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Design, Synthesis and Pharmacological Evaluation of Novel 7-Azaindole Sderivatives

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Abstract: Coumarin and its derivatives are a prominent class of benzopyrone compounds known for diverse pharmacological properties, including anticancer, antimicrobial, and antioxidant activities. The present study focuses on the synthesis, characterization, and evaluation of anticancer activity of novel coumarin derivatives. The synthesis was carried out through Pechmann condensation and subsequent functional modifications at various positions of the coumarin nucleus to enhance biological efficacy. The synthesized compounds were characterized by FT-IR, ¹H-NMR, ¹³C-NMR, and Mass spectroscopic techniques to confirm their structural integrity and purity. The anticancer potential of the synthesized derivatives was assessed using MTT assay against selected human cancer cell lines, such as MCF-7 (breast cancer), HeLa (cervical cancer), and A549 (lung cancer). Results revealed that several derivatives exhibited significant cytotoxicity, which may be attributed to the presence of electron-donating or withdrawing substituents influencing the coumarin core. The findings suggest that structural modification of coumarin scaffolds can lead to promising lead molecules for future anticancer drug development.

Keywords: Coumarin derivatives, synthesis, characterization, anticancer activity, MTT assay, structure–activity relationship.

INTRODUCTION

Azaindoles are synthetic analogs of indoles in which the carbon atom(s) in the phenyl ring have been replaced with basic nitrogen(s). Azaindoles, as indolic bioisosteres, have a wide range of applications in bio-organic and medicinal chemistry due to the customizable positioning of the extra aromatic nitrogen

Azaindoles have presented a unique challenge to synthetic chemists. Starting with substituted pyridines and building up the annulated pyrrole ring has been a popular strategy for azaindole synthesis. However, the electron-deficient structure of the pyridine ring modifies the electronics of the -system in such a way that many standard indole development methods either do not work or are inefficient when directly applied to the synthesis of azaindole analogues. Azaindoles are an important class of nitrogen containing heterocyclic compounds and were identified as the most active and potent classes of compounds with wide range of biological and pharmacological activities like sedative hypnotics, Anti-microbial activity, anti-cancer activity, anti-Alzheimer's activity. Cancer is defined as the uncontrolled proliferation of aberrant cells, and it is the second-leading cause of death after cardiovascular disease (Siegel et al., 2017). Every year, around 9.6 million people pass away by cancer (Bray et al., 2018). Despite tremendous progress in anticancer pharmaceutical research, there are still various barriers to treating cancer, such as low efficacy, high toxicity, and drug resistance, which have had a significant detrimental influence on the ability of cancer patients to lead normal lives (Akhtar et al., 2017).

As a result, one of the most crucial areas of modern cancer research is the search for potent, safe anticancer drugs with high selectivity (Fadeyi et al., 2008). According to various studies (Cacchi et al., 2005; Humphrey et al., 2006; Kochanowska-Karamyan et al., 2010; Jacob et al., 2013; Florence et al., 2007), the indole nucleus is an important component of many physiologically active natural and synthetic chemicals.

Alzheimer's disease (AD) is one of the main causes of dementia among elderly people. Prevalence of AD by 2050 is expected to be 14 and 130 million people in Europe and worldwide, respectively (WHO Association 2018). It is characterized by progressive impairment of memory cognitive functions due to the degeneration of synapses and to the death of neurons, especially in hippocampus (Scheff et al. 2006). The most promising approach to the symptomatic treatment of AD is the inhibition of acetylcholinesterase (AChE) which catalyzes the hydrolysis of acetylcholine to choline and acetate. A stiff three-atom structure including a hydrogen-bond donor and acceptor in a C4, C5, C6, or C7 position of an aza indole is produced when a sp²-hybridized nitrogen is substituted there (Song et al., 2007). 7-azaindole (1H-pyrrolo[2,3-b]pyridine) is an organic compound that naturally has an electron-deficient pyridine ring and an electron-rich pyrrole ring bonded to a [4,3]-bicyclic indene skeleton (Kaushiket al., 2013).

3.2. NEED FOR THE PRESENT WORK

Design and synthesis of heterocyclic compounds, such as 4-azaindoles, 5-azaindoles, 7-azaindoles, and azaindole complexes, based on the indole and azaindole scaffold have produced a number of potent anticancer compounds, some of which have shown inhibitory activity on cancer cell lines or target at nanomolar concentration range with minimal side effects and toxicity. The fact that the NH group in indoles and azaindoles frequently forms the key hydrogen bonds with the corresponding receptor. More than ten interesting anticancer lead compounds containing indole or azaindole skeleton were found and several of them were identified to effectively inhibit the growth of solid tumors and metastasis of cancer cells. The remarkable anticancer lead compounds developed by designing indole or azaindole hybrids with other active moiety and azaindole heterocyclic scaffolds targeting on specific target are unquestionably highlights of the past few years; these accomplishments offer helpful strategies and hints for the development of better antitumor activity agents. However, it still falls short of our expectations and needs. For instance, many compounds still exert their effects at the cellular level, the majority of their mechanisms remain at the molecular docking level, and the precise binding sites between active molecules and receptors are still unknown, hence we need to design new indole and azaindole derivatives targeting growth factors, receptors, enzymes, and kinases into a trend. also have been elucidated as far as possible.

3.3. Design of new azaindole derivatives (10a-o):

Naaz et al., (2020) reported the synthesized two series of topsentin clubbed with 1,3,4-oxadiazoles and 1,2,4-triazoles and were investigated for anticancer activity against various cell lines. Compounds **(I)** revealed the activity against breast (MCF-7, T47D) cancers cell lines with IC₅₀ values 2.54 µM. Feng et al.,(2018) reported a new series of 2,3,4,5-tetrahydro-1H-pyrido-[4,3-b] indole derivatives was carried out by against HeLa, A549, HepG2, and MCF-7 cancer cell lines by the MTT assay. The compound **(II)** exhibited most potent anticancer activity with IC₅₀ values with 9.42-15.06 µM. Song et al.,(2019) synthesized the novel N-arylsulfonyl-3-substituted indole derivatives compound **(III)** was found to be most active and aminoguanidine and arylsulfonylindole moieties were verified as good options to develop anticancer agents with IC₅₀ values with 4.54 and 1.51 µM against lung cancer A590 and Gastric cancer SGC 7950 cell lines. Ma et al. (2015) reported the (E)-4- (6-((dimethylamino)methyl)benzothiazol-2-yl)-1-((1-(4-chlorobenzyl)-1H-indol-3-yl)methylene) semi -carbazine **(IV)** displayed more appreciable anticancer activity with IC₅₀ values varying from 0.024 to 0.88 µM.

In view of the above, it is planned to synthesize new azaindole derivatives as shown in **General Structure-I** and evaluate them for their anticancer activities by standard protocol.

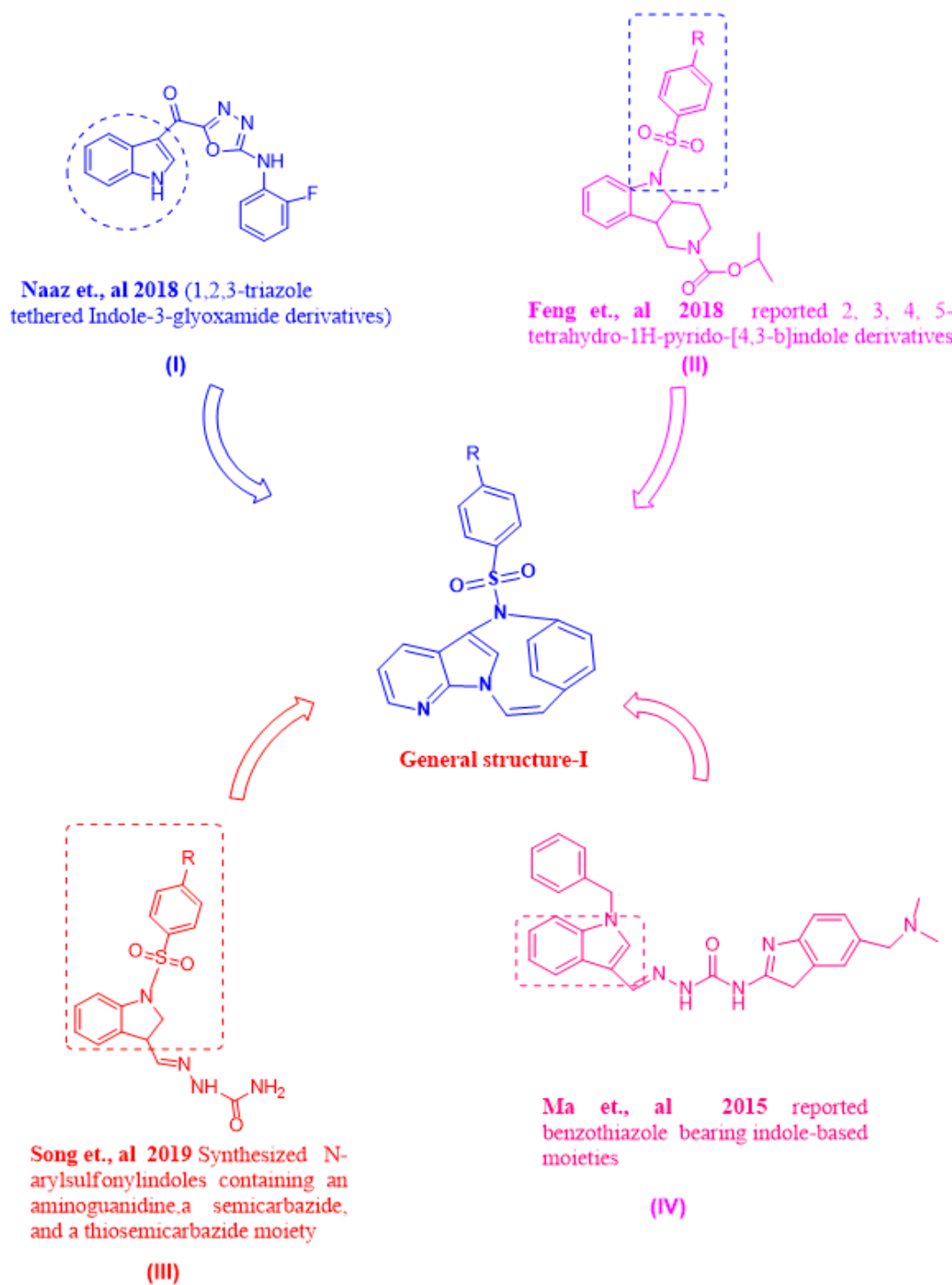


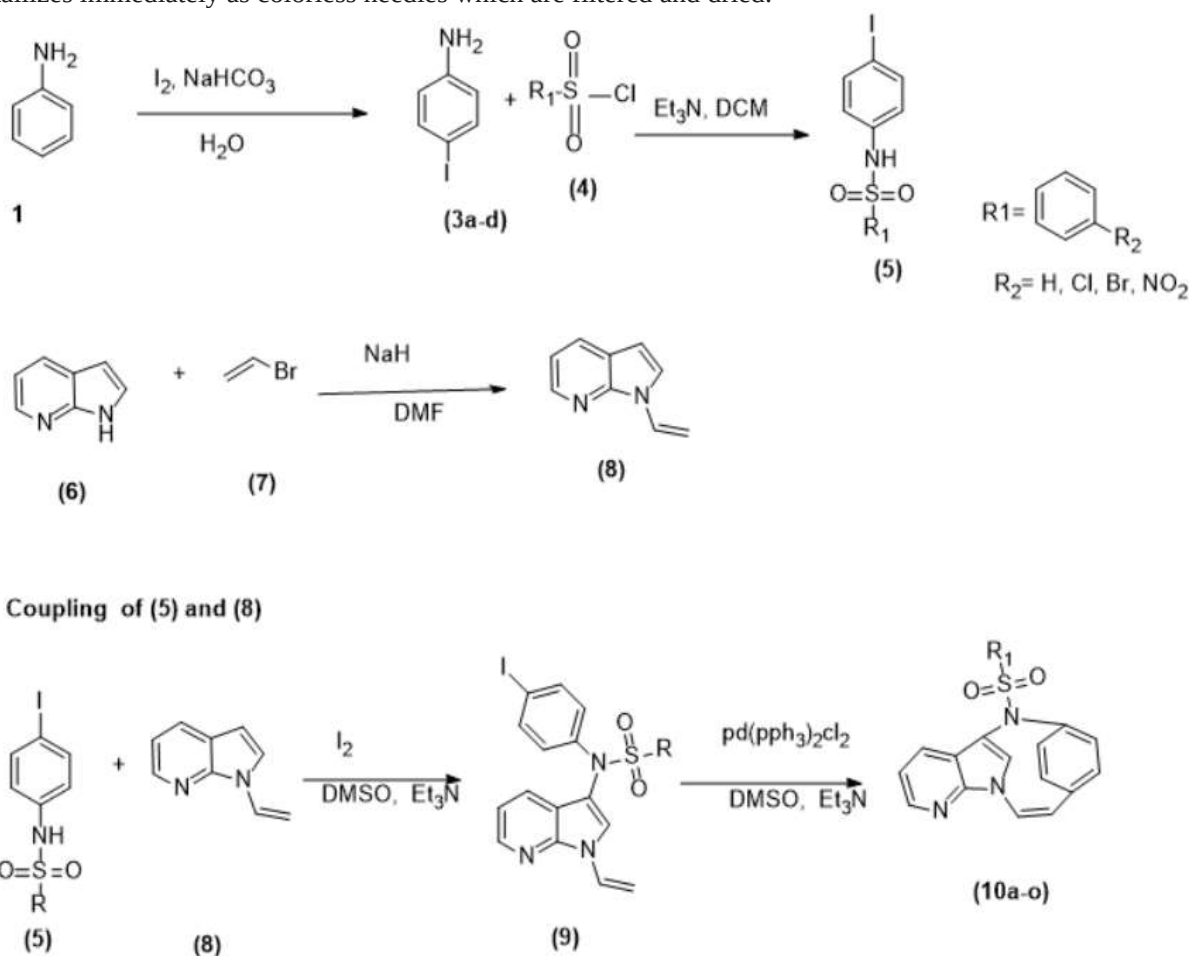
Fig-3.1.1: Design of novel Azaindole derivatives (10a-o):

MATERIAL AND METHODS

General procedure for preparation of *p*-Iodoanilines (3a-d):

Take 11ml of Aniline (**1**; 1.2mmoles), 15mg of Sodium bicarbonate(1.8mmoles), taken into round bottom flask and reflux for 3hours. The reaction mixture is cooled to 12 – 15°C by addition of ice with stirring. Powdered Iodine (**2**) is added 15-20mg portion at interval of 2-3 minutes. Stirring is continued for 20 -30 minutes. Color of free iodine solution disappeared when reaction is complete. The crude P-Iodoaniline separates out as dark crystalline mass. The crude product is purified by adding gasoline in a flask. Flask is fitted with reflux condenser and heated in water bath at temperature of 75-80°C. Flask should be shaken

frequently about 15min should be allowed for saturation. Gasoline solution decanted. P-Iodo aniline crystallizes immediately as colorless needles which are filtered and dried.



SCHEME-I

3.1.3.2. General protocol for synthesis of tosylated p-Iodoanilines (5):

To a solution of 4-iodoaniline (**3a-d**) (9mmol) in anhydrous pyridine (20ml) was added p-toluene sulfonyl chloride (**4**) (10.8mmol). The reaction mixture was stirred for 10hrs at room temperature under nitrogen atmosphere, extracted with ethyl acetate. The combined organic phases were washed with brine(45ml), dried over sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography, using a mixture of ethylacetate and hexane(1:10) as eluent to afford the products as white solid.

3.1.3.3. General procedure for coupling of vinyl indole with tosylated p-iodoaniline:

A mixture of vinyl azaindole (0.5mmol) (**8**), tosylated P-iodo aniline (**5**) (1mmol), Iodine and triethyl amine at 250 °C for 8hrs. The reaction mixture was quenched with saturated solution of Na₂S₂O₃ (5ml) and extracted with ethyl acetate. The combined organic phases were washed with brine solution (45ml), dried over sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography, using a mixture of ethyl acetate and hexane(6:4) as eluent, to afford the products as white solid as N-(4-chloro-2-iodophenyl)-N-(1-vinyl-1H-pyrrolo[2,3-b]pyridin-3-yl) sulfonamides (**9a-p**).

3.1.3.4. General procedure for synthesis of (Z)-6-(sulfonyl)-6H-7,10-etheno-5,13-(metheno)pyrido[2,3-b][1,5]diazacycloundecine derivatives (10a-p):

Compounds N-(4-chloro-2-iodophenyl)-N-(1-vinyl-1H-pyrrolo[2,3-b]pyridin-3-yl) sulfonamides (**9a-p**)(0.7 mmol), and Bis (triphenyl phosphane) palladium (0.06 gm or 0.4 mmol) in ethanol (30 mL) were refluxed for 9 hour. The residue resulted after the removal of ethanol was dissolved in dichloromethane and then washed with water. Volatilities were removed under vacuum to afford the title compounds (**10a-p**) which were purified by flash chromatography, using a mixture of ethyl acetate and hexane(6:4).

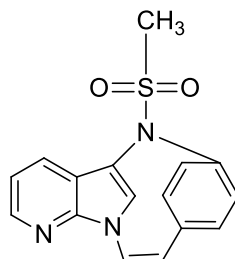
Table-3.1. Physical data of new Azaindole derivatives (10a-o)

S.No	Compound	M. Form	M.Wt	M.P	Rf*	% Yield
1	10a	C ₁₆ H ₁₃ N ₃ O ₂ S	311	235-237	0.7	57
2	10b	C ₁₆ H ₁₂ ClN ₃ O ₂ S	345	268-270	0.4	60
3	10c	C ₁₆ H ₁₂ BrN ₃ O ₂ S	390	203-205	0.6	75
4	10d	C ₁₆ H ₁₂ N ₄ O ₄ S	356	210-212	0.5	68
5	10e	C ₂₁ H ₁₅ N ₃ O ₂ S	373	198-200	0.8	52
6	10f	C ₂₁ H ₁₄ ClN ₃ O ₂ S	407	248-250	0.6	61
7	10g	C ₂₁ H ₁₄ BrN ₃ O ₂ S	452	263-265	0.4	38
8	10h	C ₂₁ H ₁₄ N ₄ O ₄ S	418	270-272	0.5	78
9	10i	C ₁₉ H ₁₃ N ₃ O ₂ S ₂	379	169-171	0.7	57
10	10j	C ₁₉ H ₁₂ ClN ₃ O ₂ S ₂	413	188-190	0.5	72
11	10k	C ₁₉ H ₁₂ BrN ₃ O ₂ S ₂	458	230-232	0.5	84
12	10l	C ₁₉ H ₁₂ N ₄ O ₄ S ₂	424	195-197	0.8	65
13	10m	C ₂₁ H ₁₃ Cl ₂ N ₃ O ₂ S	441	263-265	0.4	59
14	10n	C ₂₁ H ₁₃ Br ₂ N ₃ O ₂ S	531	190-192	0.8	81
15	10o	C ₂₁ H ₁₃ N ₅ O ₆ S	463	214-216	0.5	48

* Mobile phase: hexane: ethyl acetate

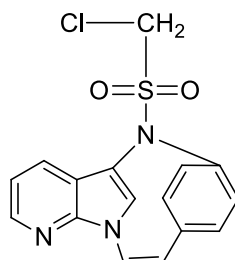
3.1.4. SPECTRAL DATA OF SYNTHESIZED COMPOUNDS:

3.1.4.1. (Z)-6-(methylsulfonyl)-6H-7,10etheno-5,13-(metheno)-pyrido[2,3b][1,5]di-azacycloundecine (10a) Compound 10a obtained as yellowish orange solid



¹H NMR (400MHz DMSO, δ ppm): 7.906-7.927 (d, 2H, $J=8.4$ Hz, Ar-H) 7.606-7.724 (t, 1H, $J=4.0$ Hz, Ar-H), 7.284-7.586 (m, 6H, Ar-H), 5.370 (s, 1H, ethylene), 2.85 (s, 3H, methyl). ¹³C NMR (100MHz, DMSO): 142.18, 132.54, 129.35, 129.09, 128.56, 128.41, 126.88, 125.27, 124.48, 114.02, 42.18, MASS spectrum m/z : 313.14[M+2]⁺, 443.25[M+4]⁺, Calc. for C₁₆H₁₃N₃O₂S; CHN: 61.72; H, 4.21; N, 13.50; S, 10.30; Found: 61.70; H, 4.26; N, 13.55; S, 10.25. IR (KBr, cm⁻¹): 3058.56 (C-H, Aromatic), 2976.58 (C-H, Aliphatic), 1660 (C=N), 1591.59 (C=C), 1222.35 (S=O), 1080.34 (C-N).

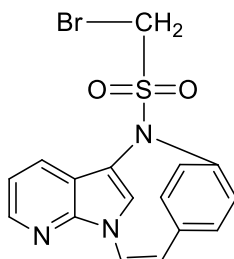
3.1.4.2. (Z)-6-((chloromethyl)sulfonyl)-6H-7,10-etheno-5,13-(metheno)pyrido[2,3-b][1,5]di-azacycloundecine (10b) : Compound 10b obtained as white solid.



¹H NMR (400MHz DMSO, δ ppm): 7.906-7.927 (d, 2H, $J=8.4$ Hz, Ar-H), 7.703-7.724 (d, 2H, $J=4.0$ Hz, Ar-H), 7.567-7.606 (t, 1H, Ar-H), 7.370-7.408 (m, 4H, Ar-H), 4.689 (s, 1H, ethylene), 3.066 (s, 2H,

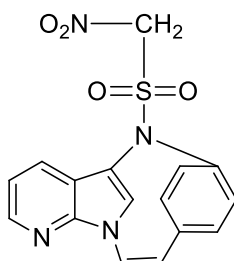
methylene). ¹³C NMR (100MHz, DMSO): 153.78, 152.34, 143.63, 142.44, 139.59, 138.74, 136.03, 129.96, 129.40, 127.96, 127.65, 126.73, 125.01, 119.01, 122.27, 119.01, 48.15, 38.66. MASS spectrum m/z: 347.14[M+2]⁺, Calc. for C₁₆H₁₂ClN₃O₂S; CHN: C, 55.57; H, 3.50; N, 12.15; O, 9.25; S, 9.27; Found: C, 55.50; H, 3.55; N, 12.18; O, 9.20; S, 9.22; IR (KBr, cm⁻¹): 3081.56 (C-H, Aromatic), 2924.43 (C-H, Aliphatic), 1638.12 (C=N), 1572.70(C=C), 1236.28 (S=O), 1136.97 (C-N).

3.1.4.3. (Z)-6-((bromomethyl)sulfonyl)-6H-7,10-etheno-5,13-(metheno)pyrido[2,3-b][1,5]diazacycloundecine (10c): Compound 10c obtained as yellowish orange solid.



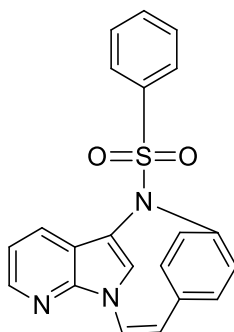
¹H NMR (400MHz DMSO, δ ppm): 7.928-7.923 (d, 2H, *J*=8.4 Hz, Ar-H) 7.659-7.724 (t, 1H, *J*=4.0 Hz, Ar-H), 7.234-7.596 (m, 6H, Ar-H), 5.371 (s, 1H, ethylene), 2.35 (s, 2H, methylene). ¹³C NMR (100MHz, DMSO): 161.32, 140.18, 130.54, 126.35, 126.03, 125.16, 124.41, 123.88, 122.27, 116.48, 113.02, 48.18, MASS spectrum m/z: 392.14[M+2]⁺, Calc. for C₁₆H₁₃N₃O₂S; CHN: 49.24; H, 3.10; N, 10.77; S, 8.22; Found: 49.53; H, 3.10; N, 10.70; S, 8.20. IR (KBr, cm⁻¹): 3062.56 (C-H, Aromatic), 2986.18 (C-H, Aliphatic), 1650 (C=N), 1585.19 (C=C), 1228.15 (S=O), 1082.34 (C-N).

3.1.4.4. (Z)-6-((nitromethyl)sulfonyl)-6H-7,10-etheno-5,13-(metheno)pyrido[2,3-b][1,5]diazacycloundecine (10d): Compound 10d obtained as yellowish white solid.



¹H NMR (400MHz DMSO, δ ppm): 7.918-7.921 (d, 3H, *J*=8.4 Hz, Ar-H), 7.661-7.664 (t, 1H, *J*=4.0 Hz, Ar-H), 7.230-7.595 (m, 5H, Ar-H), 5.370 (s, 1H, ethylene), 2.30 (s, 2H, methylene). ¹³C NMR (100MHz, DMSO): 150.25, 135.10, 128.54, 125.35, 124.03, 123.16, 120.41, 119.18, 115.27, 113.40, 110.02, 45.18, MASS spectrum m/z: 392.14[M+2]⁺, Calc. for C₁₆H₁₃N₃O₂S; CHN: 49.24; H, 3.10; N, 10.77; S, 8.22; Found: 49.53; H, 3.10; N, 10.70; S, 8.20. IR (KBr, cm⁻¹): 3062.56 (C-H, Aromatic), 2986.18 (C-H, Aliphatic), 1650 (C=N), 1585.19 (C=C), 1228.15 (S=O), 1082.34 (C-N).

3.1.4.5. (Z)-6-(phenylsulfonyl)-6H-7,10-etheno-5,13-(metheno)pyrido[2,3-b][1,5]diazacycloundecine (10e): Compound 10e obtained as orange solid.



¹H NMR (400MHz DMSO, δ ppm): 7.926-7.928 (d, 2H, *J*=8.4 Hz, Ar-H) 7.668-7.721 (t, 1H, *J*=4.0 Hz, Ar-H), 7.238-7.535 (m, 6H, Ar-H), 5.376 (s, 1H, ethylene), 2.28 (s, 2H, methylene). ¹³C NMR (100MHz, DMSO): 140.18, 138.12, 130.54, 126.35, 126.03, 125.16, 124.41, 123.88, 122.27, 116.48, 113.02, 48.18, MASS spectrum m/z: 392.14[M+2]⁺, Calc. for C₁₆H₁₃N₃O₂S; CHN: 49.24; H, 3.10; N, 10.77; S, 8.22;

Found: 49.53; H, 3.10; N, 10.70; S, 8.20. **IR (KBr, cm⁻¹):** 3051.89 (C-H, Aromatic), 2922.61 (C-H, Aliphatic), 1722.32 (C=O), 1588.12 (C=C, Aromatic), 1253.72(C-O).

3.1.4.6. Z)-6-((4-chlorophenyl)sulfonyl)-6H-7,10-etheno-5,13-(metheno)pyrido[2,3-b][1,5]diazacycloundecine (10f): Compound 10f obtained as yellowish white solid.

RESULTS AND DISCUSSION

The potential anti-cancer activity of synthetic compounds was demonstrated in in-vitro tests, and among the substances, compound 10m demonstrated encouraging anti-cancer activity. These findings compelled us to carry out docking analyses to gain insight into the way synthetic chemicals bind to HeLa and MCF-7 binding pocket. The Schrodinger package's LigPrep was used to further prepare each ligand structure once it had been constructed using maestro. The Protein Preparation Wizard in the Schrodinger software was used to make the necessary corrections to the protein structure after it was retrieved from the Protein Data Bank (PDB ID: MCF-7-3HY3 and HeLa -6I2I). The docking procedure was confirmed by docking the cocrystal ligand, which produced an RMSD of docked conformation and cocrystal ligand pose of 0.6. Docking tests were carried out using the Glide docking software. the ways that substances bind to HeLa and MCF-7 cells. have been listed in **Table- 3.1.2, Figure-3.1.1, 3.1.2, 3.1.3 and 3.1.4**

A docking study was conducted, and binding poses of synthetic molecules with HeLa and MCF-7 cells revealed that these molecules bind strongly within the enzyme's binding pocket. The chemical with strong anti-cancer action 10m has displayed the highest binding score out of all the produced compounds (-7.584 and -6.252 against HeLa and MCF-7). As seen in Figure-5.2.1, the aromatic ring coincided with the skeleton of the cocrystal ligand in the superimposed posture of a 10 m cocrystal ligand. The enzyme hydrogen bonding interactions involving 4,5-dichlorophenyl and compound 10m, which has the highest binding score. interactions between phenyl and Phe85. While phenyl formed hydrogen bonds with Phy and Trp83, OH formed hydrogen bonds with THR 276 and SER277.

3.1.7. BIOLOGICAL EVALUATION:

3.1.7.1. ANTICANCER ACTIVITY

3.1.7.1.1. MTT ASSAY:

Procurement of cell line: The HeLa and MCF-7 cell line with passage number 45 was procured from NCCS, Pune.

Media Preparation: Pour 20-30 ml MEM media in centrifuge. To this add 10ml of bovine serum, 0.5 ml antibiotic solution, 1.25ml HEPES and make up volume up to 50ml by appropriate media. Mix it and store at 208°C (for up to 4 weeks).

Sub culturing cells: Take above solution and remove the media and wash with PBS. Remove PBS and add 1ml trypsin-EDTA solution. Incubate the flask at 37°C in CO₂ incubator.

Table -3.1.3. Protocol for MTT assay:

Day 1 (Cells Suspended)	Day 2 (Drug Treatment)	Day 3 (MTT Assay)
Washing with PBS	Removed the plate from incubator to remove the media from each well	25 µl of freshly prepared MTT added in each well
Trypsinization	Drug treatment (10,25, 50,100,200 and 300µM)	Keep in incubator at 37°C for 2-3h
Cell Counting	Untreated / DMSO	Media was removed
Filled 96 well Plate (10000 cells/well)		DMSO (100 µl) was added
Incubation (24h)	Incubation (24h)	Kept the plate in Incubator at 37°C for overnight and recorded plate at 570nm in ELISA plate reader

Traditionally, the determination of cell growth is done by counting viable cells after staining with a vital dye. Several approaches have been used in the past. Trypan blue staining is a simple way to evaluate cell membrane integrity (and thus assume cell proliferation or death) but the method is not sensitive and cannot

be adapted for high throughput screening. Measuring the uptake of radioactive substances, usually tritium-labelled thymidine, is accurate but it is also time-consuming and involves handling of radioactive substances. Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. The absorbance of this coloured solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer.

The absorption max is dependent on the solvent employed. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion can be directly related to the number of viable (living) cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced, through the production of a dose-response curve. Solutions of MTT solubilized in tissue culture media or balanced salt solutions, without phenol red, are yellowish in colour. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple MTT formazan crystals which are insoluble in aqueous solutions. The crystals can be dissolved in acidified isopropanol. The resulting purple solution is spectrophotometrically measured. An increase in cell number results in an increase in the amount of MTT formazan formed and an increase in absorbance. The use of the MTT method does have limitations influenced by:

(1) the physiological state of cells.

(2) Variance in mitochondrial dehydrogenase activity in different cell types.

Nevertheless, the MTT method of cell determination is useful in the measurement of cell growth in response to mitogens, antigenic stimuli, growth factors and other cell growth promoting reagents, cytotoxicity studies, and in the derivation of cell growth curves.

3.1.7.1.2. RESULTS AND DISCUSSIONS

4,5 Dichloro phenyl substituted compound **10m** showed both HELa and MCF-7 with IC_{50} value of 0.78 and 1.31 μ M. Most active compound among the series was found to be **10n** with IC_{50} of **0.92** and **1.91** μ M and compared to 0.68, 1.65 μ M of standard drug that is cisplatin against HELa and MCF-7 respectively. In general, increasing the electronegative group increasing the anti-cancer activity was found to increase significantly. Effect of the nature of aryl group and substituents on HELa and MCF-7 inhibitory activity was studied to understand the structure activity relationship. The simple phenyl group in **10e** when replaced with Cl resulted in marginal decrease in both HELa and MCF-7 inhibitory activity. Substitution with 3-chloro group as in enhanced HELa and MCF-7 inhibitory activity significantly and compound **10f** with Cl substituted moiety showed potent activity with IC_{50} value of 1.36 and 4.26 μ M against HELa and MCF-7 cell lines respectively.

Insertion of electron withdrawing group (4-nitro) as in **10d**, **10h**, **10l**, **10** resulted in increasing HELa and MCF-7 cell lines inhibitory activity when compared with their corresponding unsubstituted compounds, **10a**, **10e**, **10i** which indicated that electron withdrawing groups enhance the HELa and MCF-7 cell inhibitory activity. To explore further the SAR, compounds with two Cl groups have been synthesized (**10m**) and to our expected observations. Three compounds with 4,5 di bromo phenyl groups as in **10n** have also shown enhanced activity over unsubstituted compounds, in both HELa and MCF-7 cell lines. Anti-cancer activity of all the compounds have been listed in **Table 3.1.4** and graphically depicted in **Figure 3.1.5** and **3.1.6**.

Table -3.1.4: Anti cancer activity data against MCF-7 and HeLa cell lines

S.No	Compound	M. Form	MCF-7 $IC_{50}(\mu$ M)	HELa $IC_{50}(\mu$ M)
1	10a	$C_{16}H_{13}N_3O_2S$	1.10 $\pm$ 0.02*	6.17 $\pm$ 0.02
2	10b	$C_{16}H_{12}ClN_3O_2S$	1.52 $\pm$ 0.04	5.02 $\pm$ 0.04
3	10c	$C_{16}H_{12}BrN_3O_2S$	1.12 $\pm$ 0.02	2.78 $\pm$ 0.04
4	10d	$C_{16}H_{12}N_4O_4S$	1.90 $\pm$ 0.02	4.62 $\pm$ 0.02
5	10e	$C_{21}H_{15}N_3O_2S$	2.80 $\pm$ 0.02	5.35 $\pm$ 0.03
6	10f	$C_{21}H_{14}ClN_3O_2S$	1.36 $\pm$ 0.03	4.26 $\pm$ 0.03
7	10g	$C_{21}H_{14}BrN_3O_2S$	1.35 $\pm$ 0.02	3.45 $\pm$ 0.03

8	10h	C ₂₁ H ₁₄ N ₄ O ₄ S	1.08±0.03	2.38±0.03
9	10i	C ₁₉ H ₁₃ N ₃ O ₂ S ₂	2.90±0.02	7.64±0.02
10	10j	C ₁₉ H ₁₂ ClN ₃ O ₂ S ₂	2.10±0.08	5.64±0.02
11	10k	C ₁₉ H ₁₂ BrN ₃ O ₂ S ₂	2.02±0.04	5.59±0.02
12	10l	C ₁₉ H ₁₂ N ₄ O ₄ S ₂	1.65±0.03	3.24±0.03
13	10m	C ₂₁ H ₁₃ Cl ₂ N ₃ O ₂ S	0.78±0.03***	1.31±0.02**
14	10n	C ₂₁ H ₁₃ Br ₂ N ₃ O ₂ S	0.92±0.03**	1.91±0.02*
15	10o	C ₂₁ H ₁₃ N ₅ O ₆ S	1.12±0.05	1.95±0.03
16	Cisplatin	-	0.68±0.02	1.65±0.02

Results were presented as mean±SD, (n=6) analyzed by ANOVA (one-way) followed by tukey's multiple comparison test with *p<0.05 as significant.

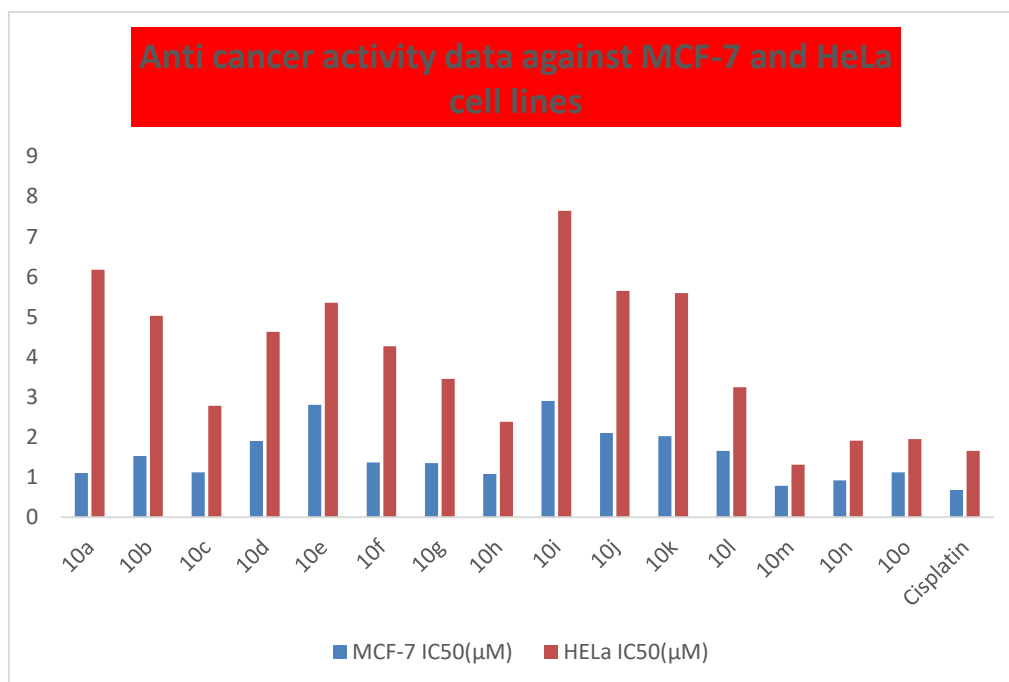


Fig: 3.1.5: Graphical representation of New Azaindole derivatives anti-cancer activity data against MCF-7 and HeLa cell lines (IC₅₀ μM). The values represent the mean±SD μM from

3.1.8. ANTI-ALZHEIMER'S ACTIVITY:

The tests were conducted in compliance with CPCSEA guidelines and received IAEC/40/SURA/HYD/2018 approval from the institutional animal ethical committee. 5-6 Weeks Swiss albino male mice weighing 20-25 g were used for behavioral studies. The animals were maintained in conventional environmental conditions, including a temperature of 20–25 °C, a 12-hour light/dark cycle, and a standard pellet meal, as well as water available at all times. The animals were given a week to get used to the lab environment before the test. Thereafter, they were randomly separated into five groups of six animals each as follows:

Group 1: (vehicle control): Animals received Phosphate Buffer Solution, per oral

Group 2: (negative control): Mice administered with β-amyloid peptide by cerebroventricular injection.

Group 3: Mice injected with β-amyloid peptide and rivastigmine (per oral) 5 mg/kg.

Group 4: Mice injected with β-amyloid peptide and treated with 200 mg/kg and 400 mg/kg of test compounds (per oral)

Macha B et al. (2021) identified the bregma site in the skull using stereotaxic equipment and delivered 10 μL containing 10 μg Aβ (25-35) peptide to all but one of the groups to induce neurotoxicity at

intra-cerebroventricular injection (INCO, India). Test chemicals and common medications were given on the fourteenth day after the β -amyloid peptide was started. Behavior studies and metabolic markers are examined on days 7 and 21 following β -amyloid peptide administration.

3.1.8.1. In vitro AChE and BuChE inhibitory studies (Baswaraju M et al., 2021):

The investigation of AChE and BuChE was carried out using standard described procedures and ready-to-use kits, all of which were supplied by Sigma Aldrich. The test chemicals were dissolved in DMSO and 1% bovine serum before being diluted with phosphate buffer. Ellman's reagent (5, 5'-dithiobis- 2-nitrobenzoic acid, DTNB) solution (5, 5'-dithiobis- 2-nitrobenzoic acid, DTNB) was produced by solubilizing 2 mg of DTNB in 200 mL of buffer. The modified Ellman method for determining the IC_{50} of test compounds involves the formation of thiocholine by the action of AChE on the substrate, acetylthiocholine iodide. Thiocholine immediately reacts with DTNB to produce yellow colour, and the intensity of the colour is proportional to the enzyme activity in the presence of test chemicals when evaluated spectroscopically at 412 nm. In a test tube, the assay mixture of 10 mL AChE and 30 μ L DTNB solution was mixed with intermittent shaking. After adding concentrations (5 μ M & 10 μ M and 25 μ M) of test compounds and standards, the solution was incubated for 20 minutes before adding 30 μ L of DTNB reagent and 30 μ L of substrate acetyl thiocholine iodide. The intensity of the yellow hue so formed was measured in triplicate at 412 nm one minute gap at 37 °C. The amount of acetyl thiocholine hydrolyzed in the enzymatic reaction is indicated by the intensity of yellow colour produced in the stoichiometric reaction. The aforementioned methodology was carried similarly for BuChE activity using butyrylthiocholine iodide as substrate. Linear regression plot drawn between % inhibition vs log concentration of test compounds on MS Excel determines the IC_{50} of compounds. The results are presented as mean $\pm$ standard deviation of the triplicates.

3.1.8.2. In vitro estimation of Brain Cholinesterases:

The Ellman's method was further followed to estimate brain AChE and BuChE levels in experimental animals. Rat brain was employed to ascertain the cholinesterase inhibitory activity of test compounds in which animals were sacrificed in order to collect the brain and homogenate was prepared 0.1M phosphate buffer (pH 8.0). To a test tube containing 2.6 mL of phosphate buffer was added 0.4 mL brain homogenate and contents were mixed thoroughly. 100 μ L of DTNB chromophore reagent was transferred in to the above solution and swirled for proper mixing along with air bubbling. Absorbance of resultant color developed was measured at 412 nm, when absorbance reaches a stable value, it was recorded as the basal reading. Acetyl thiocholine substrate 20 μ L was then transferred into test tube and alternation in absorbance was noted for a period of 10 minutes at intervals of 2 minutes and later change in the absorbance per minute was determined.

3.1.8.3. In vivo Behavior Pharmacological Activities:

3.1.8.3.1. Jumping Avoidance Box (Barros et al.2000):

A Plexiglas partition in a box was used to create two equal chambers, and a gate was installed to allow the animals access to the adjacent compartment through a 14*17 cm area. The test animals are exposed for 30 seconds to light which is followed by 10 seconds sound stimulus and a single low intensity foot shock (0.5 mA) for three seconds in each session. Each mouse in each group received 15 such trials each day, with a 15-second break between successive trials, which was repeated for five days.

3.1.8.3.2. Rectangular Maze Test (Shearet al.1998):

Rectangular Maze test apparatus consists of three interconnected chambers A, B, and C, with chamber B containing the maze used to assess test animals' memory capacity. All hungry mice were allowed to move from chamber A to chamber C via chamber B. Chamber C held reward food for the animal as it moved from chamber A to chamber C, as indicated by the pilot light. Every test animal was trained daily to collect the reward food, and the time duration was recorded. The animals were termed trained when the time required to complete each maze remained consistent for three consecutive days, and the time to traverse for maze completion was then recorded for each animal before and after treatment with test compounds and standard medication.

CONCLUSION:

The present study successfully achieved the synthesis, characterization, and biological evaluation of novel coumarin derivatives. The synthesized compounds were confirmed by spectroscopic analyses such as FT-

IR, ^1H -NMR, ^{13}C -NMR, and Mass spectrometry, ensuring structural integrity and purity. The in vitro anticancer screening revealed that several derivatives exhibited promising cytotoxic activity against selected human cancer cell lines, indicating the strong potential of coumarin as a core scaffold for anticancer drug design. Structure–activity relationship (SAR) analysis suggested that substituent variations on the coumarin ring significantly influence biological activity, guiding future optimization strategies. Overall, this work highlights coumarin derivatives as potential lead molecules for the development of safer and more effective anticancer agents, warranting further pharmacological and molecular docking studies to explore their mechanism of action and therapeutic potential.

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