the synthesis

Compound	Substitution	Smile Notation	Log P
Isoniazid	--	O=C(NN)c1ccncc1	-0.65
Ethambutol	--	CCC(NCCNC(CC)CO)CO	-0.41
Mechlorethamine	--	C1CCN(C)CCC1	0.91
Cyclophosphamide	--	N1CCCOP1(=O)N(CCC1)CCC1	0.97
AZ-2		Clc4ccccc4C3C(Cl)C(=O)N3/N=C2\C(=O)Nc1ccccc12	3.533
AZ-3		Oc1ccc(cc1)C4C(Cl)C(=O)N4/N=C3\C(=O)Nc2ccccc23	2.423
AZ-5		Oc1ccc(cc1OC)C4C(Cl)C(=O)N4/N=C3\C(=O)Nc2ccccc23	2.242
AZ-8		[O][N+](=O)c1ccc(cc1)C4C(Cl)C(=O)N4/N=C3\C(=O)Nc2ccccc23	2.862
AZ-9		Fc1ccc(cc1)C4C(Cl)C(=O)N4/N=C3\C(=O)Nc2ccccc23	3.066
AZ -10		Brc4cc(C3C(Cl)C(=O)N3/N=C2\C(=O)Nc1ccccc12)c(O)cc4	3.628
AZ-12		Clc1ccc(cc1)C4C(Cl)C(=O)N4/N=C3\C(=O)Nc2ccccc23	3.581

AZ-13		Oc4ccccc4C3C(Cl)C(=O)N3/N=C2\C(=O)Nc1ccccc12	2.843
AZ-14		COc1ccc(cc1)C4C(Cl)C(=O)N4/N=C3\C(=O)Nc2ccccc23	2.959
AZ-15		[O][N+](=O)c1cccc(c1)C4C(Cl)C(=O)N4/N=C3\C(=O)Nc2ccccc23	2.838

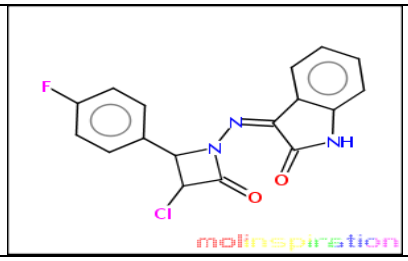
	Molinspiration property engine v2011.04 miLogP 3.066 TPSA 65.535 natoms 24.0 MW 343.745 nON 5 nOHNH 1 nviolations 0 nrotb 2 volume 272.305	Molinspiration bioactivity score v2011.06 GPCR ligand -0.30 Ion channel modulator -0.45 Kinase inhibitor -0.05 Nuclear receptor ligand -0.66 Protease inhibitor -0.50 Enzyme inhibitor -0.30

Fig. 1: Molinspiration score of compound AZ-9

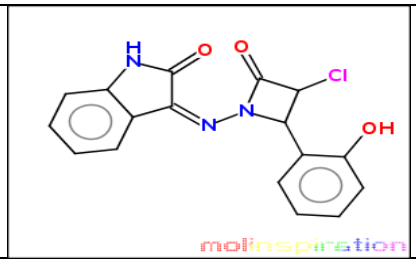
	Molinspiration property engine v2011.04 miLogP 2.843 TPSA 85.763 natoms 24.0 MW 341.754 nON 6 nOHNH 2 nviolations 0 nrotb 2 volume 275.391	Molinspiration bioactivity score v2011.06 GPCR ligand -0.31 Ion channel modulator-0.42 Kinase inhibitor -0.09 Nuclear receptor ligand-0.58 Protease inhibitor -0.49 Enzyme inhibitor -0.25

Fig. 2: Molinspiration score of compound AZ-13

Table 4: Comparison of microwave and conventional synthetic methods

Compound	Microwave		Conventional	
	Time (min)	Yield (%)	Time (min)	Yield (%)
AZ-2	3.5	77.00%	420	63.5%
AZ-3	3.5	78.00 %	420	64.5%
AZ-5	3.5	71.00 %	420	59.00%
AZ-8	3.5	78.00 %	420	64.5%
AZ-9	3.5	80.5%	420	68.00%
AZ-10	3.5	71.00%	420	59.00%
AZ-12	3.5	87.00%	420	70.00%
AZ-13	3.5	80.5%	420	68.00%
AZ-14	3.5	77.00%	420	63.5%
AZ-15	3.5	87.00%	420	70.00%

Purity of the synthesized compounds was ascertained by TLC and melting point determination by open capillary tube method. The results are shown in table 5.

Table 5: Physical characterization data of synthesized compounds

Compound	Mol. formula	Mol. Wt.	Melting point (°C)	R_f
AZ-2	$C_{17}H_{11}Cl_2N_3O_2$	360.2	236-238°C	0.75
AZ- 3	$C_{17}H_{12}ClN_3O_3$	341.754	243-246°C	0.91
AZ-5	$C_{18}H_{14}ClN_3O_4$	370.78	237-239°C	0.95
AZ-8	$C_{17}H_{11}ClN_4O_4$	370.752	254-256°C	0.91
AZ-9	$C_{17}H_{11}ClFN_3O_2$	343.745	255-257°C	0.72
AZ-10	$C_{17}H_{11}BrClN_3O_3$	420.65	226-229°C	0.95
AZ-12	$C_{17}H_{11}Cl_2N_3O_2$	360.2	256-259°C	0.80
AZ-13	$C_{17}H_{12}ClN_3O_3$	341.754	243-245°C	0.72
AZ-14	$C_{18}H_{14}ClN_3O_3$	355.781	240-242°C	0.75
AZ-15	$C_{17}H_{11}ClN_4O_4$	370.752	251-253°C	0.80

The synthesized compounds were characterized by IR, ^{1}HNMR and Mass spectroscopic methods. The IR spectra of the synthesized compounds are presented in table 6, ^{1}HNMR report for compound AZ -12 is shown in Graph 1 and the mass spectrum report for compound AZ – 13 is shown in Graph 2.

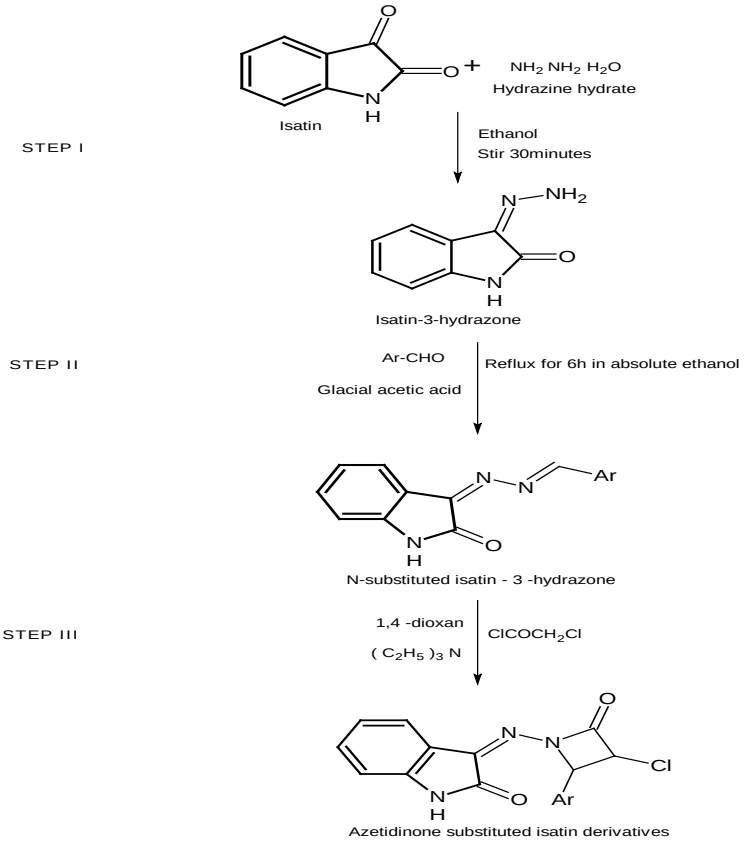


Fig. 3: General scheme for the synthesis of azetidinone containing isatin derivatives

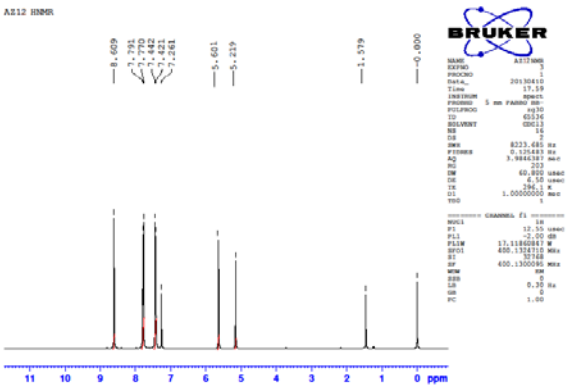
The results showed that the microwave assisted synthetic method took a minimum reaction time but gave high yield comparing with conventional method. The results are shown in table 4.

Table 6: IR spectra of synthesized compounds

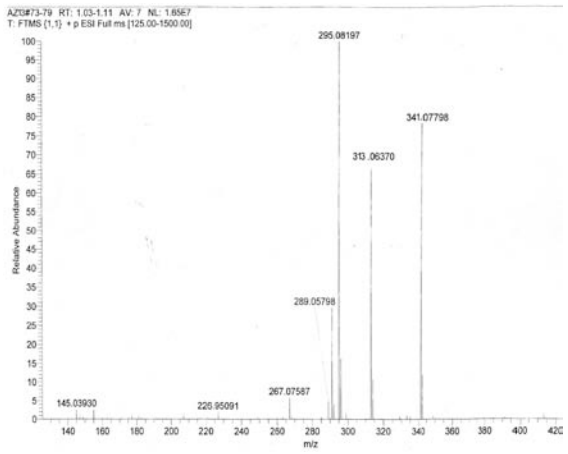
Compound	IR Peaks (cm^{-1})
AZ-2	3488.41 (N-H str), 3034.8 (Ar C-H str), 1716.99(C=O),1647.64 (C=N str), 1485.82(CH-N str), 760.138 (C-Cl str)
AZ-3	3568.72(OH str), 3450.55 (N-H str),3031.12(Ar-CH), 1712.2 (C=O str), 1642.02 (C=N str), 1489.3(CH-N str),740.82(C-Cl)
AZ-5	3650.37(OH str),3460.16 (N-H str), 3035.13 (Ar C-H str), 1710.34(C=O str), 1635.02(C=N str), 1470.85(CH-N str), 1135.54(C-O-C str), 780.384(C-Cl)
AZ-8	3450.05(N-H str), 3035.66(Ar-CH str), 1721.41(C=O str), 1652.33 (C=N str),1550.2 (N=O), 1465.78(CH-N str),981.93(N-O),755.99(C-Cl)
AZ-9	3410.225(N-H Str), 3032.145(Ar-CH Str), 1725.625(C=O Str), 1650.23(C=N Str), 1340.35(C-F Str),757.134(C-Cl)
AZ-10	3590.237(O-H Str), 3465.12(N-H Str), 3030.005(Ar-CH), 1712.36(C=O Str), 769.65(C-Cl), 595.2(C-Br)
AZ-12	3470.56(N-H Str), 3029.70(Ar-CH), 1713.41(C=O Str), 1645.13(C=N Str), 1483.79(CH-N Str), 7580352(C-Cl)
AZ-13	3550.72(O-H Str), 3400.55(N-H Str), 3032.44(Ar-CH Str), 1715.16(C=O), 1647.88(C=N), 1470.03(CH-N), 761.58(C-Cl)
AZ-14	3465.22(N-H Str), 3040.37(Ar-CH Str), 1710.34(C=O Str), 1635.02(C=N), 1470.85(CH-N Str), 1135.54(C-O-C), 780.384(C-Cl)
AZ-15	3456.26(N-H Str), 3032.76(Ar-CH), 1741.21(C=O), 1656.69(C=N), 1556.32(N=O), 982.33(N-O), 760.11(C-Cl)

Table 7: Schrodinger Glide XP scores of synthesized compounds for anticancer activity

Target	PDB ID	Compound	Glide score
GAMMA-TUBULIN	1Z5W		
		AZ-13	-8.94
		AZ-5	-7.35
		AZ-3	-6.56
		AZ-9	-6.37
		AZ-10	-6.34
		AZ-14	-6.11
		AZ-15	-5.52
		AZ-12	-5.43
		AZ-8	-5.37
		AZ-2	-5.33



Graph 1: ^{1}HNMR report for compound AZ -12



Graph 2: Mass spectrum report for compound AZ – 13

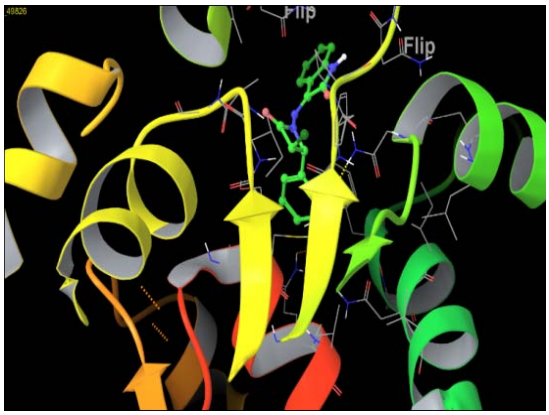


Fig. 4: Docking image of AZ-13 to Gamma tubulin – 1Z5W

The tested derivatives showed cytotoxic activity against the tested cancer cell lines. But the compound AZ-13 showed significant anticancer activity particularly against L929 cell line. The results are shown in table 8.

Based on the Schrodinger Glide XP score, the compounds AZ-13, AZ-5 and AZ-3 were selected for *in vitro* anticancer evaluation against MCF7 and L929 cell lines by MTT assay method. Docking scores for anticancer activity are presented in table 7 and the docking image of AZ-13 is shown in fig based on the Schrodinger Glide XP score, the compounds AZ-9, AZ-14 and AZ-3 were selected for antitubercular evaluation.

Docking scores for antitubercular activity are presented in table 9 and the docking image of AZ-9 is shown in figure 5. All the selected compounds showed anti-tubercular activity. But the compound AZ-9 showed significant anti-tubercular activity comparing with other selected compounds. The results are shown in table 10. gure 4.

Table 8: *In vitro* cytotoxicity of selected compounds

Con. (µg/ml)	Percentage inhibition									
	AZ 3		AZ 5		AZ9		AZ13		Doxorubicin	Paclitaxel
	MCF7	L929	MCF7	L929	MCF7	L929	MCF7	L929	MCF7	L929
1	17.40	35	33.18	37	13.42	33	36.75	41	57.17	52.28
10	22.12	43	36.84	45	19.25	39	43.62	52	62.76	62.1
100	34.45	50	42.76	52	32.33	46	45.83	60	63.70	75.69
250	42.34	56	47.44	59	36.53	53	49.24	69	65.37	82.61
500	49.52	64	51.43	67	41.24	60	54.35	74	69.54	88.37

Con. – Concentration

Table 9: Schrodinger Glide XP scores of synthesized compounds for anti tubercular activity:

Target	PDB ID	Compound	Glide score
MYCOBACTERIUM TUBERCULOSIS-CYTOCHROME P450 14 ALPHA-STEROL DEMETHYLASE (CYP51)	1EA1	AZ-9	-9.43
		AZ-14	-8.82
		AZ-3	-8.62
		AZ-10	-8.1
		AZ-13	-7.97
		AZ-5	-7.79
		AZ-12	-7.35
		AZ-8	-7.17
		AZ-15	-7.2
		AZ-2	-6.95

DISCUSSION

In the present study, the *in-silico* molecular modeling studies were carried out for the selection of suitable drug candidates prior to wet lab synthesis. *In-silico* studies were performed on 26 analogues by means of ACD Lab ChemSketch 12.0, Marvin Sketch, Molinspiration, PASS, and Schrodinger. Of the proposed 26 analogues, 10 candidates were chosen for wet lab synthesis.

These compounds were synthesized by both conventional and microwave methods. The later one took minimum reaction time but gave maximum yield comparing with conventional method. The

synthesized compounds were subjected to TLC, melting point determination, IR, ^{1}HNMR and Mass spectroscopic studies. All these evaluation ensured the synthesized compounds. On docking with Gamma-Tubulin (1Z5W), the compounds AZ-13, AZ-5 and AZ-3 showed good glide score, these compounds were subjected to *in vitro* anticancer evaluation against MCF7 and L929 cell lines by MTT assay method. The results showed that the compound AZ-13 having significant anticancer activity particularly against L929 cell line. Of course this compound needs further studies such as toxicity and *in vivo* evaluation. On docking with Cytochrome P450 14 Alpha-Sterol Demethylase (CYP51), the compounds AZ-9, AZ-14 and AZ-3 showed good glide score, these compounds were selected for antitubercular study by REMA method. The compound AZ-9 showed significant antitubercular activity comparing with other selected compounds. This compound also needs further study. So it is clear that further works needed to be done in the future for the development of clinically useful chemotherapeutic agents.

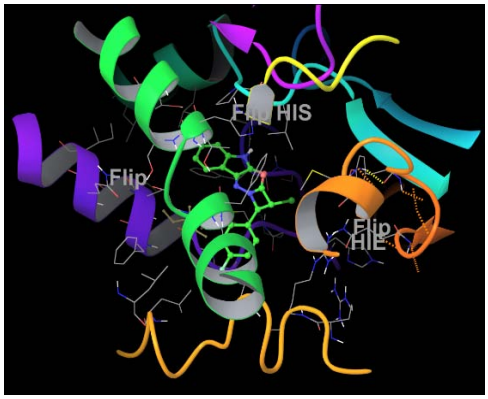


Fig. 5: Docking image of AZ- 9 to CYP51 - 1EA1

Table 10: Anti tubercular activity of selected compounds

Conc.	Percentage Inhibition			
µg/ml	AZ 3	AZ 9	AZ 10	AZ 14
0	0	0	0	0
50	41	47	39	44
100	50	55	47	52
150	61	66	59	64
200	72	77	68	75
250	76	83	73	80

Conc. – Concentration

CONCLUSION

In summary, the prime objective of the present work was to design, synthesize and biologically screen some of the azetidinone containing isatin derivatives. The present work involved the preliminary *in-silico* screening of various analogues to analyze for their molecular descriptors using computational softwares. Derivatives with desired physicochemical properties, obeying Lipinski Rule of Five and those with no violations were chosen for wet lab synthesis. Synthesis of ten analogues was performed and the purity of the same was ascertained by consistency in melting point and R_f value. The compounds were characterized by IR, NMR and Mass spectral studies. Selected derivatives were screened for antimycobacterial and anticancer activity. Activities of the screened analogues were comparable, reflecting the novelty of azetidinone analogues with improved biological activity. Azetidinone and its analogues have established themselves as good antibiotics. This work has proved the biological action of some of the synthesized analogues as antimycobacterial and anticancer agents. The analogues can be subjected to further detail pharmacological screening for consideration as drug candidates.

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COMPETING INTERESTS

None

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None

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