DESIGN, SYNTHESIS, CHARACTERIZATION AND INSILICO EVALUATION OF NOVEL FLAVONE DERIVATIVES AS ANTICANCER AGENTS

A Dissertation submitted to

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY
CHENNAI – 600 032

In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY

IN

BRANCH II (PHARMACEUTICAL CHEMISTRY)

SUBMITTED BY

MANI MEGALAI. V

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CERTIFICATE

This is to certify that the project entitled **"DESIGN, SYNTHESIS, CHARACTERIZATION AND INSILICO EVALUATION OF NOVEL FLAVONE DERIVATIVES AS ANTICANCER AGENTS''** as submitted by **V. MANI MEGALAI (261915006),** in partial fulfillment for the award of the degree of **Master of Pharmacy** in the **academic year 2019-2021**. It was carried out at **C.L. BAID METHA COLLEGE OF PHARMACY, CHENNAI – 600097** under the guidance and supervision of **K. DHUNMATI, M.Pharm, Asst. Prof.,** Professor and Head in the Department of Pharmaceutical Chemistry during the academic year 2019 – 2021.

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DECLARATION

I do hereby declare that the thesis entitled **"DESIGN, SYNTHESIS, CHARACTERIZATION AND INSILICO EVALUATION OF NOVEL FLAVONE DERIVATIVES AS ANTICANCER AGENTS"** by **V. MANIMEGALAI (261915006),** in partial fulfillment of the degree of **MASTER OF PHARMACY** was carried out of **C.L. BAID METHA COLLEGE OF PHARMACY, CHENNAI-600 097** under the guidance and Supervision of **K. DHUNMATI, M.PHARM., Asst, Prof.,** during the academic year 2019-2021. The work embodied in this thesis is original & is not submitted in part or full for any other degree of this or any other university.

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Date:

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MANIMEGALAI. V

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LIST OF ABBREVIATIONS

EGFR – Epidermal Growth Factor Receptor

RTK – Receptor Tyrosine Kinase

TKI – Tyrosine Kinase Inhibitors

PDB – Protein Data Bank

RCSB – Research Collaboratory for Structural Bioinformatics

DMSO – Dimethyl Sulphoxide

DNA – Deoxyribonucleic Acid

°C – Degree Celsius

KOH – Potassium Hydroxide

K_2CO_3 – Potassium Carbonate

TLC – Thin Layer Chromatography

IR – Infrared

^1H NMR – Proton Nuclear Magnetic Resonance

MS – Mass Spectra

M.p – Melting point

MCF – Michigan Cancer Foundation

IC_{50} – Half Maximal Inhibitory Concentration

INTRODUCTION

DESIGN, SYNTHESIS, CHARACTERIZATION AND INSILICO EVALUATION OF NOVEL FLAVONE DERIVATIVES AS ANTICANCER AGENTS

I. INTRODUCTION:

1.1 INTRODUCTIN OF MEDICINAL CHEMISTRY

Medicinal chemistry is a discipline that encloses the design, development, and synthesis of pharmaceutical drugs. Medicinal/Pharmaceutical chemistry deals with the discovery, design, development and both pharmacological and analytical characterization of drug substances. The use of plants, minerals, and animal parts as medicines has been recorded since the most ancient civilizations. With the evolution of the knowledge the means for drug discovery also evolved.

New molecules with potential pharmaceutical interest, "hits', are natural products, or compounds generated by computational chemistry, or compounds from a screening of chemical libraries, from combinatorial chemistry, and from pharmaceutical biotechnology. The “hit” compound is improved for its pharmacologic, pharmacodynamic and pharmacokinetic properties by chemical or functional group modifications, transforming it into a lead compound.

A lead compound should have a known structure and a known mechanism of action. The lead compound is further optimized to be a drug candidate that is safe to use in human clinical trials. Thus, Medicinal chemistry is the field of pharmaceutical sciences which applies the principles of chemistry and biology to certain of knowledge leading to the introduction of new therapeutic agents.

Thus, Medicinal chemistry is the field of pharmaceutical sciences which applies the principles of chemistry and biology to certain of knowledge leading to the introduction of new therapeutic agents.

Medicinal chemistry covers three critical steps.

A discovery step, involves the choice of the therapeutic target (receptor, enzyme, transport group, cellular, or *in-vivo* model) and the identification (or discovery) and production of new active substances interacting with the selected target. Such compounds are usually called as lead compounds; they can originate from synthetic organic chemistry, from natural sources, or from biotechnological process. Drug design aims at the development of the drugs with high specificity and therapeutic indeed.

An optimization step, which deals with the improvement of the lead structures. The optimization process takes primarily in to account the increase in potency, selectivity and toxicity. Its characteristics are the establishment of the molecular mode of action. However, an assessment of the pharmacokinetic parameters such as absorption, distribution, metabolism, excretion and oral bioavailability is almost systematically practiced at an early stage of the development in order to eliminate unsatisfactory candidates.

A development step, whose purpose is the continuation of the improvement of the pharmacokinetic properties and the fine tuning of the pharmaceutical properties (chemical formulation) of the active substances in order to render them suitable for clinical use. These chemical formulations, and of water-soluble derivatives or in the elimination of properties related to patient's compliance (causticity, irritation, painful injections and undesirable organoleptic properties).

Molecular modeling, or more generally, computational chemistry, has become a well-established part of drug development. In particular, medicinal chemistry in its most common practice-focusing on small organic molecules-encompasses synthetic organic chemistry and aspects of natural products and computational chemistry in close combination with chemical biology, enzymology and structural biology, together aiming at the discovery and development of new therapeutic agents.

1.2 FLAVONOIDS

- Flavonoids are an important class of natural products: particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits, vegetables and certain beverages.
- They have miscellaneous favorable biochemical and antioxidant effects associated with various diseases such as cancer. Alzheimer's disease (AD), atherosclerosis, etc., (1-3).
- Flavonoids are associated with a broad spectrum of health-promoting effects and are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications.
- This is because of their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme functions. They are also known to be potent inhibitors for several enzymes, such as xanthine oxidase (XO), cyclo-oxygenase (COX), lipoxygenase and phosphoinositide 3-kinase (4–6).
- In nature, flavonoid compounds are products extracted from plants and they are found in several parts of the plant.
- Flavonoids are used by vegetables for their growth and defense against plaques (7). They belong to a class of low-molecular-weight phenolic compounds that are widely distributed in the plant kingdom.
- Flavonoids are also abundantly found in foods and beverages of plant origin, such as fruits, vegetables, tea, cocoa and wine; hence they are termed as dietary flavonoids.
- Flavonoids have several subgroups, which include chalcones, flavones, flavonols and isoflavones. These subgroups have unique major sources. For example, onions and tea are major dietary sources of flavanols and flavones.

1.3 STRUCTURE OF FLAVONOIDS

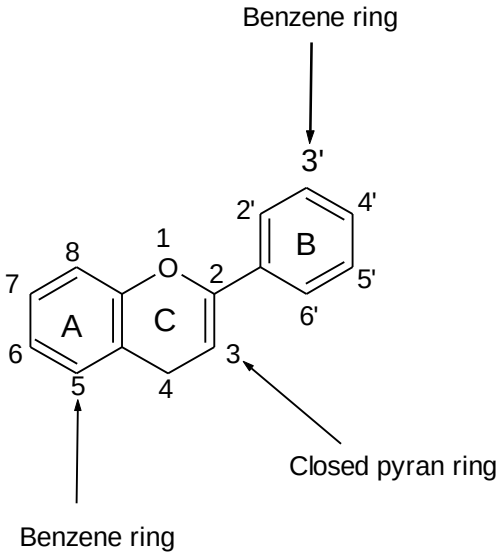


Figure 1: Structure of flavonoids

Their basic structure is a skeleton of diphenyl propane, namely, two benzene rings (ring A and B) linked by a three-carbon chain that forms a closed pyran ring (heterocyclic ring containing oxygen, the C ring) with benzenic A ring.

Therefore, their structure is also referred to as C_6-C_3-C_6 is most cases, B ring is attached to position 2 of C ring, but it can also bind in position 3 or 4; this, together with the structural features of the ring B and the patterns of glycosylation and hydroxylation of the three rings, makes the flavonoids one of the larger and more diversified groups of phytochemicals.

1.4 CLASSIFICATION AND CHEMISTRY OF FLAVONOIDS

Flavonoids are classified in six major subgroups depending on the carbon of the C ring on which B ring is attached and the degree of unsaturation and oxidation of the C ring. Flavonoids in which B ring is linked in position 3 of the ring C are called isoflavones; those in which B ring is linked in position 4, neoflavanoids, while those in which the B ring is

linked in position 2 can be further subdivided into several subgroups on the basis of the structural features of the C ring. These subgroup are: flavones, flavonols, flavanones, flavanonols, flavanols or catechins and anthocyanins.

Flavones

Flavones are one of the important subgroups of flavonoids. Flavones are widely present in leaves, flowers and fruits as glucoside. They have a double bond between positions 2 and 3 and a ketone in position 4 of the C ring.

Eg: Apigenin, luteolin

Flavonols

Flavonols are flavonoids with a ketone group. Flavonols occur abundantly in a variety of fruits and vegetables. Compared with flavones, flavonols have a hydroxyl group in position 3 of the C ring, which may also be glycosylated.

Eg: kaempferol, rutin, myricetin, quercetin

Flavanones

Flavanones are another important class which is generally present in all citrus fruits such as oranges, lemons and grapes. Hesperitin, naringenin and eriodictyol are examples of this class of flavonoids. Flavanones, also called dihydroflavones, have the C ring saturated; therefore, unlike flavones, the double bond between positions 2 and 3 is saturated and this is the only structural difference between the two subgroups of flavonoids.

Eg: hesperetin, hespereidin, naringenin

Flavanonols

Flavanonols, also called dihydroflavonols or catechins, are the 3-hydroxy derivatives of flavanones. They are a highly diversified and multisubstituted subgroup.

Eg: taxifolin, silymarin

Flavanols

Flavanols are also referred to flavan-3-ols as the hydroxyl group is always bound to position 3 of the C ring. Unlike many flavonoids, there is no double bond between positions 2 and 3. Eg: Catechin

Anthocyanidins:

Anthocyanins are pigments responsible for colours in plants, flowers and fruits. The color of the anthocyanin depends on the pH and also by methylation or acylation at the hydroxyl groups on the A and B rings.

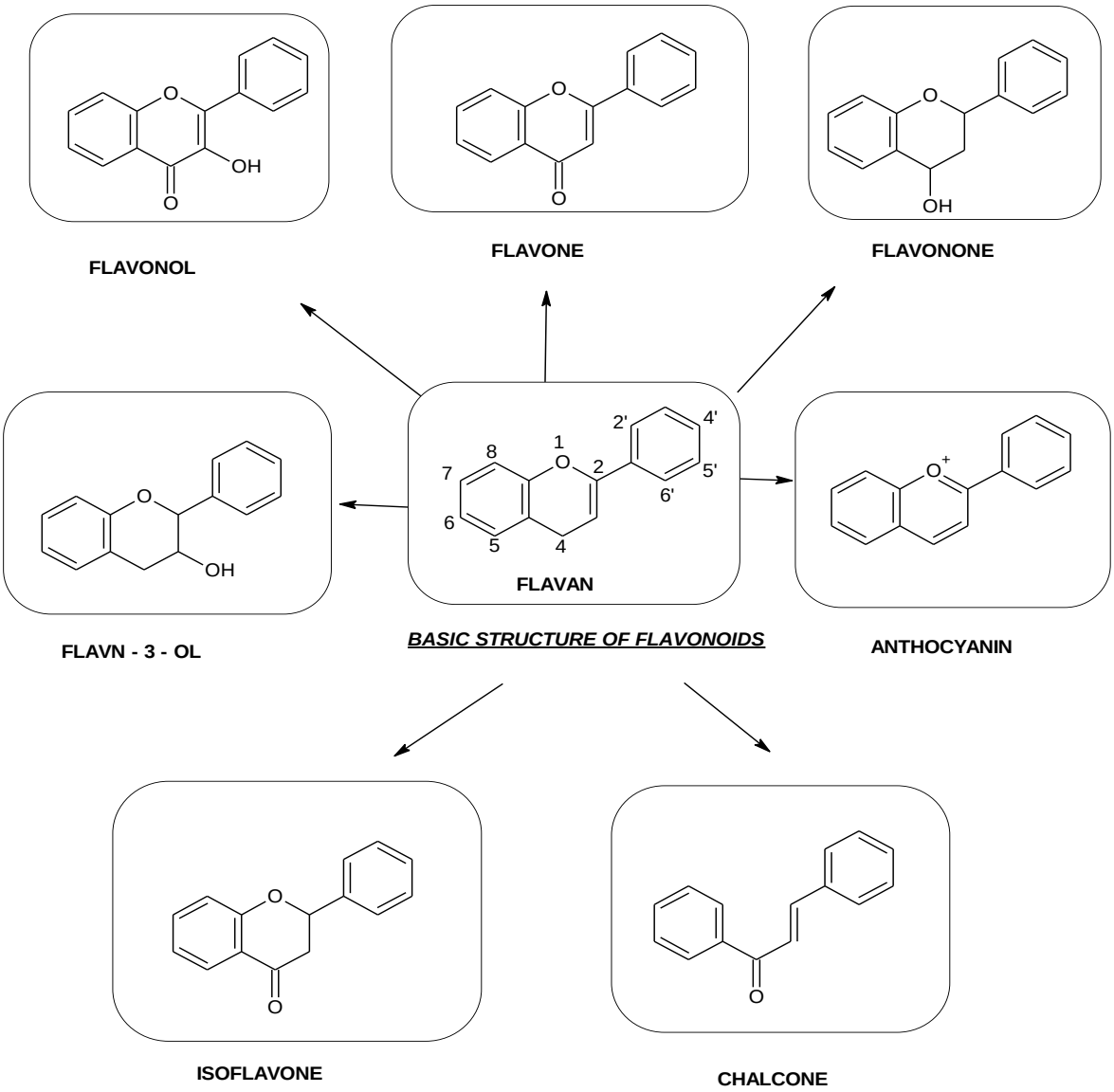
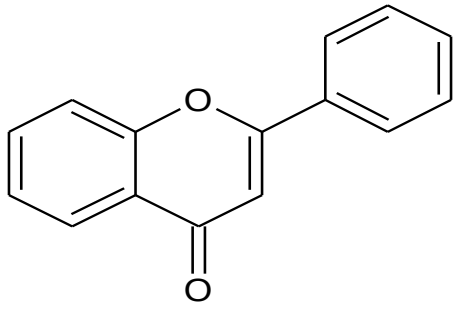


Figure 2: Classification of flavonoids

1.5 FLAVONE

Flavones are a class of flavonoids based on the backbone of 2-phenylchromen-4-one. Flavones are important scaffold known to be associated with several biological activities including antioxidant [9, 10], cytotoxic [11, 12], antibacterial [13,14], antimalarial [15], anti-inflammatory [16,17,18], anti-HIV [19], antitumor [20]



Molecular Formula: $C_{15}H_{10}O_2$

Formula Weight: 222.2387

Composition: C(81.07%) H(4.54%) O(14.40%)

Molar Refractivity: 64.20 ± 0.3 cm^3

Molar Volume: 179.2 ± 3.0 cm^3

Parachor: 475.7 ± 6.0 cm^3

Index of Refraction: 1.635 ± 0.02

Surface Tension: 49.5 ± 3.0 dyne/cm

Density: 1.239 ± 0.06 g/cm^3

Dielectric Constant: Not available

Polarizability: 25.45 ± 0.5 $10^{-24}cm^3$

RDBE: 11

Monoisotopic Mass: 222.06808 Da

Nominal Mass: 222 Da

Average Mass: 222.2387 Da

M+: 222.067531 Da

M-: 222.068628 Da

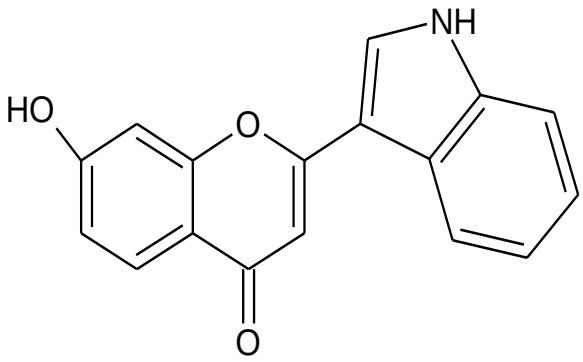
[M+H]+: 223.075356 Da

[M+H]-: 223.076453 Da

[M-H]+: 221.059706 Da

[M-H]-: 221.060803 Da

1.6. INDOLE-3-CARBOXALDEHYDE FLAVONE



Molecular Formula: $C_{17}H_{11}NO_3$

Formula Weight: 277.27414

Composition: C(73.64%) H(4.00%) N(5.05%) O(17.31%)

Molar Refractivity: 78.36 ± 0.3 cm^3

Molar Volume: 190.1 ± 3.0 cm^3

Parachor: 557.2 ± 6.0 cm^3

Index of Refraction: 1.761 ± 0.02

Surface Tension: 73.7 ± 3.0 dyne/cm

Density: 1.458 ± 0.06 g/cm^3

Dielectric Constant: Not available

Polarizability: 31.06 ± 0.5 $10^{-24}cm^3$

RDBE: 13

Monoisotopic Mass: 277.073893 Da

Nominal Mass: 277 Da

Average Mass: 277.2741 Da

M+: 277.073345 Da

M-: 277.074442 Da

[M+H]+: 278.08117 Da

[M+H]-: 278.082267 Da

[M-H]+: 276.06552 Da

[M-H]-: 276.066617 Da

1.7 DOCKING

- Docking is a procedural method to predict the preferred orientation of one molecule to another when bound forming a stable complex.
- Docking is important in Drug designing which is used for calculating the binding alignment of small molecular drugs or inhibitors to their protein targets and can predict affinity and activity of complex formed.
- Molecular docking is an attractive scaffold to understand drug biomolecular interactions for the rational drug design and discovery, as well as in the mechanistic study by placing a molecule (ligand) into the preferred binding site of the target specific region of the DNA/protein (receptor) mainly in a non-covalent fashion to form a stable complex of potential efficacy and more specificity.
- The information obtained from the docking technique can be used to suggest the binding energy, free energy and stability of complexes. At present, docking technique is utilized to predict the tentative binding parameters of ligand-receptor complex beforehand.
- The main objective of molecular docking is to attain ligand-receptor complex with optimized conformation and with the intention of possessing less binding free energy.
- Molecular docking can demonstrate the feasibility of any biochemical reaction as it is carried out before experimental part of any investigation.
- There are some areas, where molecular docking has revolutionized the findings. In particular, interaction between small molecules (ligand) and protein target (may be an enzyme) may predict the activation or inhibition of enzyme. Such type of information may provide a raw material for the rational drug designing. Some of the major applications of molecular docking are Lead optimization**,** hit identifications, Drug- DNA interaction.

1.8 DRUGFILTERS ChemSketch

ACD/ChemSketch Freeware is a drawing package that allows you to draw chemical structures including organics, organometallics, polymers and Markush structures. It also includes features such as calculation of molecular properties (e.g. molecular weight, density, molar refractivity, etc.),2D and 3D structure cleaning and viewing, functionality for naming structures (fewer than 50 atoms and 3 rings), and prediction of log P.

Swiss ADME

A free webtool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. It allows to compute physicochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties, druglike nature and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery.

ProTox II

ProTox-II, a virtual lab for the prediction of toxicities of small molecules. The prediction of compound toxicities is an important part of the drug design development process. ProTox-II incorporates molecular similarity, fragment propensities, most frequent features and (fragment similarity-based CLUSTER cross-validation) machine-learning, based a total of 33 models for the prediction of various toxicity endpoints such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways and toxicity targets.

PreADMET:

PreADMET is a web-based application for predicting ADME data and building drug- like library using *in silico* method. It predicts permeability for Caco-2 cell, MDCK cell and BBB (blood-brain barrier), HIA(human intestinal absorption), skin permeability and plasma protein binding.

REVIEW OF LITERATURE

I. REVIEW OF LITERATURE

- Zhu-ping Xiao *et al* 2014, designed and synthesized twenty-one fluoroquinolone-flavonoid hybrids derived from naringenin. The antibacterial *invitro* was evaluated for the synthesized compounds using ciprofloxacin as a typical drug, within which the compound **[**Figure: **1]** naringenin-ethylidene-ciprofloxacin have an excellent activity against *E. coli, B. subtilis, S. aureus* and *Candida albicans,* with MIC values of 0.71, 0.062, 0.29, and 0.14 µg/ml. respectively. [22]

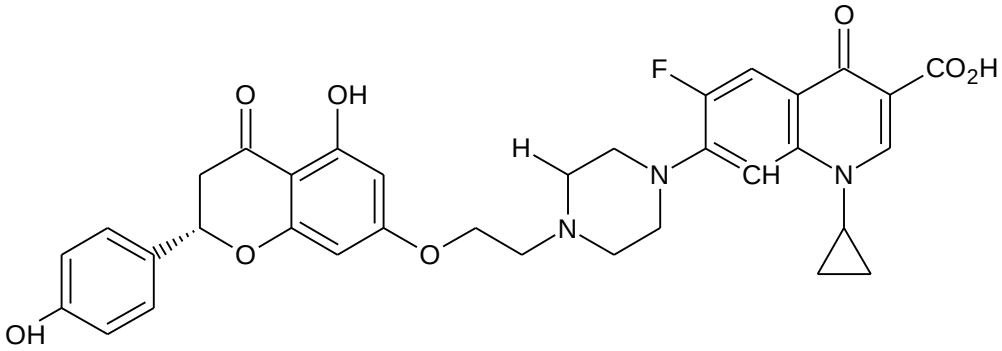


Figure 1: Fluoroquinolone-flavonoid hybrids derivative.

- Angel Amnesty *et al.* In 2018, twenty-eight 4-substituted 1,2,3-Triazole-coumarin derivatives were synthesized by using a Huisgen 1,3, dipolar cycloaddition reaction, catalyzed by copper (I) within the antimicrobial activity of the compound **[**Figure: **2]** showed the foremost active agent in Enterococcus faecalis with variable MICs of 12.5% with a 2-OMe-ph group connected to the triazole nucleus associated to an -OCH_2-linker respectively. [23]

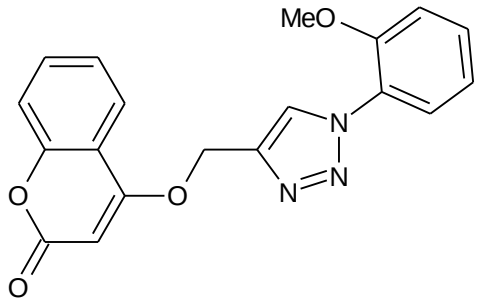


Figure 2: 1,2,3-Triazole-coumarin 4-substituted flavonoid derivative.

- Some chalcones and flavones were synthesized by the microwave-assisted Claisen-Schmidt condensation technique (MWI) by A. Rahim *et al* 2020. The antibacterial activity of the synthesized compounds was tested *invitro* **[**Figure: **3]** 4'-Methylflavone showed a yield of 98% with a MIC value of *B. cereus, S. aureus, P. aeruginosa*, and *Vibrio cholera* was 64 and Salmonella typhi was 32 µg/ml. [24]

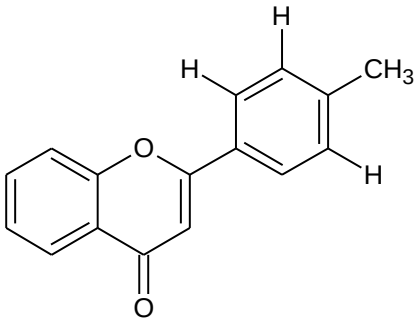


Figure 3: Derivative of microwave-assisted Claisen-schmidt condensation technique of flavone.

- Harbinder Singh *et al* 2019 used ^{13}C NMR and ^{1}H NMR to characterize Indolinedione coumarin hybrid molecules that they developed and synthesized. Compound **[**Figure: **4]** have showed the strongest inhibitory zone of 2.5 and 1.3cm against *S. aureus* and *S. enterica*, the MIC value was found to be 312 µg/ml within the studied molecular docking figure 7. Compound **[**Figure: **4]** can sufficiently block DHFR activity to prevent the substrate from binding to its active site within the studied molecular docking figure 7. [25]

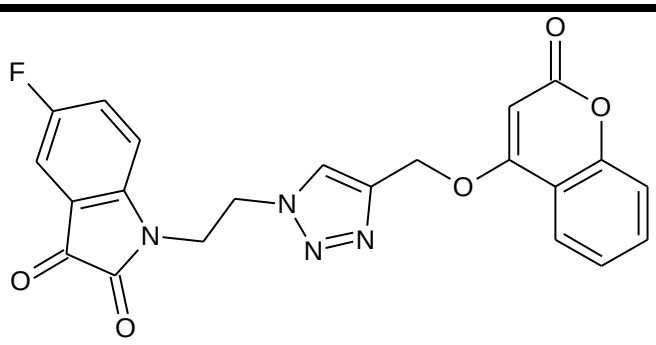


Figure 4: Indanedione coumarin hybrid derivative.

- Xian-Hai Lv *et al* developed and synthesized flavone mannich base derivatives with benzylamine moiety. When compared to novobiocin, the synthesized compounds **[**Figure: **5]** and **[**Figure: **6]** showed the strongest activity against *staphylococcus aureus* and *salmonella gallinarum* with MIC values of 2, 0.25 mg/L [26]

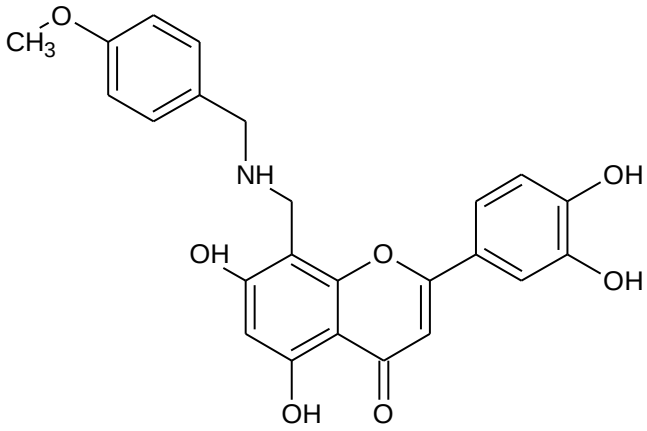


Figure 5: Mannich base derivative of flavonoid.

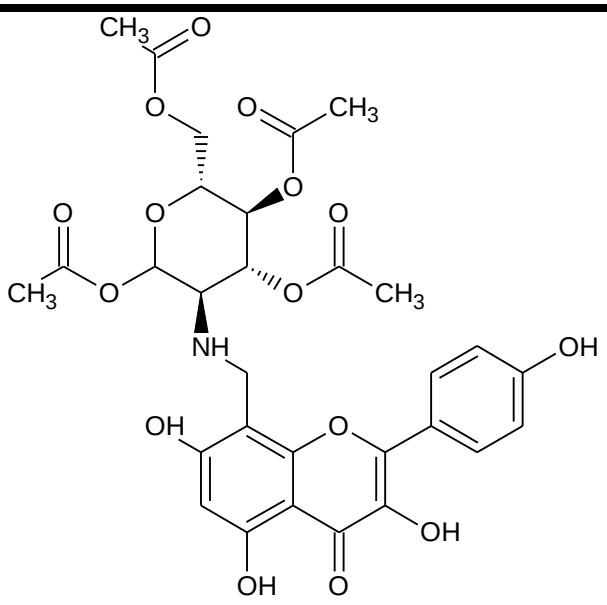


Figure 6: Substitution of flavone mannich base.

- Harbinder Singh *et al* synthesized bromo substitution in the fifth position of isatin, substitution of para-cholo in the case of curcumin-isatin and para-methoxy in the case of curcumin hybrids of coumarin in the A ring are examined for their antibacterial activity against the hybrid molecules of the compound **[**Figure: **7]** and **[**Figure: **8]** showed potent inhibitory zones of strong 29 and 31mm inhibition with MIC values of 12.50 µg/ml and 6.25 µg/ml. [27]

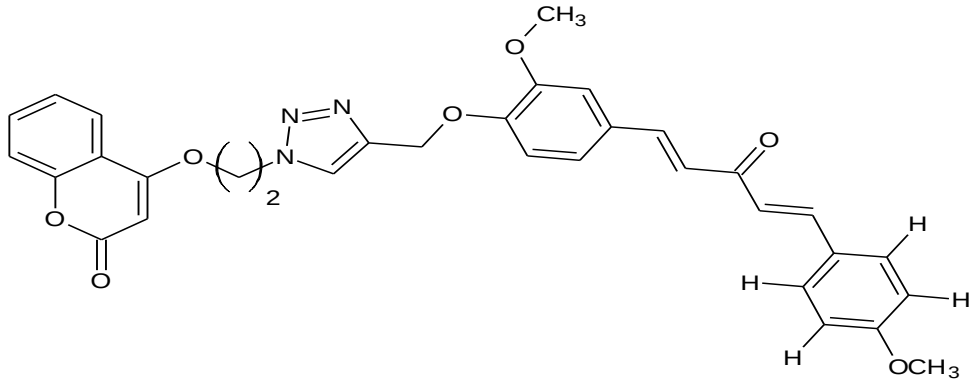


Figure 7: Bromo substitution of flavonoid derivative.

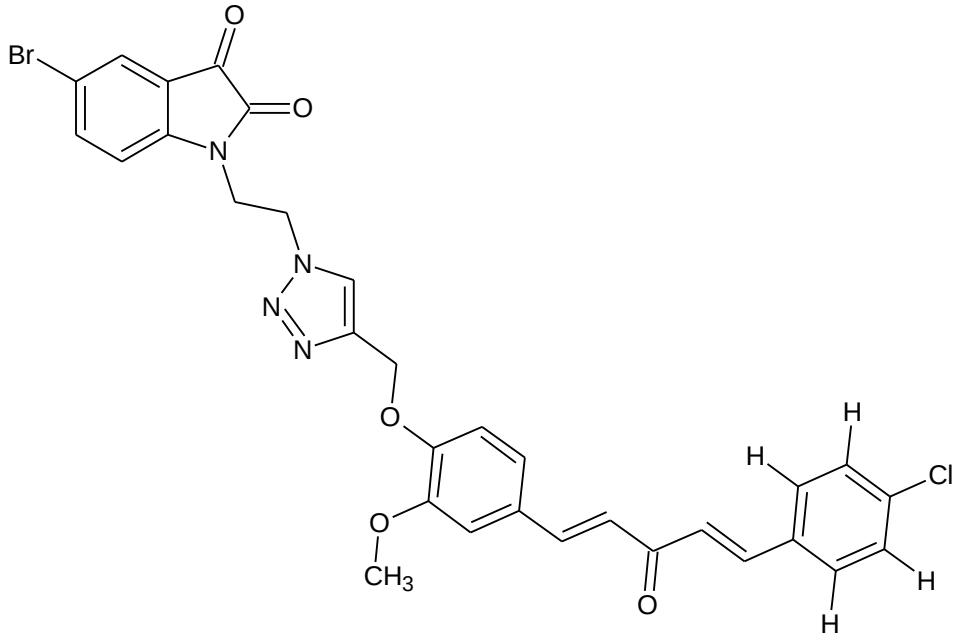
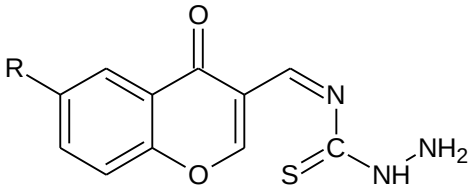


Figure 8: Derivative of bromo substitution.

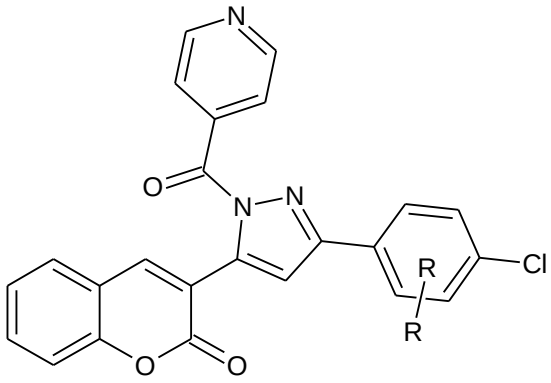
- Gurpinder Singh *et al* synthesized hydrazone **[**Figure: **9]** in pure form provides significant yields. In the antibacterial activity of compound have showed the most promising activity in which the hydrazone derivatives of **[9a]** (hydrogen), **[9b]** (methyl), **[9c]** (chloro) **[9d]** (fluoro) at 50µmol and 100µmol concentration against *E. coli*. [28]



Compound	**R**
9a	H
9b	CH_3
9c	Cl
9d	F

Figure 9: Substituted by hydrazone flavonoid.

- The antibacterial activity of a series of eleven synthetic compounds **[**Figure: **10]** obtained from derivatives by Prashant Aragade *et al* was evaluated for their *invitro*. Compounds **[10a]** with R = 2,4-$(Cl)_2$**, [10b]** with R = 4-F, **[10c]** with 4-OH showed excellent activity with MIC values of 0.5-2µg/ml, 0.25-1µg/ml, 0.25-0.5µg/ml compared to the standard drug like ampicillin. [29]



Compound	R
10a	2,4-$(Cl)_2$
10b	4-F
10c	4-OH

Figure 10: Derivative of 3-[3-(substituted phenyl)-1-isonicotinoyl-1H-pyrazol-5-yl]-2H-chromen-2-one.

- Ghadamali Khodarahmi *et al* synthesized a novel hybrid containing quinazolinone, benzofuran, and imidazole are evaluated. In the antibacterial activity of synthesized compounds **[**Figure: **11]** showed the most active against gram-positive bacteria, particularly aureus, but had less action against *E. coli* in gram-negative bacteria. [30]

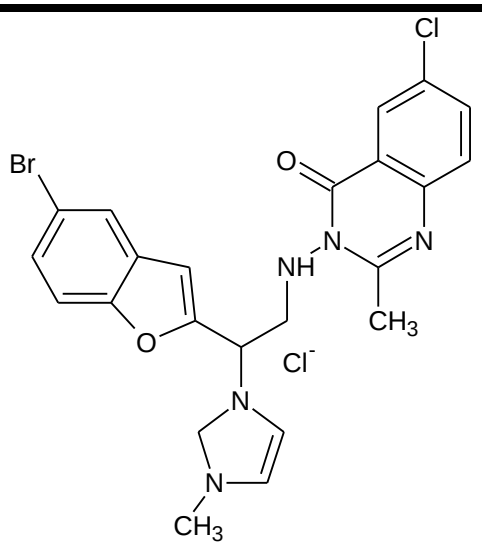
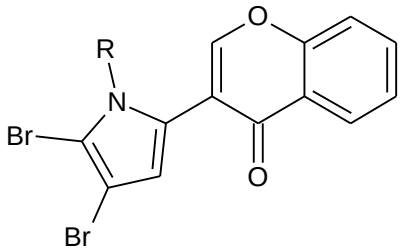


Figure 11: Substitution of benzofuran, quinazolinone, and imidazole of flavonoid derivative.

- Rajesh A. Rane *et al* 2013 synthesized and evaluated twenty-three hybrids of bromo pyrrole alkaloids **[**Figure: **12]** for the anticancer activity of compound **[12a]** with hydro and **[12b]** with methyl have showed the most promising activity against cancer cell lines PA1 and KB403 with MIC values of 0.41 µM and 1.28 µM respectively. $IC_{50} < 1.00$ µM were found in nearly all of them, indicating promising anticancer action. [31]



Compound	R
12a	H
12b	CH_3

Figure 12: Hybrids of bromo pyrrole derivative.

- Designed a novel of 3-arylcoumarins was synthesized by Yong Zou *et al* 2010. The anticancer activity of the compound **[**Figure: **13]** with a 7,8-dihydroxy group or 7,8-diacetyloxy group was characterized, and the compound with an IC_{50} value of 5.18 mmol/L against cell lines KB exhibited the most promising activity. [32]

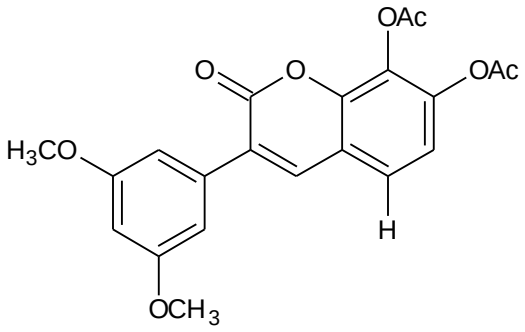
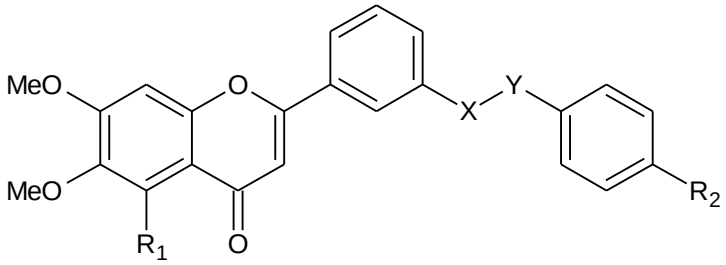


Figure 13: Substitution of 3-arylcoumarins by flavonoids derivative.

- Yong Sup Lee *et al* 2019 synthesized and evaluated thirty-one flavone-based arylamide **[**Figure: **14]**. Compounds **[14a]** with X-Y = CONH, R_1 = MeO, R_2 = 4-CH_3, and **[14b]** with X-Y = CONH, R_1 = MeO, R_2 = 4-Cl and showed the most promising anticancer activity. (33)



Compound	**X-Y**	**R_1**	**R_2**
14a	CONH	MeO	4-CH_3
14b	CONH	MeO	4-Cl

Figure 14: Derivative of flavone-based arylamide

- In the case of Christian G. Hartinger *et al* when the reaction was applied, it was observed that a rearrangement reaction leads to 1'(alkylamino) aurones. The i*nvitro* anticancer activity of the compound **[**Figure: **15]** have showed moderate activity in cell lines tested. [34]

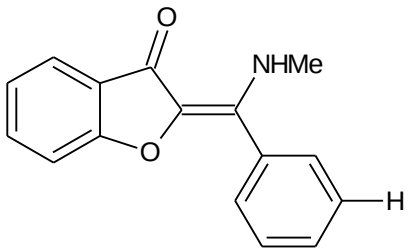


Figure 15: A rearrangement reaction of 1'(alkylamino) aurones derivatives.

- Yoongho Lim *et al* 2012 designed and synthesized a C-7 methoxy or hydroxy substituent with bulky substituents that can enhance the inhibitory effect on human HCT116 colon cancer cells, an *invitro* CDK2 binding assay, and an *insilico* CDK2 docking study for naringenin. In these derivatives of compound **[**Figure: **16]** (4.35 µM) showed more inhibitory activity than the naringenin *invitro* CDK2 binding assay. [35]

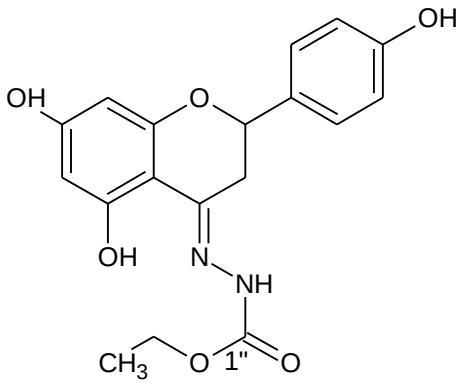


Figure 16: Bulky substituents of C-7 methoxy or hydroxy substituent in flavonoid derivative.

- Sumit Kumar *et al* synthesized a novel class of flavone and flavone tanaproget units linked by an alkyl alkenyl chain spacer and an alkyl group substitution at the C-3 position on flavone molecules. *Invitro* antiproliferative activity of compounds **[**Figure: **17]** and **[**Figure: **18]** showed excellent cytotoxicity with IC_{50} values of 1.67 $\pm$ 0.143 and 1.84 $\pm$ 0.167 compared to IC_{50} value of doxorubicin of 2.25 $\pm$ 0.095 against HeLa cells. [36]

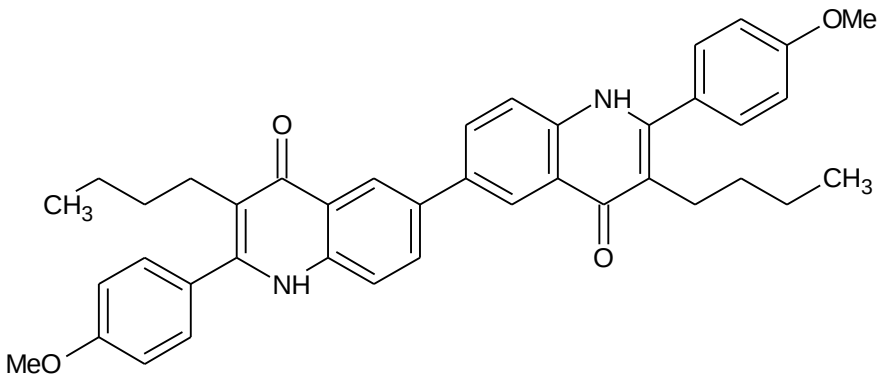


Figure 17: Flavone and Flavone tanaproget derivative.

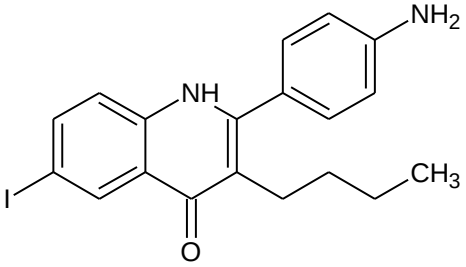
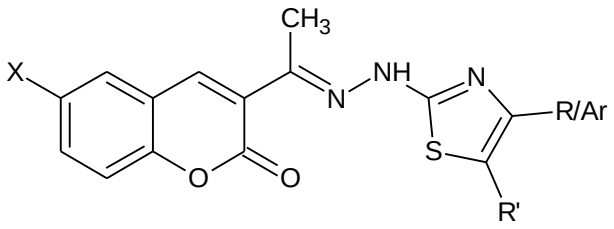


Figure 18: Substitution of flavone and flavone tanaproget derivative.

- Yasmin M. Syam *et al,* developed a new series of thiazole-2-yl-hydrazono-chromen-2-one against the Hela cell line **[**Figure: **19]**. *In-vitro* anticancer activity of compound **[19a]** and **[19b]** with X=Br, R/Ar=CH_3, R'=COOEt, **[19c]** with X=Cl, R/Ar=CH_3, R'=COOEt, and **[19d]** with X=Br, R/Ar=CH_3, R'-COOEt showed the strongest IC_{50} value of 0.0091-0.0654 µM. [37]



Compound	X	R/Ar	R’
19a	Br	CH_3	$COCH_3$
19b	H	CH_3	COOEt
19c	Cl	CH_3	COOEt
19d	Br	CH_3	COOEt

Figure 19: Derivative of thiazole-2-yl-hydrazono-chromen-2-one.

- Xiong-Li Liu *et al* synthesized and designed chrysin-chromene-spiro oxindole hybrids have been demonstrated for the anticancer activity *in-vitro* of compound **[**Figure: **20]** promises cytotoxicity towards A549 with an IC_{50} value of 3.15 $\pm$ 0.51 and MRC-5 cells. [38]

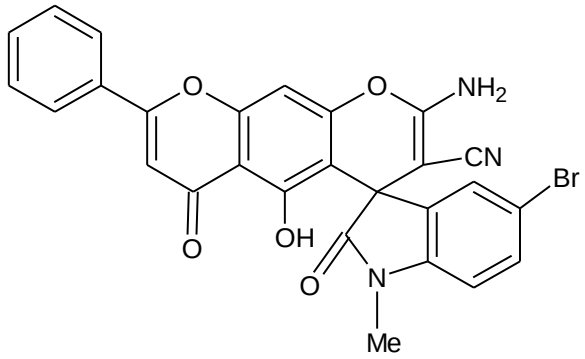


Figure 20: Chrysin-chromene-spiro oxindole hybrids of flavonoid derivative.

- Jiefu Wang *et al* 2020 designed and synthesized the flavone and isoflavones structure of twenty-eight potential HDCAs inhibitors. *In vivo* and *in vitro* compound **[**Figure: **21]** showed stronger anti-proliferative activity. [39]

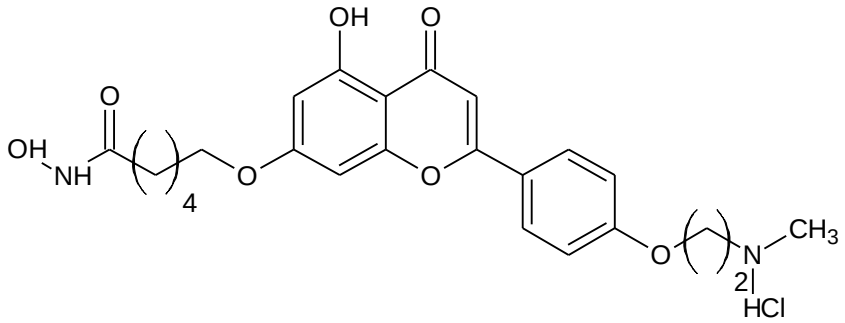


Figure 21: Flavone and isoflavones structure of potential HDCAs inhibitors.

- Alameqdad Y. Habashneh *et al* 2014 the antitumor activity of 6-flavone substituted amidrazones, which are a newly synthesized the compound, was characterized by NMR and MS spectra. Compound **[**Figure: **22]** with IC_{50} values of 5.18 and 2.89 µM showed the most active against leukemic cancer (K562) and breast cancer (MCF-7) cancer cell lines respectively. [40]

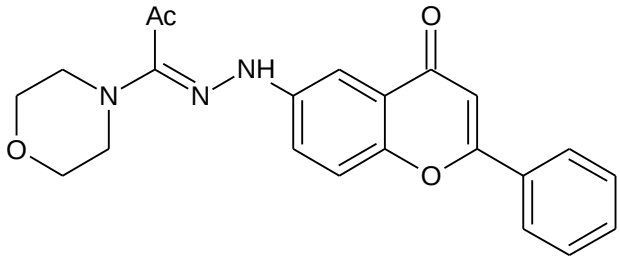


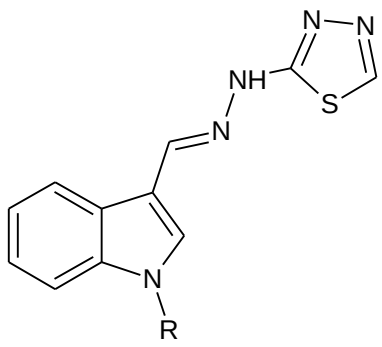
Figure 22: Derivative of 6-flavone substituted amidrazones

- Medha J Gunaratna et al. In 2021, a series of synthesis and anti-isomers of N-substituted indole-3-carboxide oxime derivatives were synthesized via a Schiff base reaction of the appropriate carbaldehyde derivative with hydroxylamine hydrochloride. The urease inhibitory activity of these derivatives in vitro was

assessed using a modified Berthelot reaction to Macrotyloma uniflorum urease. Of the compounds tested, compound **23** and **24** were identified as derivatives with strong urease inhibitory activity compared to thiourea. In addition, insilico studies were performed on all oxime compounds to investigate the binding interactions of urease enzymes with the active site compared to thiourea. In addition, the drug likeness of the synthesized oxime compound was predicted.

Figure 23: Derivatives of N-substituted indole-3-carbaldehyde oxime derivatives

- 2013 Kobra Rostamizadeh et al Different analytical and spectroscopic approaches were used to confirm the structures of a series of novel thiocarbohydrazones of s ubstituted indoles and their corresponding thiadiazole derivatives. The derivatives were made using a sequential synthetic procedure that included replacement of various aliphatic and benzylic substituents at the N1 position of the indole ring, condensation with thiocarbohydrazide, and ultimately cyclization with triethyl orthoformate. The derivatives were evaluated for antimycobacterial activity against Mycobacterium bovis BCG, and the results revealed that thiadiazol e derivatives **24a, b, c, d, e,** and **f** had the highest activity among the synthesised compounds, with an IC50 value of 3.91 lg/mL. The findings suggest that the thiadi azole moiety is critical for antimycobacterial action.



compound	R
24a	Butyl
24b	Pentyl
24c	4-Fluorobenzy
24d	4-Chlorobenzyl
24e	4-Bromobenzyl
24f	4-Methoxybenzyl

Figure 24: Substitution of indoles and thiadiazole derivatives

AIM AND OBJECTIVES

II. AIM AND OBJECTIVE

- ❖ Cancer is one of the most life-threatening diseases, with more than 100 different types occurring due to some molecular changes within the cell.
- ❖ It is the third leading cause of death worldwide following cardiovascular and infectious diseases. It is estimated that 12.5% of the population dies due to cancer.
- ❖ Hence, the design and development of new drugs for cancer therapeutics remains to be an important and challenging task for medicinal chemists worldwide.

The work aimed at

- To design substituted Indole-3-carboxaldehyde flavone derivatives using chemsketch.
- **In silico screening**: To predict physicochemical parameters and ADMET for all the designed compounds using online software's such as Swiss ADME, PreADMET and Pro Tox II.
- To perform docking studies for all designed compounds with EGFR kinase using Auto dock vina 1.1.2.
- To synthesize substituted Indole-3-carboxaldehyde flavone derivatives based on docking score.
- To characterize the synthesized compounds using IR, NMR, Mass spectrometry.
- To evaluate in vitro anticancer activity of the synthesized compounds

SCOPE
AND
PLAN OF STUDY

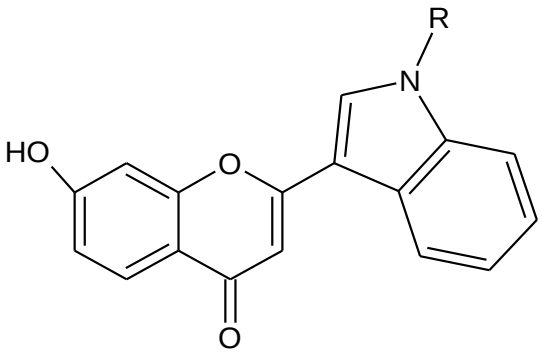
CHAPTER IV

SCOPE AND PLAN OF STUDY

4.1. OBJECTIVE OF THE PRESENT STUDY

- According to the literature survey the final compounds were planned to synthesize with assuming that these compounds will possess potent biological activities.
- The literature survey shows that indole-3-carboxaldehyde flavone derivatives were reported to contains various biological activities such as anti-microbial, anti-inflammatory, anti-oxidant, anticancer, anti-viral and anti-proliferative activity.

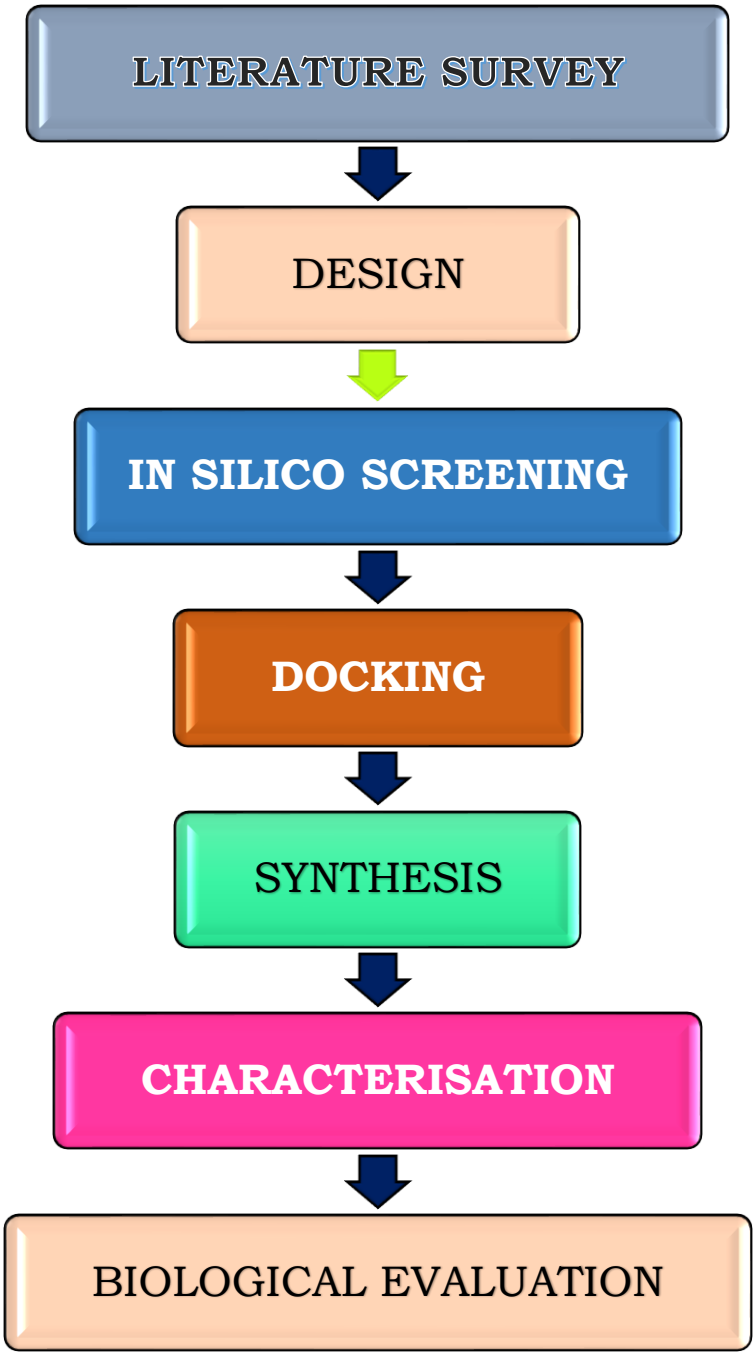
General structure of compounds to be synthesized as to follows:



Substituted Indole - 3 - carboxaldehyde flavone

Figure 3: Structure of substituted indole-3-carboxaldehyde

4.2 STEPS INVOLVED IN THE PLAN OF STUDY



MATERIALS AND METHODS

CHAPTER V

MATERIALS AND METHODS

5.1 DESIGN OF COMPOUNDS:

18 compounds were designed using Chemsketch software. Physicochemical properties were calculated for all the compounds.

Molar refractivity

Molar refractivity, A, is a measure of the total polarizability of a mole of a substance and is dependent on the temperature, the index of refraction, and the pressure.

The molar refractivity is defined as

$$A = \frac{4}{3}\pi N_A \alpha$$

Where $N_A \approx 6.02210^{23}$ is the Avogadro constant and α is the mean polarizability of the molecule.

Molar Volume

The molar volume, symbol V_m, is the volume occupied by one mole of a substance (chemical element or chemical compound) at a given temperature and pressure. It is equal to the molar mass (M) divided by the mass density (ρ). It has the SI unit cubic meters per mole (m3/mol), although it is more practical to use the units cubic decimeters per mole (dm3/mol) for gases and cubic centimeters per mole (cm3/mol) for liquids and solids. The molar volume of a substance can be found by measuring its molar mass and density then applying the relation

$$V_m = M/p$$

Index of refraction

The refractive index or index of refraction of a material is a dimensionless number that describes how light propagates through that medium.

It is defined as

$$n = c/v$$

Where, c is the speed of light in vacuum and v is the phase velocity of light in the medium.

Surface Tension

Surface tension is the elastic force of a fluid surface which makes it acquire the least surface area possible. Surface tension has the dimension of force per unit length, or of energy per unit area.

Polarizability

Polarizability is the ability to form instantaneous dipoles. It is a property of matter. Polarizabilities determine the dynamical response of a bound system to external fields and provide insight into a molecule's internal structure. In a solid, polarizability is defined as the dipole moment per unit volume of the crystal cell.

Parachor

It is an empirical constant for a liquid that relates the surface tension to the molecular volume and that may be used for a comparison of molecular volumes under conditions such that the liquids have the same surface tension and for determinations of partial structure of compounds by adding values obtained for constituent atoms and structural features called also molar parachor, molecular parachor.

Parachor is a quantity defined according to the formula

$$P = Y^{1/4} M/d$$

Where: $Y^{1/4}$ is the fourth root of surface tension

M is the molar mass

D is the density.

Density

The density, or more precisely, the volumetric mass density, of a substance is its mass per unit volume.

$$\rho = \frac{m}{V}$$

Dielectric constant

Substances have capacity to produce dipoles in another molecule. Dielectric constant is a measure of this capacity and it is a physical property. It is affected by both the attractive forces that exist between atoms and also molecules. It is denoted by E.

5.2 IN-*SILICO* SCREENING OF DESIGNED COMPOUNDS:

In silico is an expression used to mean “performed on computer or via computer simulation”. In silico drug designing is defined as the identification of the drug target molecule by employing bioinformatic tools. *In silico* approaches to predict pharmacokinetic parameters (ADME) were pioneered by Lipinski *et al*. By studying the physicochemical properties of >2000 drugs from the WDI (World Drug Index, Derwent Information, London), which can be assumed to have entered Phase II human clinical trials (and therefore must possess drug-like properties), the so-called ‘rule-of five’ was derived to predict oral bioavailability (intestinal absorption) of a compound that can be considered as the major goal of drug development.

DRUG TARGET

Generally, the “target” is the naturally existing cellular of molecular structure involved in the pathology of interest that the drug in development is meant to act on.

TARGET SELECTION

Target for mechanistic drug design usually fall into three

- Enzymes
- Receptors
- Nucleic acids

STRUCTURAL DETERMINATION

Crystal structure of target protein can be taken from the PDB database by the referring the articles.

SELECTION OF LIGANDS

- It is also called as lead identification
- Synthetic compound is carried to screen out lead compound
- Based on experimental study
- Preparing derivatives of already existing drugs.

- 3d structure of compound and target is docked and scoring function evaluates complementarity. Hits fulfill certain criteria and then selected as lead
- When lead molecules have been identified, they have to be optimized in terms of potency, selectivity, pharmacokinetics (i.e.) absorption, distribution, metabolism and excretion (ADME) and toxicology before they can become candidates for drug development.

ACTIVE SITE OF TARGET PROTEIN

The crystal structure of target-ligand complex can be prepared to determine its active site in Auto dock vina 1.1.2 software.

The ADME properties of the designed compounds were evaluated using Swiss ADME and PreADMET online software's. Toxicity of all the designed compounds further it will be carried out by using ProTox II software.

SwissADME

Lipinski's rule of five

Lipinski's rule of five also known as the Pfizer's rule of five or simply the rule of five (RO5) is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely orally active drug in humans.

The rule

Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria:

- No more than 5 hydrogen bond donors (the total number of nitrogen – hydrogen and oxygen–hydrogen bonds)
- No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
- A molecular mass less than 500 daltons
- An octanol-water partition coefficient log P not greater than 5

Ghose Filter

This filter defines drug-likeness constraints as follows:

- Calculated log P is between -0.4 and5.6
- Molecular weight is between 160 and480
- Molar refractivity is between 40 and130
- The total number of atoms is between 20 and70.

Veber Filter

The molecules fitting to these two properties have a high probability of good oral bioavailability.

- ✓ Rotatable bond: max. 12
- ✓ Polar Surface Area: max. 140A^2

Egan Rule

Predicts good or bad oral bioavailability.

- ✓ 0 ≥ TPSA ≤132
- ✓ -1 ≥ logP ≤6.

Molar Refractivity

It is a measure of the total polarizability of a mole of a substance and is dependent on the temperature, the index of refraction, and the pressure.

The molar refractivity is defined as

$$A = 4PI/3\ N_A\alpha$$

Where NA= 6.022×10^{23} is the Avogadro constant and α is the mean polarizability of a molecule

Polar surface area (PSA) or topological polar surface area (TPSA)

It is a measure of apparent polarity of a molecule is defined as the surface sum overall polar atoms, primarily oxygen and nitrogen, also including their attached hydrogen atoms. PSA is a commonly used for the optimization of a drug's ability to permeate cells. Molecules with a polar surface area of greater than 140 angstroms squared tend to be poor at permeating cell membranes.

For molecules to penetrate the blood–brain barrier (and thus act on receptors in the central nervous system), a PSA less than 90 angstroms squared is usually needed. Topological PSA (TPSA, fast 2D calculation).

ADME Guideline

- ✓ TPSA < 140 Å2 good intestinal absorption.

- ✓ TPSA < 70 Å2 good brain penetration.

Lipophilicity

Lipophilicity is the ability of a molecule to mix with an oily phase rather than with water, is usually measured as partition coefficient, *P*, between the two phases and is often expressed as log *P*. Lipophilicity has also been found to affect a number of pharmacokinetic parameters: higher lipophilicity (log*P*>5) gives, in general, lower solubility, higher permeability in the gastrointestinal tract, across the blood–brain barrier and other tissue membranes, higher affinity to metabolizing enzymes and efflux pumps, and higher protein binding. Low lipophilicity can also negatively impact permeability and potency and thus results in low BA and efficacy.

Partition coefficient, P

It is defined as a particular ratio of the concentrations of a solute between the two solvents (a biphase of liquid phases), specifically for un- ionized solutes, and the logarithm of the ratio is thus **log *P***. When one of the solvents is water and the other is a non-polar solvent, then the log *P* value is a measure of lipophilicity or hydrophobicity.

- ❖ log $P_{oct/wat}$= log[solute]unionized octanol / [solute] unionized water
- ❖ log $P_{oct/wat}$=log CO/CW

Lipophilicity not only impacts solubility but also influences permeability, potency, selectivity, absorption, distribution, metabolism, and excretion (ADME) properties and toxicity. A desired log P value (octanol-water partition coefficient) is no more than 5.

Water Solubility

Water solubility is a measure of the amount of chemical substance that can dissolve in water at a specific temperature. Solubility is common physicochemical parameter for drug discovery compounds. Determination of the aqueous solubility of the drug candidate is an important analysis as it reflects the bioavailability of the compound.

Log S

- The aqueous solubility of a compound significantly affects its absorption and
- distribution characteristics. Typically, a low solubility goes along with a bad absorption and therefore the general aim is to avoid poorly soluble compounds.
- Log S value is a unit stripped logarithm (base10) of the solubility measured in mol/liter. Log S value should be greater than -4.

Rotatable Bonds

The bioavailability of a drug like molecule is related with it rotatable bond number. Less than seven rotatable bonds are essential for good bioavailability. Many highly potent molecules carried more than 10 rotatable bonds and still administered through oral route.

Hydrogen bond acceptors and donors

12 or fewer H-bond donors and acceptors will have a high probability of good oral bioavailability.

PreADMET Drug-Likeliness

Drug likeness is a qualitative concept used in drug design for how "druglike" a substance is with respect to factors like bioavailability. It is estimated from the molecular structure before the substance is even synthesized and tested. The most well-known rule relating the chemical structures to their biological activities is Lipinski's rule and it is called the 'rule of five'. Another well-known rule is the Lead- like rule. PreADMET contains drug-likeness prediction module based on these rules.

ADME Prediction

Numerous *in vitro* methods have been used in the drug selection process for assessing the intestinal absorption of drug candidates. Among them, Caco2-cell model and MDCK (Madin-Darby canine kidney) cell model has been recommended as a reliable in vitro model for the prediction of oral drug absorption. In absorption, this module provides prediction models for in vitro Caco2-cell and MDCK cell assay. Additionally, *in silico* HIA (human intestinal absorption) model and skin permeability model can predict and identify potential drug for oral delivery and transdermal delivery.

In distribution, BBB (blood brain barrier) penetration can give information of therapeutic drug in the central nervous system (CNS), plasma protein binding model in its disposition and efficacy. In order to build these QSAR models, genetic functional approximation is used to select relevant descriptors from all 2D descriptors that calculated by Topomol module, followed by Resilient back- propagation (Rprop) neural network to develop successful nonlinear model.

Toxicity prediction

In silico toxicity prediction will have more and more importance in early drug discovery since 30% of drug candidates fail owing to these issues.

ProTox II

ProTox II, a virtual lab for the prediction of toxicities of small molecules. The prediction of compound toxicities is an important part of the drug design development process. Computational toxicity estimations are not only faster than the determination of toxic doses in animals, but can also help to reduce the amount of animal experiments.

ProTox II incorporates molecular similarity, fragment propensities, most frequent features and (fragment similarity based CLUSTER cross- validation) machine-learning, based a total of 33 models for the prediction of various toxicity endpoints such as acute toxicity,

hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways and toxicity targets.

Toxic doses and Toxicity classes

Toxic doses are often given as LD50 values in mg/kg body weight. The LD50 is the median lethal dose meaning the dose at which 50% of test subjects die upon exposure to a compound.

Toxicity classes are defined according to the globally harmonized system of classification of labelling of chemicals (GHS). LD50 values are given in [mg/kg]:

- Class I: fatal if swallowed ($LD50 \leq 5$)
- Class II: fatal if swallowed ($5 < LD50 \leq 50$)
- Class III: toxic if swallowed ($50 < LD50 \leq 300$)
- Class IV: harmful if swallowed ($300 < LD50 \leq 2000$)
- Class V: may be harmful if swallowed ($2000 < LD50 \leq 5000$)
- Class VI: non-toxic ($LD50 > 5000$)

5.3 DOCKING

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions.

In this study, Auto dock vina 1.1.2 is used to perform docking studies. Docking studies were performed with the active site of Epidermal growth factor receptor (EGFR) Kinase (PDB ID: 2j5f). Protein was downloaded from Research collaborator for structural bioinformatics (RCSB) and used for docking analysis. This molecular docking study was performed using the selected protein from the protein data bank (PDB ID: 2j5f).

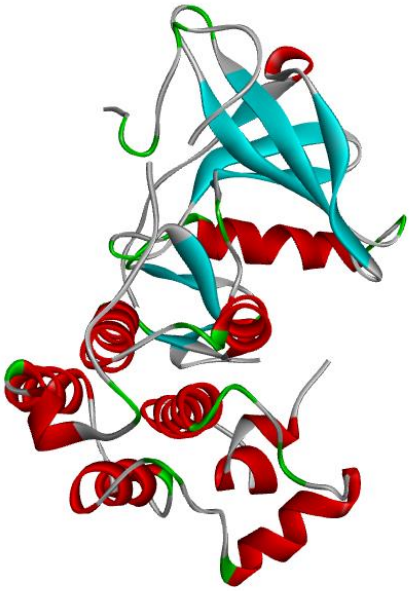
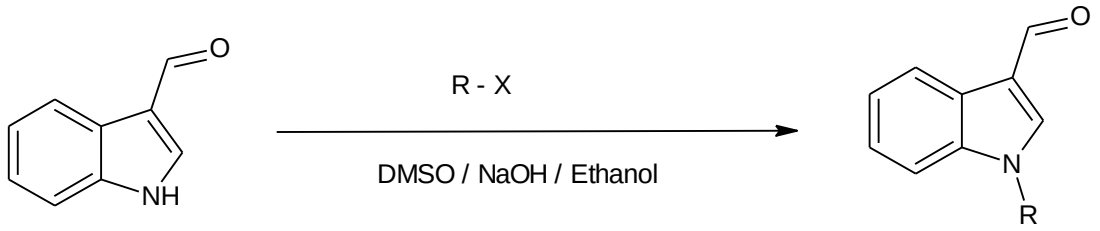


Figure 4: 3D View of 2j5f

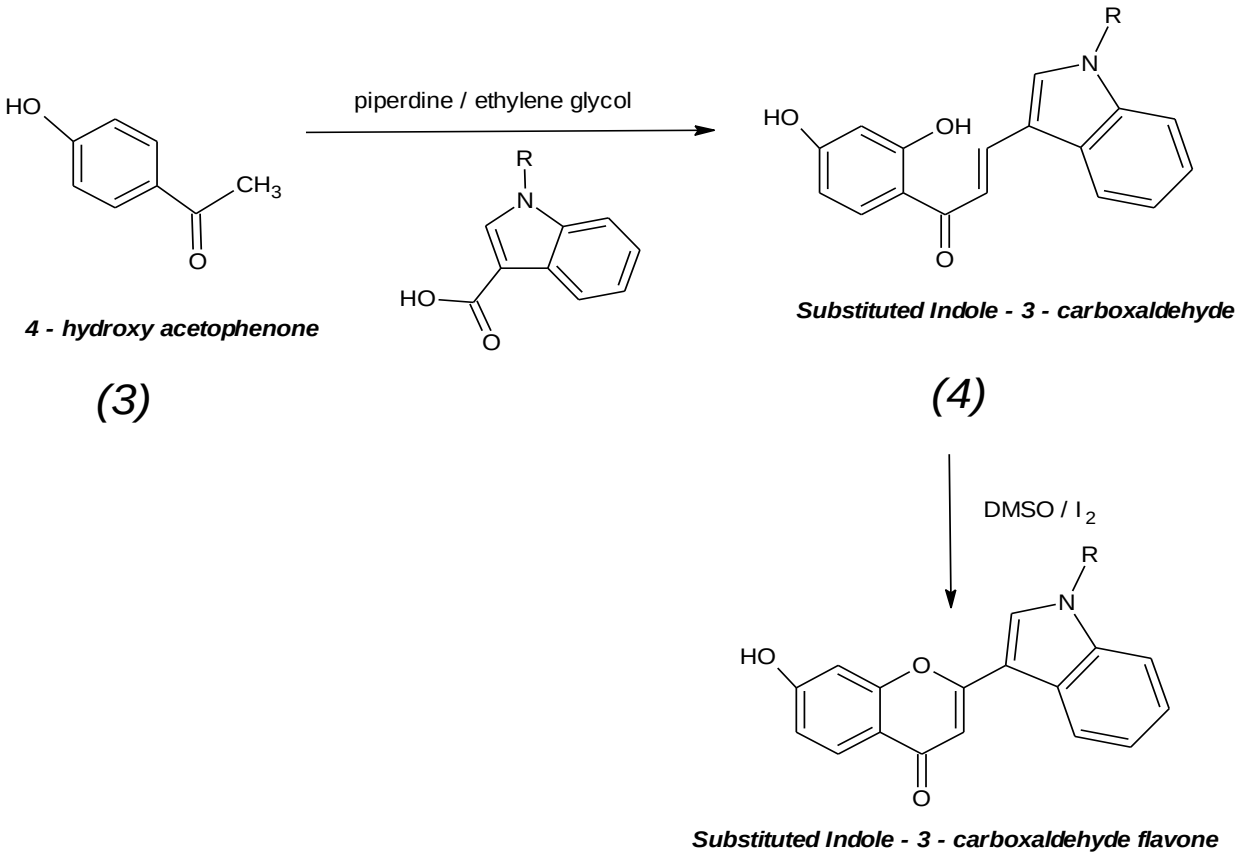
5.4. SYNTHETIC SCHEME

Synthesis of Indole-3-carboxaldehyde



(1) *(2)*

Synthesis of Substituted Indole-3-carboxaldehyde flavone



4 - hydroxy acetophenone

(3)

Substituted Indole - 3 - carboxaldehyde

(4)

Substituted Indole - 3 - carboxaldehyde flavone

(5a-r)

Figure 5: Synthetic Scheme

Table:1 Details of the -R represented in the synthetic scheme

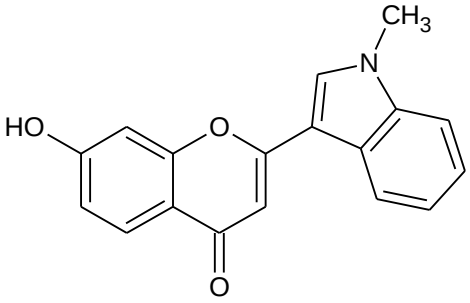
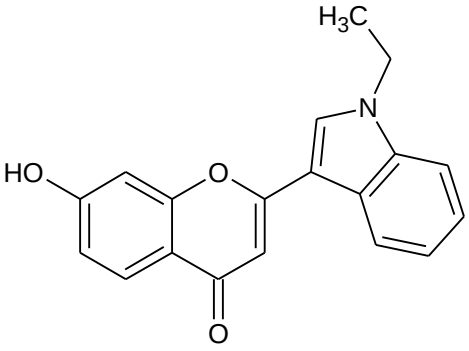
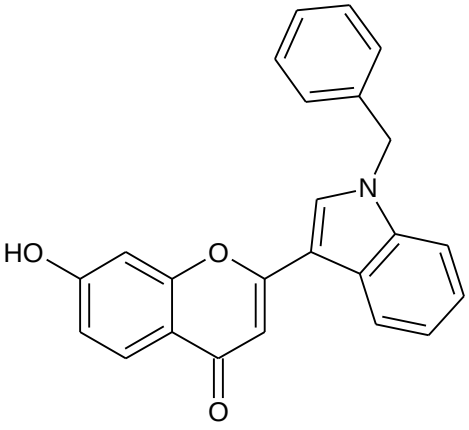
S.NO	COMPOUNDS	R
1	5a	CH_3
2	5b	CH_2CH_3
3	5c	CH_2Ph
4	5d	CH_2PhF_4
5	5e	COPh
6	5f	SO_2CH_3
7	5g	SO_2Ph
8	5h	$COCH_3$
9	5i	$COOCH_2CH_3$
10	5j	$CH_2CH_2(CH_3)_2$
11	5k	$CH_2CH_2CH_2CH_3$
12	5l	CH_2-CH-CH_2CH_2
13	5m	CH_2-CH-$CH_2CH_2CH_2$
14	5n	CH_2-CH-C_5H_{10}
15	5o	CH_2-$C_6H_5OCH_3$
16	5p	$CH_2CH_2CH_3$
17	5q	$CH_2C_6H_4$-2F
18	5r	$CH_2C_6H_4$-4F

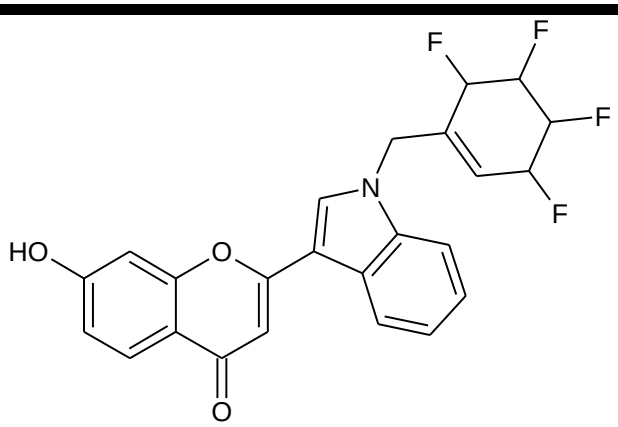
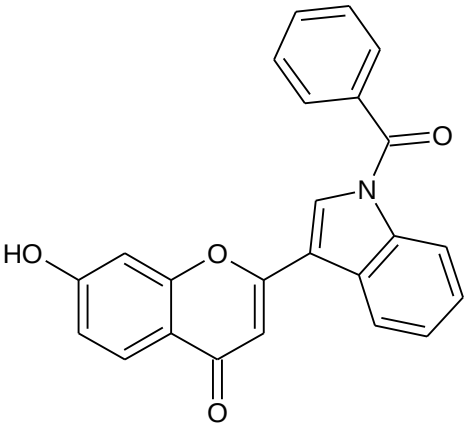
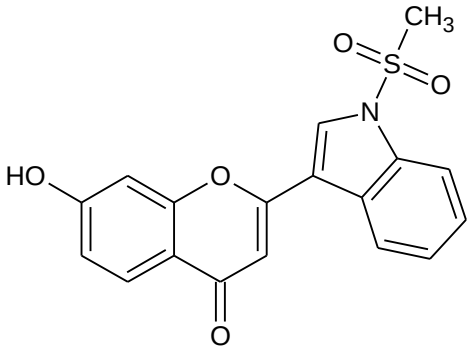
All reagents were purchased from commercial suppliers and were used without further purification. Melting points (M. p.) were determined on Sigma melting point apparatus and are uncorrected. IR spectra were recorded on Fourier Transform infrared spectrophotometer (Shimadzu IR Tracer-100). 1H NMR spectra was recorded on a Bruker spectrometer. Mass spectra were recorded on an Agilent Mass spectrometer.

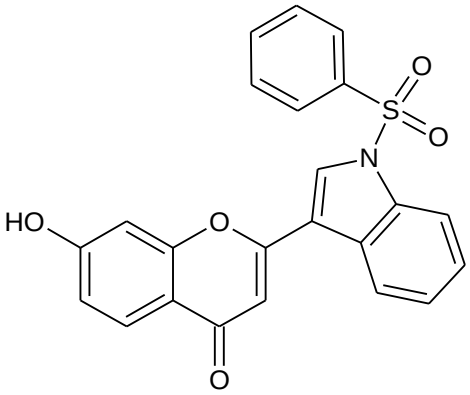
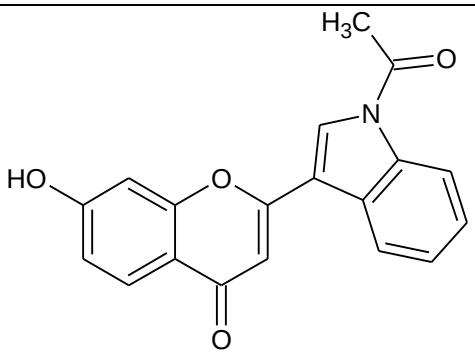
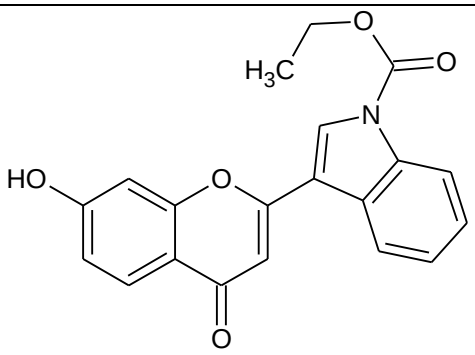
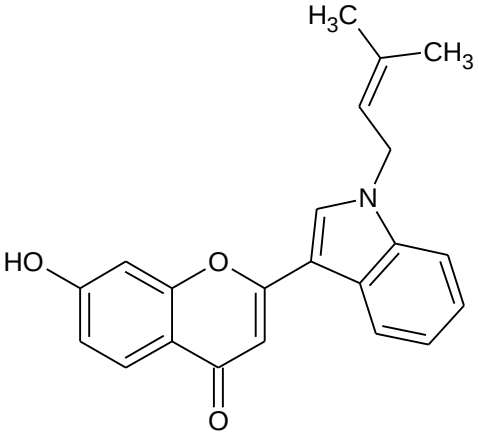
5.5 LIST OF COMPOUNDS TO BE SYNTHESIZED

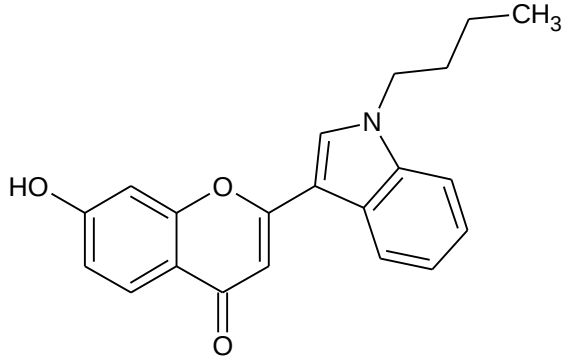
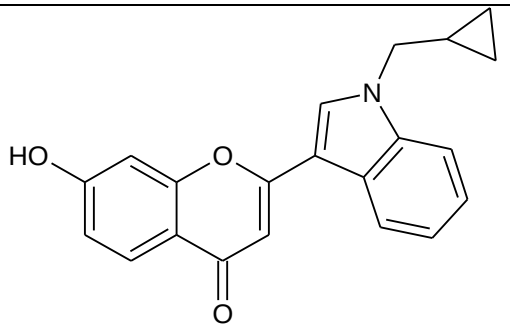
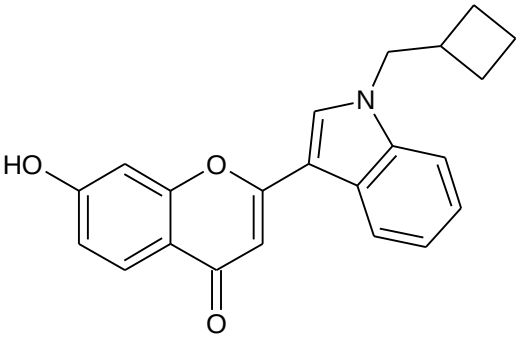
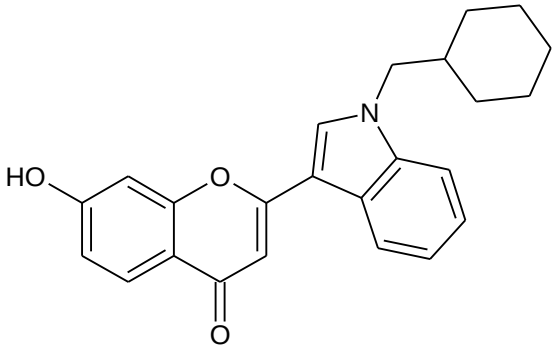
18 compounds were designed by using ChemSketch and their structures were tabulated in Table No.2 &3. Their physicochemical properties were calculated.

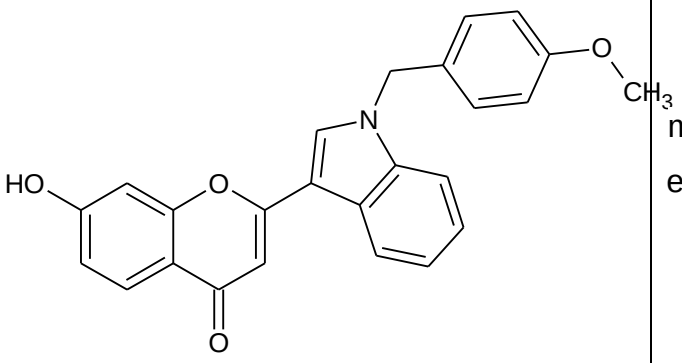
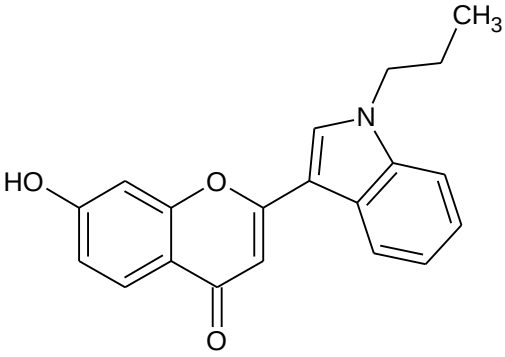
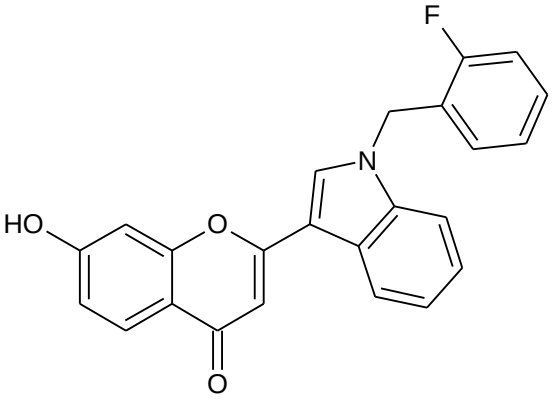
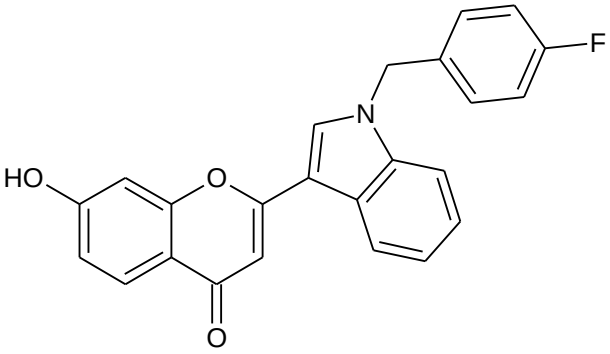
Table 2: Structure of designed compound

COMPOUND NO.	STRUCTURE	IUPAC NAME
5a		7-Hydroxy-2-(1-methyl-3-indolyl)-4-chromenone
5b		2-(1-Ethyl-3-indolyl)-7-hydroxy-4-chromenone
5c		2-(1-Benzyl-3-indolyl)-7-hydroxy-4-chromenone

5d		7-Hydroxy-2-{1-[(3,4,5,6-tetrafluoro-1-cyclohexen-1yl)methyl]-3-indolyl}-4-chromenone
5e		2-(1-Benzoyl-3-indolyl)-7-hydroxy-4-chromenone
5f		7-Hydroxy-2-(1-mesyl-3-indolyl)-4-chromenone

5g		7-Hydroxy-2-[1-(phenylsulfonyl)-3-indolyl]-4-chromenone
5h		2-(1-Acetyl-3-indolyl)-7-hydroxy-4-chromenone
5i		Ethyl 3-(7-hydroxy-4-oxo-2-chromenyl)-1-indolecarboxylate
5j		7-Hydroxy-2-[1-(3-methyl-2-butenyl)-3-indolyl]-4-chromenone

5k		2-(1-Butyl-3-indolyl)-7-hydroxy-4-chromenone
5l		2-[1-(Cyclopropylmethyl)-3-indolyl]-7-hydroxy-4-chromenone
5m		2-[1-(Cyclobutylmethyl)-3-indolyl]-7-hydroxy-4-chromenone
5n		2-[1-(Cyclohexylmethyl)-3-indolyl]-7-hydroxy-4-chromenone

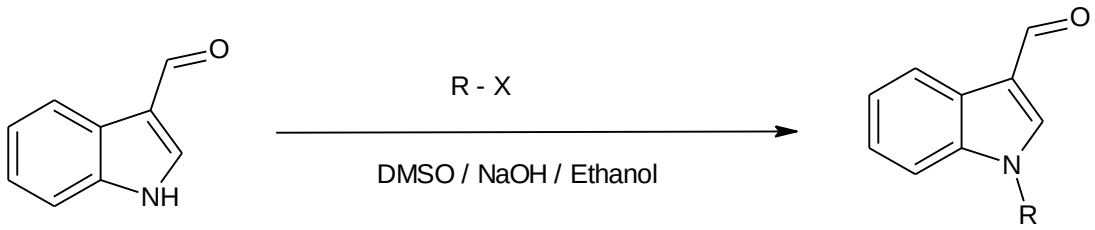
5o		7-Hydroxy-2-{1-[(p-methoxyphenyl)methyl]-3-indolyl}-4-chromenone
5p		7-Hydroxy-2-(1-propyl-3-indolyl)-4-chromenone
5q		2-{1-[(o-Fluorophenyl)methyl]-3-indolyl}-7-hydroxy-4-chromenone
5r		2-{1-[(p-Fluorophenyl)methyl]-3-indolyl}-7-hydroxy-4-chromenone

5.6. GENERAL PROCEDURE FOR SYNTHESIS

STEP 1: Synthesis of N-substituted indole-3-carboxaldehyde derivatives

Microwave irradiation procedure:

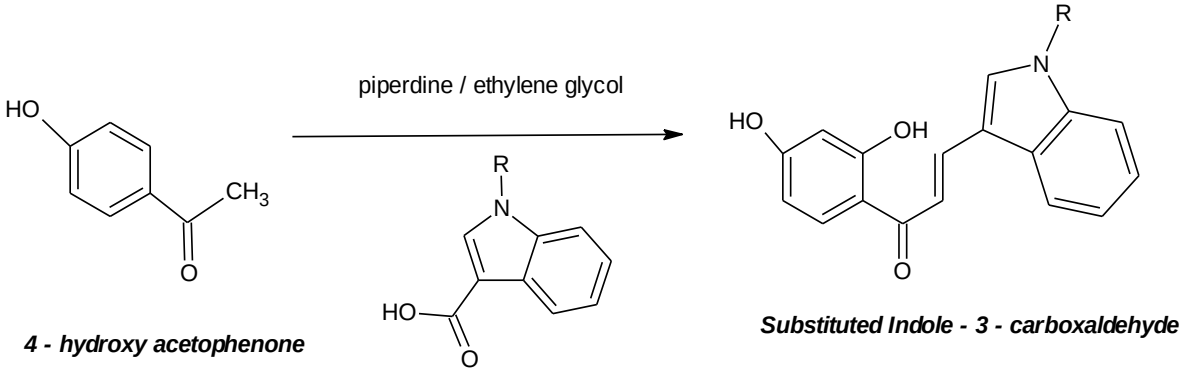
A mixture of indole-3-carboxaldehyde (1) 1mmol, the appropriate alkylating reagent 1 mmol, KOH (4 mmol), anhydrous K_2CO_3 (4 mmol) and dimethyl formamide (1ml) in an open pyrex-glass vessel was subjected to microwave irradiation at 350 W. Irradiation was carried out in successive 3o s periods to avoid overheating of the solvent and the reaction miture left another 30 sec, at room temperature as a time gap between every successive irradiation period. After completion of the reaction as monitored by TLC, the reaction mixture was colled, and poured onto water. The precipitated solid was filtered off, washed with water, dried and recrystallized from ethanol.



STEP 2: Synthesis of indolyl chalcone derivatives

Microwave irradiation procedure:

A mixture of indole-3-carboxaldehyde (1 mmol), 4-hydroxyacetophenone (1mmol), ethylene glycol (1ml) and piperdine (0.5ml) in an open pyrex-glass vessel was subjected to microwave irradiation at 750 W. Irradiation was carried out in successive 30 S periods to avoid over heating of the solvent and the reaction mixture left another 30 s at room temperature as a time gap between every successive irradiation period. After completion of the reaction as monitored by TLC, the reaction mixture was cooled, and poured onto water (10ml). The precipitated solid as filtered off, washed with water, dried and recrystallized from ethanol.

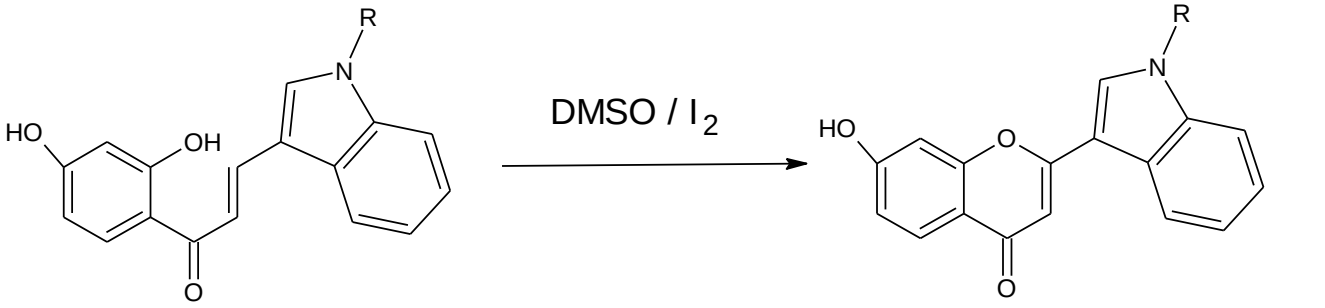


4 - hydroxy acetophenone

Substituted Indole - 3 - carboxaldehyde

STEP 3: Synthesis of indolyl flavone

The chalcone (0.01 mol) was suspended in dimethyl sulfoxide (DMSO, 6 ml) and iodine (0.01 mol, 1.27 g) was added to it. The mixture was refluxed for 20-50 min in an oil bath until the completion of reaction. Then the reaction mixture was poured in crushed ice and the solid separated was filtered and washed with 20% aq. Sodium thiosulfate until product becomes colorless. It was further purified by column chromatography using hexane-ethyl acetate (80:20 v/v) as eluting solvent.



Substituted Indole - 3 - carboxaldehyde

Substituted Indole - 3 - carboxaldehyde flavone

BIOLOGICAL STUDIES

MTT Cell Viability Assay:

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/mL using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100µL of the diluted cell suspension (1 x 10^4 cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100µL of different concentrations of test samples were added on to the partial monolayer in microtiter plates. The plate was then incubated at 37ºC for 24 h in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 20µL of MTT (2 mg/mL of MTT in PBS) was added to each well. The plate was incubated for 4 h at 37ºC in 5% CO_2 atmosphere. The supernatant was removed and 100µL of DMSO was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage of viability was calculated using the following formula, % of viability = Sample abs/Control abs x 100

SPECTRAL STUDIES

VI SPECTRAL STUDIES

The objective of the spectral studies is to confirm the chemical structures of the synthesized compounds, and the various functional groups with the final compounds. Also, it is helpful in the nature of the functional groups attached in the synthesized compounds.

i) Infrared Spectroscopy

The infrared spectroscopy is one of the most powerful analytical techniques, this offers the possibility of chemical identification. The most important advantages of infrared spectroscopy over the other usual methods of structural analysis are that it provides useful information about the functional groups present in the molecule quickly. The technique is based upon the simple fact that a chemical substance show marked selectable absorption in the infrared region. After absorbing IR radiations, the molecules of a chemical compound exhibit small vibrations, giving rise to closely packed absorption bands called as IR absorption spectrum which may extend over a wide wavelength range. Various bands will be present in IR spectrum which corresponds to the characteristic functional groups and bonds present in a chemical substance. Thus an IR spectrum of a chemical compound is a fingerprint for its identification.

ii) Nuclear Magnetic Resonance Spectroscopy

It is the branch of spectroscopy in which radiofrequency waves induces transitions between magnetic energy levels of nuclei of a molecule. The magnetic energy levels are created by keeping nuclei in a magnetic field. Without the magnetic field the spin states of nuclei are degenerated i.e., possess the same energy and the energy level transition is not possible. The energy level transition is possible with the application of external magnetic field which requires different Rf radiation to put them into resonance. This is a measurable phenomenon. It is a powerful tool for the investigation of nuclei structure. ^{1}HNMR and ^{13}CNMR Spectra's of the prepared derivatives were done by using 400-MHz and 500-MHz Bruker spectrometer using internal standard as tetra methyl silane. ^{1}H and ^{13}C NMR Spectral were taken with dimethyl sulphoxide (DMSO) as a solvent and the data of chemical shift were shown as delta values related

to trimethyl silane (TM) in ppm.

iii) Mass spectroscopy

Mass spectrometer performs three essential functions. First, it subjects molecules to bombardment by a stream of more amounts of energy electrons, converting some of the molecules to ions, which are then accelerated in a field of electric. Second, the ions which are accelerated are divided according to their ratios of mass to charge in an electric or magnetic field. Finally, the ions that have particular mass-to-charge ratio are detected by a device which can count the number of ions striking it. The detector's output is amplified and fed to a recorder. The trace from the recorder is a mass spectrum a graph of particles detected as a function of mass-to- charge ratio. The Mass spectra of the synthesized compounds were taken using Agilent spectrometer

IR SPECTRAL DATA OF THE SYNTHESIZED COMPOUNDS

Synthesis of 2-(1-Acetyl-3-indolyl)-7-hydroxy-4-chromenone (5h)

The 1-Acetyl-3-indolyl chalcone (0.01 mol) was suspended in dimethyl sulfoxide (DMSO, 6 ml) and iodine (0.01 mol, 1.27 g) was added to it. The mixture was refluxed for 20-50 min in an oil bath until the completion of reaction. Then the reaction mixture was poured in crushed ice and the solid separated was filtered and washed with 20% aq. Sodium thiosulfate until product becomes colorless. It was further purified by column chromatography using hexane-ethyl acetate (80:20 v/v) as eluting solvent.

Dark Yellow solid; Yield: 78 %; M. p: 180-195°C. Solubility: DMSO, FT-IR (KBr, cm-1): 3445.48 (Phenyl OH), 1614.20 (C=O), 1634.65 (Amide C=O), Elemental analysis for $C_{19}H_{13}NO_4$ calculated C (71.47%), H (4.1%), N (4.39%), O (20.04%) found C (71.26%), H (3.5%), N (4.20%), O (19.04%) M+ calculated for $C_{19}H_{13}NO_4$ 319.31 found to 319.31.

Synthesis of 2-(1-Butyl-3-indolyl)-7-hydroxy-4-chromenone (5k)

The 1-Butyl-3-indolyl chalcone (0.01 mol) was suspended in dimethyl sulfoxide (DMSO, 6 ml) and iodine (0.01 mol, 1.27 g) was added to it. The mixture was refluxed for 20-50 min in an oil bath until the completion of reaction. Then the reaction mixture was poured in crushed ice and the solid separated was filtered and washed with 20% aq. Sodium thiosulfate until product becomes colorless. It was further purified by column chromatography using hexane-ethyl acetate (80:20 v/v) as eluting solvent.

Yellow solid; Yield: 81.5 %; M. p: 184 – 200, Solubility: DMSO, FT-IR (KBr, cm-1): 3418.96 (phenyl OH), 1630.52 (C=O), 2820.14 (Aliphatic OH), Elemental analysis for $C_{21}H_{19}NO_3$ calculated C (75.66%), H (5.74%), N (4.2%), O (14.4%) found C (75.06%), H (4.74%), N (3.5%), O (14.4%) M+ calculated for 333.38 found to 335.38.

Synthesis of 2-[1-(Cyclohexylmethyl)-3-indolyl]-7-hydroxy-4-chromenone (5n)

The 1-(cyclohexylmethyl)-3-indolyl chalcone (0.01 mol) was suspended in dimethyl sulfoxide (DMSO, 6 ml) and iodine (0.01 mol, 1.27 g) was added to it. The mixture was refluxed for 20-50 min in an oil bath until the completion of reaction. Then the reaction mixture was poured in crushed ice and the solid separated was filtered and washed with 20% aq. Sodium thiosulfate until product becomes colorless. It was further purified by column chromatography using hexane-ethyl acetate (80:20 v/v) as eluting solvent.

Yellow solid; Yield: 82.2 %; m. p: 185 – 210, Solubility: DMSO, FT-IR (KBr, cm-1): 3443.98 (phenyl OH), 1634.58 (C=O), 2979.43 (Aromatic OH) Elemental analysis for $C_{24}H_{23}NO_3$ calculated C (77.19%), H (6.21%), N (3.75%), O (12.85%) found C (76.22%), H (6.05%), N (2.75%), O (12.15%).

Synthesis of 2-{1-[(o-Fluorophenyl)methyl]-3-indolyl}-7-hydroxy-4-chromenone (5q)

The 1-[(o-Fluorophenyl)methyl]-3-indolyl chalcone (0.01 mol) was suspended in dimethyl sulfoxide (DMSO, 6 ml) and iodine (0.01 mol, 1.27 g) was added to it. The mixture was refluxed for 20-50 min in an oil bath until the completion of reaction. Then the reaction mixture was poured in crushed ice and the solid separated was filtered and washed with 20% aq. Sodium thiosulfate until product becomes colorless. It was further purified by column chromatography using hexane-ethyl acetate (80:20 v/v) as eluting solvent.

Yellow solid; Yield: 79.8 %; M. p: 180 – 195, Solubility: DMSO, FT-IR (KBr, cm-1): 3445.97 (phenyl OH), 1654.72 (C=O), 2940.56 (Aromatic OH) Elemental analysis for $C_{24}H_{16}NO_3$ calculated for C (74.8%), H (4.18%), N (3.63%), F (4.93%), O (12.45%) found C (73.8%), H (3.19%), N (3.13%), F (3.93%), O (12.15%).

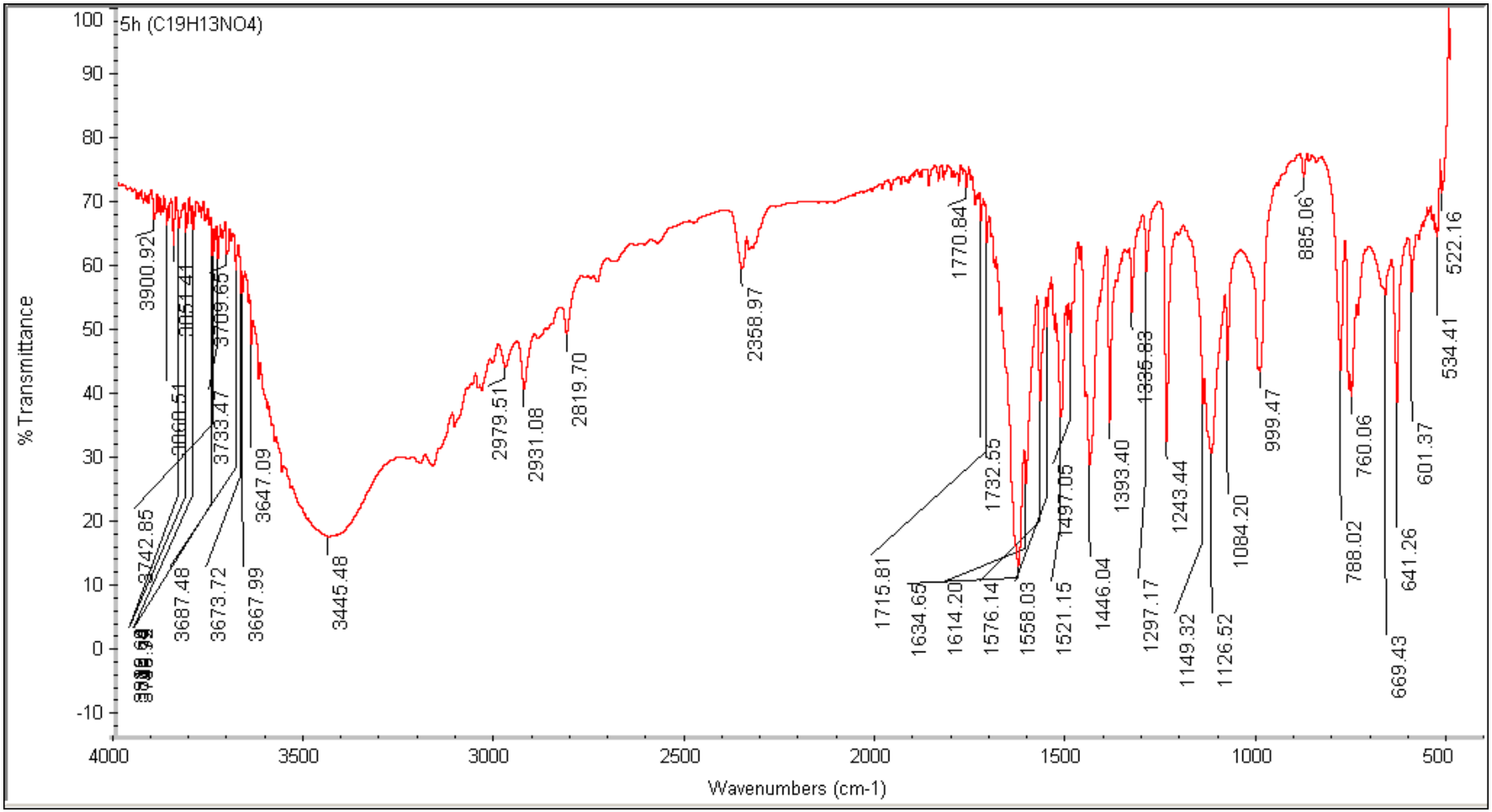
Synthesis of 2-{1-[(p-Fluorophenyl)methyl]-3-indolyl}-7-hydroxy-4-chromenone (5r)

The 1-[(p-Fluorophenyl)methyl]-3-indolyl chalcone (0.01 mol) was suspended in dimethyl sulfoxide (DMSO, 6 ml) and iodine (0.01 mol, 1.27 g) was added to it. The mixture was refluxed for 20-50 min in an oil bath until the completion of reaction. Then the reaction mixture was poured in crushed ice and the solid separated was filtered and washed with 20% aq. Sodium thiosulfate until product becomes colorless. It was further purified by column chromatography using hexane-ethyl acetate (80:20 v/v) as eluting solvent.

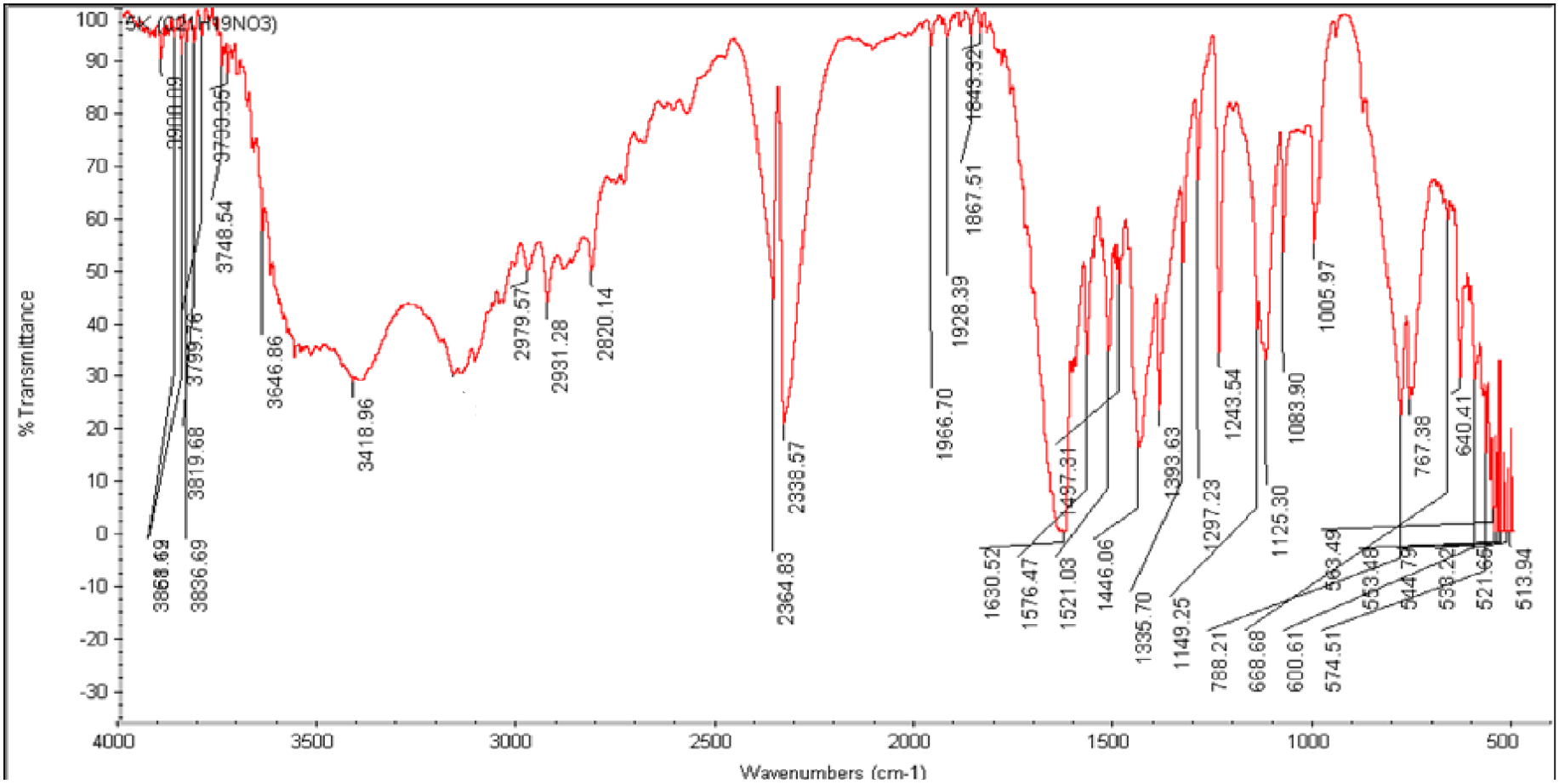
Yellow solid; Yield: 85.6 %; M. p: 182 – 205, Solubility: DMSO, FT-IR (KBr, cm-1): 3443.92 (phenyl OH), 1656.00 (C=O), 2812.88 (Aromatic OH) Elemental analysis for $C_{24}H_{16}NO_3$ calculated for C (74.8%), H (4.18%), N (3.63%), F (4.93%), O (12.45%) found C (73.8%), H (3.19%), N (3.13%), F (3.93%), O (12.15%).

IR SPECTRAL DATA

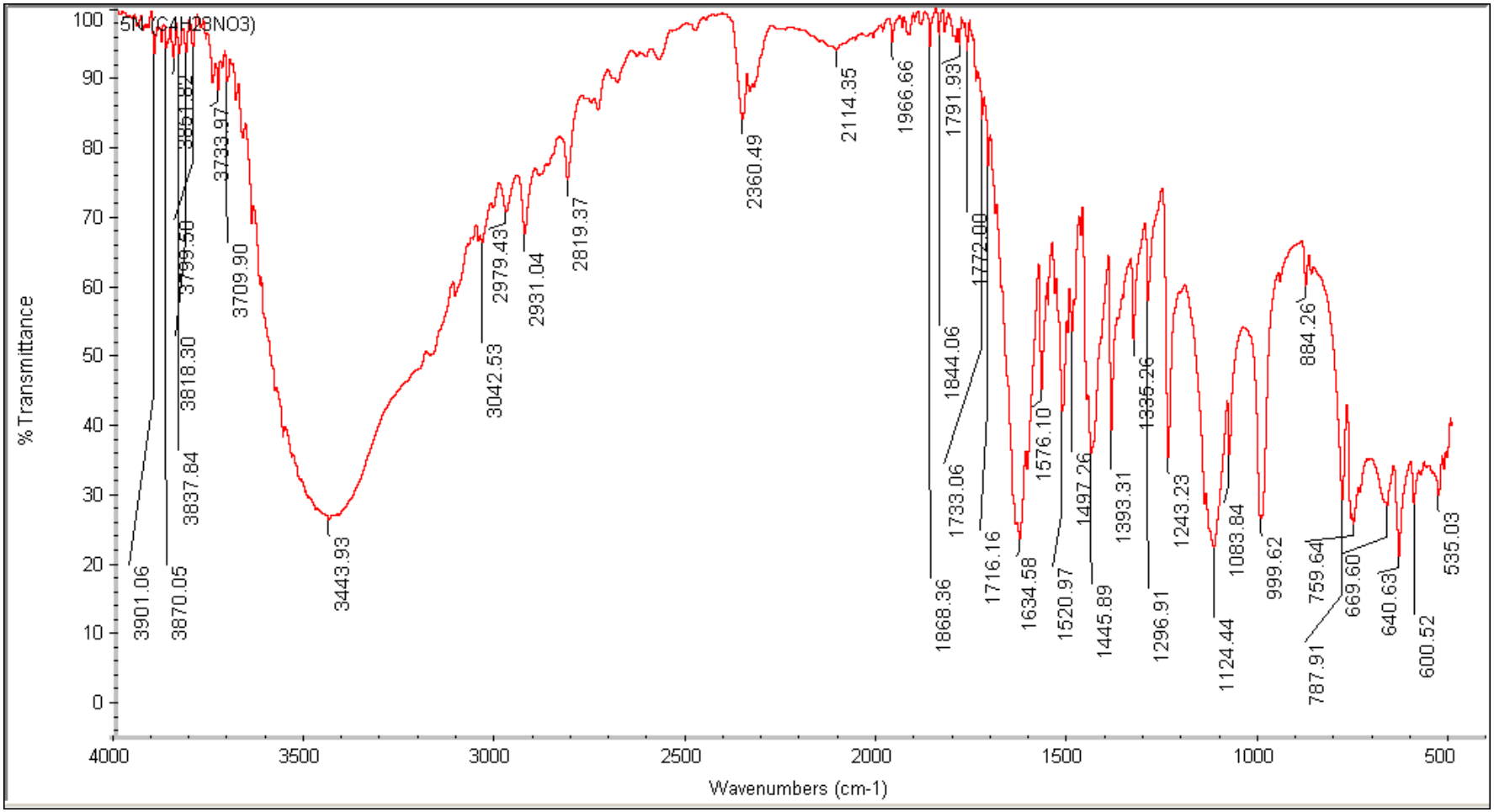
5h



5k


5K (C21H19NO3)
% Transmittance
Wavenumbers (cm-1)
3836.69
3819.68
3799.76
3748.54
3646.86
3418.96
2979.57
2931.28
2820.14
2364.83
2338.57
1966.70
1928.39
1867.51
1630.52
1576.47
1521.03
1497.31
1446.06
1393.63
1335.70
1297.23
1243.54
1149.25
1125.30
1083.90
1005.97
788.21
767.38
668.68
640.41
600.61
574.51
513.94

5n


5N (C4H28NO3)
% Transmittance
Wavenumbers (cm-1)
100
90
80
70
60
50
40
30
20
10
0
4000
3500
3000
2500
2000
1500
1000
500
3901.06
3870.05
3837.84
3818.30
3799.50
3733.97
3709.90
3443.93
3042.53
2979.43
2931.04
2819.37
2360.49
2114.35
1966.66
1868.36
1844.06
1791.93
1772.00
1733.06
1716.16
1634.58
1576.10
1520.97
1497.26
1445.89
1393.31
1336.26
1296.91
1243.23
1124.44
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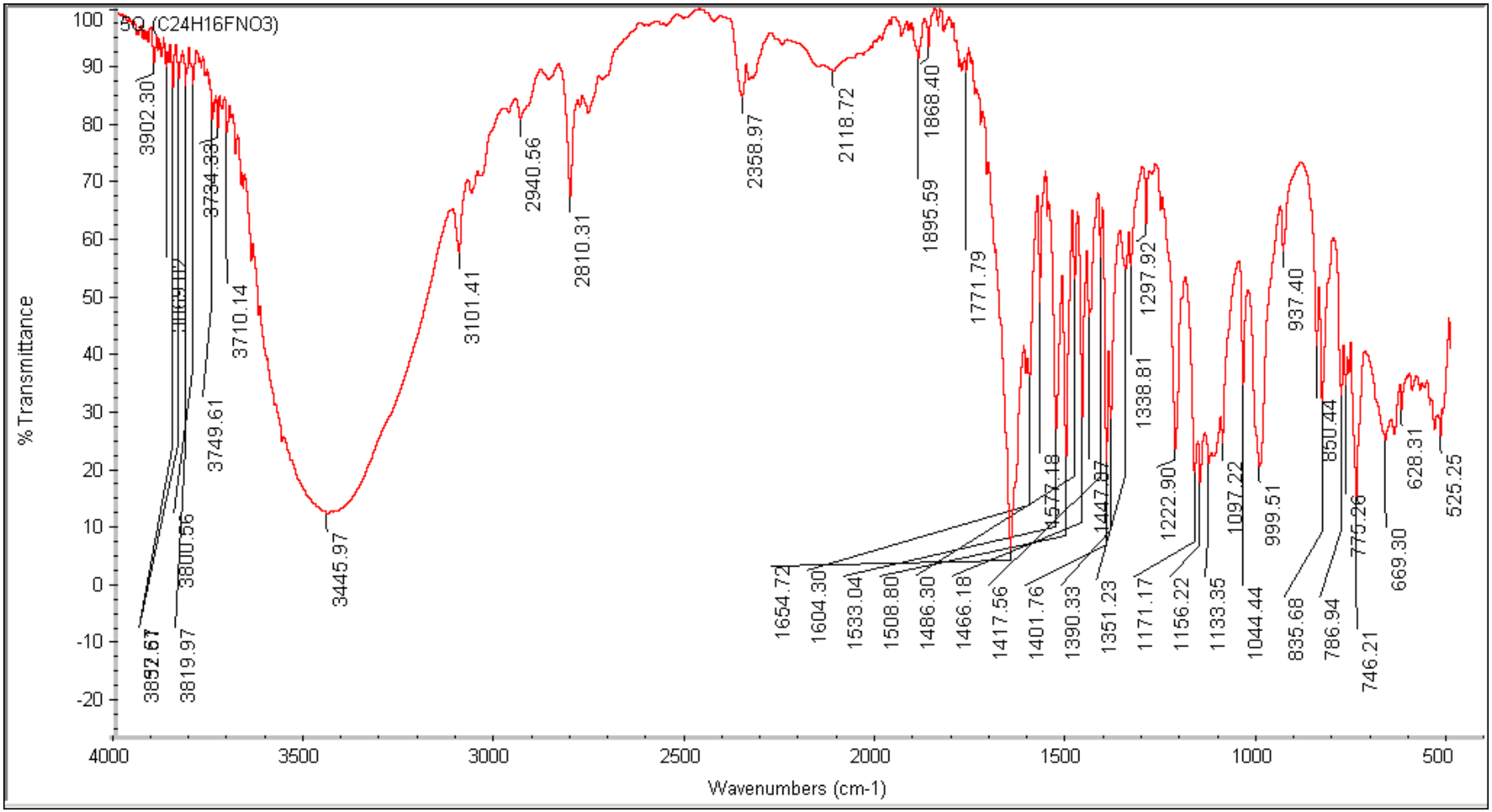
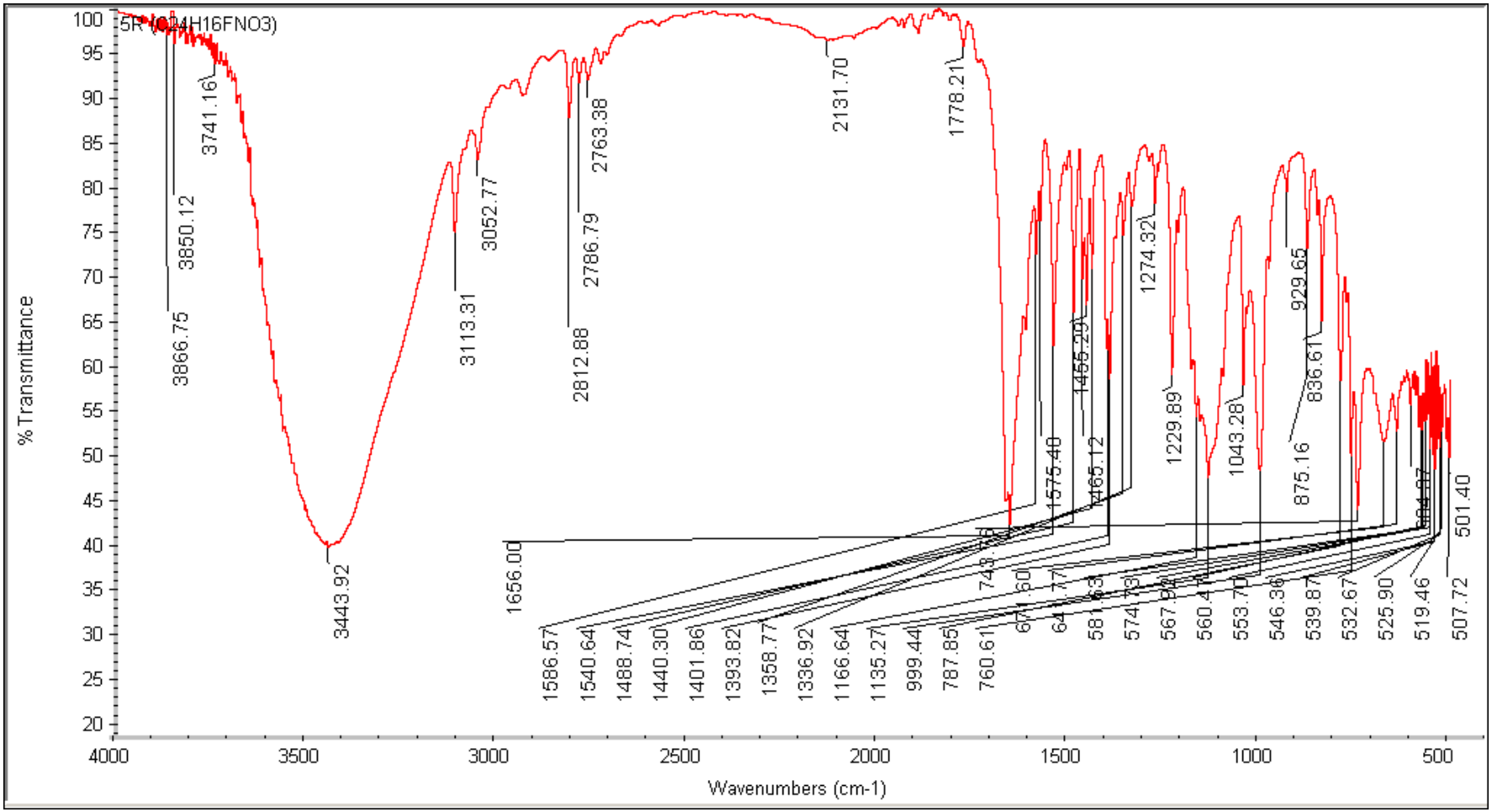
999.62
884.26
787.91
759.64
669.60
640.63
600.52
535.03

5q


5Q (C24H16FNO3)
% Transmittance
Wavenumbers (cm-1)
3902.30
3734.33
3710.14
3749.61
3800.56
3819.97
3445.97
3101.41
2940.56
2810.31
2358.97
2118.72
1895.59
1868.40
1771.79
1654.72
1604.30
1533.04
1508.80
1486.30
1466.18
1417.56
1401.76
1390.33
1351.23
1338.81
1297.92
1222.90
1171.17
1156.22
1133.35
1097.22
1044.44
999.51
937.40
850.44
835.68
786.94
775.26
746.21
669.30
628.31
525.25

5r


5R (C24H16FNO3)
% Transmittance
Wavenumbers (cm-1)
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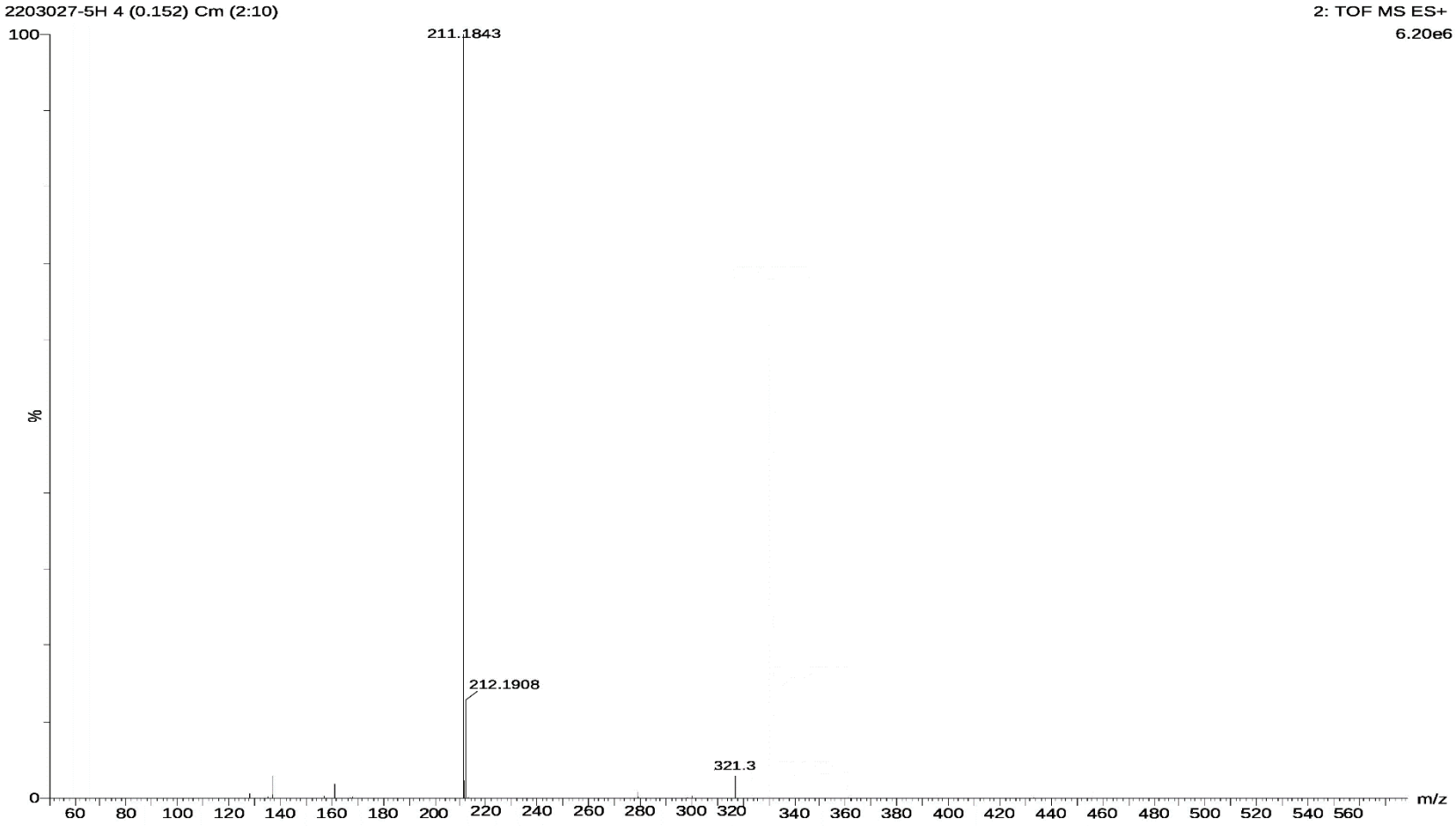
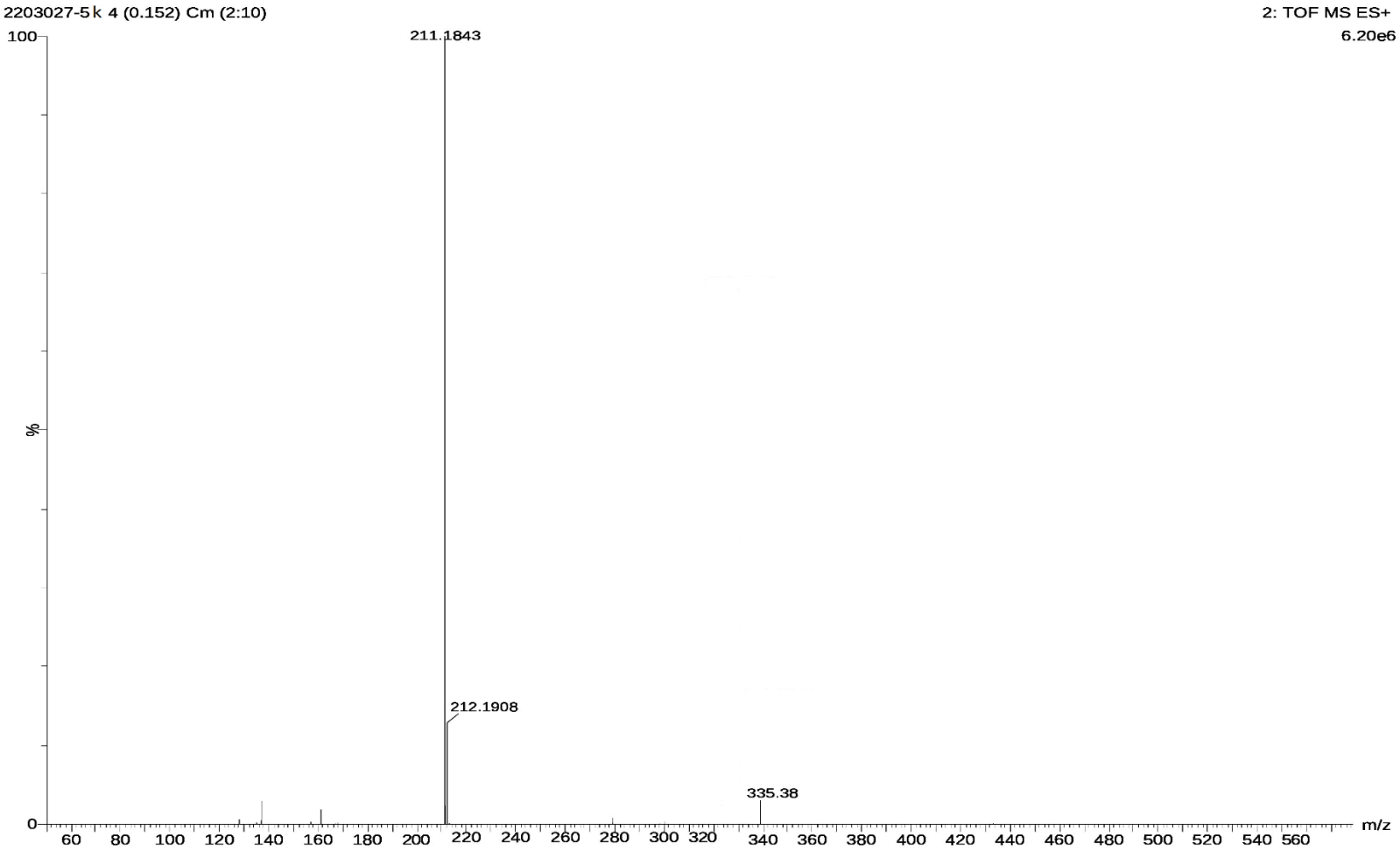
3850.12
3741.16
3443.92
3113.31
3052.77
2812.88
2786.79
2763.38
2131.70
1778.21
1656.00
1586.57
1540.64
1488.74
1440.30
1401.86
1393.82
1358.77
1336.92
1166.64
1135.27
999.44
787.85
760.61
1575.40
1465.12
1455.20
1274.32
1229.89
1043.28
929.65
875.16
836.61
581.63
574.73
567.84
560.47
553.70
546.36
539.87
532.67
525.90
519.46
507.72
501.40

MASS SPECTRA DATA

5h



5k


2203027-5k 4 (0.152) Cm (2:10)
2: TOF MS ES+
6.20e6
100
%
0
211.1843
212.1908
335.38
m/z
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540 560

CANCER:

- Cancer is the uncontrolled growth of abnormal cells anywhere in the body. These abnormal cells are termed cancer cells, malignant cells, or tumor cells. These cells can infiltrate normal body tissues.
- Many cancers and the abnormal cells that compose the cancer tissue are further identified by the name of the tissue that the abnormal cells originated from (for example, breast cancer, lung cancer, and colorectal cancer). When damaged or unrepaired cells do not die and become cancer cells and show uncontrolled division and growth - a mass of cancer cells develop.
- This process of cancer cells leaving an area and growing in another body area is termed metastatic spread or metastasis. For example, if breast cancer cells spread to a bone, it means that the individual has metastatic breast cancer to bone.

Risk Factors for Developing Cancer

- Genetic predisposition or gene mutations
- Use of tobacco
- Certain Infections like hepatitis B and C and human papillomavirus
- Exposure to ultraviolet radiations from sun and other ionizing radiations like x rays
- Environmental pollutants
- Immunosuppressive medicines

Other factors that can contribute include consumption of alcohol, poor diet, lack of physical activity and obesity. The risk of cancer increases significantly with age.

Types of Cancer

There are more than 100 types of cancer, usually named for the organs or tissues where the cancers form. In males, the most common cancers are that of lung, prostate, stomach, colon and rectum. In females, the most common cancers are that of breast, colon, rectum, lung and cervix.

Symptoms of Cancer

Cancer is a disease in which there are not many apparent r pronounced symptoms in early stages and this is the reason mostly cancers are detected when they reach a late stage of the disease.

Symptoms vary according to the location, stage and type of the cancer. Few cancer specific symptoms include abnormal lump, unexplained weight loss, fatigue, change in bowel movements, abnormal bleeding or prolonged cough. All these symptoms can also occur due to other health issues as well.

Stages of Cancer

Stage means the extent to which the disease has progressed like the size of the tumor and if it has spread to the other parts of the body

- **Stage 0:** Cancer cells are present but have not spread to surrounding tissues

- **Stage I, II and III:** Higher the number, more advanced the disease in terms of size of the tumor and invasion of surrounding organs and tissues

Treatment of Cancer

The treatment depends on the type and stage of the cancer. Treatment includes radiation therapy, chemotherapy, surgery and target therapy.

Prevention from Cancer

- Avoiding known causes of cancer like tobacco use, radiation exposure etc
- Lifestyle changes
- A diet low in fat and high in fiber, fruits, and vegetables lowers the risk of certain types of cancers
- Vaccination against hepatitis B virus and human papillomavirus
- Screening tests at regular intervals of those who are at higher risk e.g., people who have family history of cancers or who are exposed to ionizing radiations.
- Regular preventive complete body checkup

EPIDERMAL GROWTH FACTOR RECEPTOR:

- Molecular targeting strategies for cancer therapy are distinct from conventional chemotherapy and radiotherapy in their potential to provide increased tumor specificity. One particular molecular target of high promise in oncology is the epidermal growth factor receptor (EGFR).
- Growth factor receptors are involved in a wide range of cellular responses to the environment. The epidermal growth factor (EGF) receptor ErbB-1, as well as three other members of the ErbB family (ErbB-2, ErbB-3, and ErbB-4) continue to provide essential biological and molecular discoveries that are useful to the entire receptor tyrosine kinase (RTK) research.
- The four ErbB receptors recognize 11 structurally similar growth factors and mediate developmental, homeostatic, and pathological processes. Each receptor is a type I transmembrane protein with a ligand-binding ectodomain that is extensively glycosylated and disulfide-bonded, a single transmembrane domain, and a large cytoplasmic region that encodes a tyrosine kinase and many phosphorylation sites. ErbB-2 does not bind to a known ligand, but rather serves as a co-receptor for the other three receptors.
- The cytoplasmic tyrosine kinase is activated when a growth factor binds to the ectodomain, triggering signaling pathways that direct cellular responses. Dimerization events in numerous areas of the proteins trigger receptor activation. Dimerization is induced by ligand binding and is required for kinase activation, with the exception of a few constitutively active mutants. ErbB-1, -3, and -4 receptor dimerization involves both homo- and heterodimerization, especially with ErbB-2. ErbB-3 lacks an active tyrosine kinase and hence relies on ErbB-2 connection for signaling. The level of active ErbB receptors is modulated by a growing number of negative regulators and is positively influenced by other cellular components, such as adhesion molecules, in the context of a cellular environment. These modulators and their mechanisms are still a work in progress.

- In many cancer types, mutations affecting EGFR expression or activity could result in cancer.
- Epidermal growth factor receptor (EGFR, also known as ErbB-1 or HER-1) inhibitors are medicines that bind to certain parts of the EGFR and slow down or stop cell growth.

EGFR inhibitors can be classified as either:

- tyrosine kinase inhibitors (TKI) (eg, erlotinib, gefitinib): these bind to the tyrosine kinase domain in the epidermal growth factor receptor and stop the activity of the EGFR
- monoclonal antibodies (eg, cetuximab, necitumumab): these bind to the extracellular component of the EGFR and prevent epidermal growth factor from binding to its own receptor, therefore preventing cell division.

EGFR inhibitors may be used in the treatment of cancers that are caused by EGFR up-regulation, such as non-small-cell lung cancer, pancreatic cancer, breast cancer, and colon cancer.

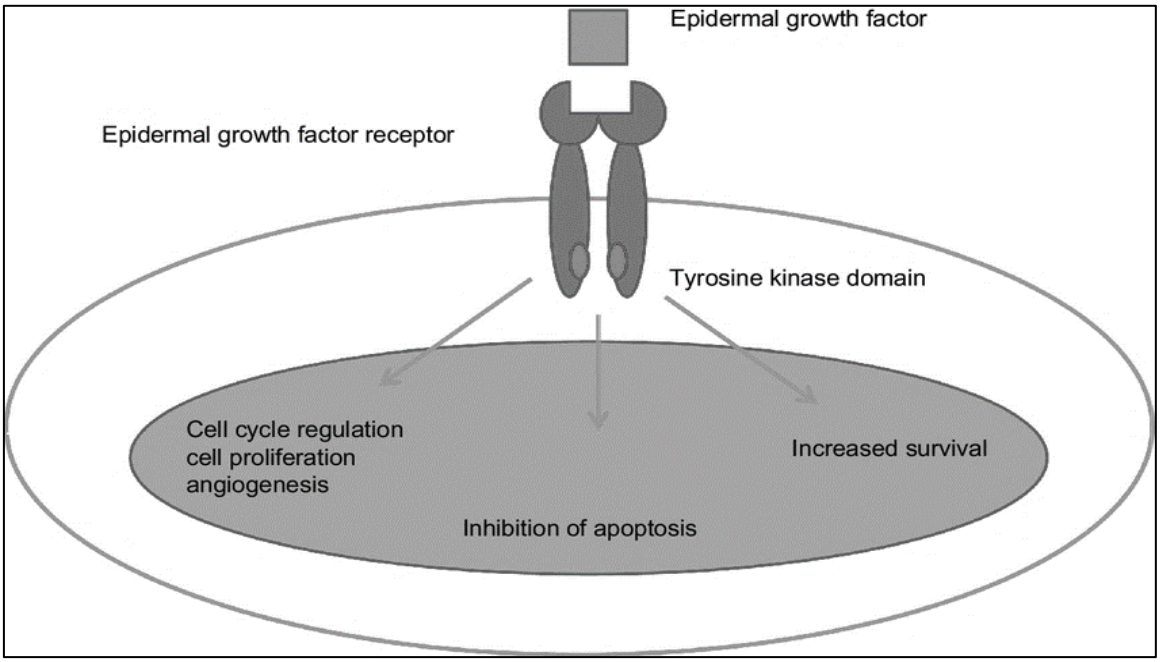


Figure 6: Structure of EGFR Kinase

RESULT AND DISCUSSION

VIII RESULT AND DISCUSSION

8.1. IN SILICO SCREENING OF DESIGNED COMPOUNDS

❖ The ADME properties of the designed compounds were evaluated using SWISS ADME software and PreADMET software, their results were tabulated (Table no: 4 & 5 respectively)

❖ Toxicity of all the designed compounds were evaluated by using ProTox II software and the results were tabulated (Table No :6).

❖ Anticancer agents generally possess greater number of hydrogen bond acceptors. All the designed compounds have less than 5 hydrogen bond donors and 6-10 hydrogen bond acceptors. This obeys Lipinski rule of rule. Molecular weight of all the designed compounds was around 500 daltons.

❖ All our designed compounds show log P value between 1- 5. It was found that lipophilicity plays a major role in determining where drugs are distributed within the body after adsorption and, as a consequence, how rapidly they are metabolized and excreted. In the biological system drug disposition depends on the ability to cross membranes, so there is a strong relationship with measures of lipophilicity. So, there is a strong lipophilic character of the molecule plays a major role in producing the antimicrobial effect.

❖ TPSA has been used as descriptor for characterizing absorption and passive transportation properties through biological membranes, allowing a good prediction of transport of candidate drugs in the intestines and through the blood-brain barrier. Compounds with TPSA values within the range 97.89 $A°^2$ have good intestinal absorption. TPSA (Total Polar Surface Area) of our designed compounds were found to be in the range of 55.37-89.52 $A°^2$. Therefore, it is expected that our compounds might possess good intestinal absorption.

❖ Synthetic accessibility score is normalized between 1 (easy synthesis) and 10 (very difficult synthesis). The designed compounds showed score between 3 to 6. This indicates the designed compounds can be synthesized in ease. BBB penetration values of all compounds were found to be less than 1. So, these

compounds are CNS inactive. Caco2 cell permeability of all compounds lies between 18-25. This range indicates all the designed compounds have moderate permeability. Plasma protein binding of all the compounds was found in the range of 94-100, which indicates the chemicals are strongly bound. HIA (Human Intestinal Absorption) values of all designed compounds were found between 92-98. This indicates that all the designed compounds are well absorbed in intestine

- The designed compounds passed Lipinski rule of five, that the drug is suitable for oral administration.

MOLINSPIRATION

Molinspiration offers broad range of cheminformatics software tools supporting molecule manipulation and processing, including SMILES and SDfile conversion, normalization of molecules, generation of tautomers, molecule fragmentation, calculation of various molecular properties needed in QSAR, molecular modelling and drug design, high quality molecule depiction, molecular database tools supporting substructure and similarity searches. Our products support also fragment-based virtual screening, bioactivity prediction and data visualization. Molinspiration tools are written in Java, therefore can be used practically on any computer platform.

Table 3: Molinspiration results for all designed compounds

S.NO	Compound	MilogP	TPSA	N atoms	Molecular weight	Number of violations	Number of rotatable bonds	Volume	GPCR ligand	Ion channel modulater	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	5a	3.45	55.37	22	291.31	0	1	253.93	0.10	-0.14	0.44	0.38	-0.29	0.40
2	5b	3.83	55.37	23	305.33	0	2	270.74	0.08	-0.23	0.40	0.33	-0.30	0.36
3	5c	5.05	55.37	28	367.40	1	3	325.58	0.16	-0.20	0.30	0.33	-0.15	0.34
4	5d	4.91	55.37	32	443.40	0	3	357.79	0.21	-0.0	0.33	0.36	0.01	0.34
5	5e	4.92	72.44	29	381.39	0	2	327.76	0.16	-0.26	0.30	0.42	-0.10	0.36
6	5f	2.83	89.52	25	355.37	0	2	285.36	0.30	-0.27	0.39	0.44	-0.16	0.40

7	5g	4.36	89.52	30	417.44	0	3	340.21	0.23	0.04	0.30	0.17	-0.07	0.37
8	5h	3.71	72.44	24	319.32	0	1	272.92	0.04	-0.59	0.28	0.22	-0.30	0.27
9	5i	3.68	81.68	26	349.34	0	3	298.70	0.15	0.06	0.03	0.47	-0.23	0.39
10	5j	5.13	55.37	26	345.40	1	3	314.71	0.11	-0.07	0.30	0.35	-0.11	0.44
11	5k	4.89	55.37	25	333.39	0	4	304.34	0.16	-0.16	0.33	0.33	-0.17	0.38
12	5l	4.32	55.37	25	331.37	0	3	293.76	0.37	-0.01	0.53	0.48	0.00	0.56
13	5m	4.50	55.37	26	345.40	0	3	310.56	0.26	-0.08	0.43	0.39	-0.03	0.43
14	5n	5.74	55.37	28	373.45	1	3	344.17	0.17	-0.09	0.32	0.34	-0.10	0.35
15	5o	5.10	64.61	30	397.43	1	4	351.13	0.11	-0.24	0.26	0.28	0.18	0.28
16	5p	4.33	55.37	24	319.36	0	3	287.54	0.15	-0.17	0.35	0.35	-0.21	0.35
17	5q	5.16	55.37	29	385.39	1	3	330.51	0.16	-0.23	0.29	0.32	-0.15	0.31
18	5r	5.21	55.37	29	385.39	1	3	330.51	0.16	-0.21	0.32	0.33	-0.17	0.31

Table 4: SWISS ADME RESULTS

Compound no	Formula	Molecular weight (g/mol)	Number of heavy atoms	Number of aromatic heavy atoms	Fraction Csp3	Number of rotatable bonds	Number of H bond acceptors	Number of H bond donors	Molar refractivity	TPSA (Å)	Log $P_{O/W}$	Log S
5a	C18H13NO3	291.30	22	19	0.06	1	3	1	86.70	55.37	2.43	-4.05
5b	C19H15NO3	305.33	23	19	0.11	2	3	1	91.51	55.37	2.64	-4.23
5c	C24H17NO3	367.40	28	25	0.04	3	3	1	111.19	55.37	3.03	-5.42
5d	C24H17F4NO3	443.39	32	19	0.21	3	7	1	113.16	55.37	3.26	-5.45
5e	C24H15NO4	381.38	29	25	0.00	3	4	1	111.61	72.44	2.90	-5.44
5f	C18H13NO5S	355.36	25	19	0.06	2	5	1	95.67	97.89	2.11	-4.09
5g	C23H15NO5S	417.43	30	25	0.00	3	5	1	114.51	97.89	2.75	-5.45
5h	C19H13NO4	319.31	24	19	0.05	2	4	1	91.93	72.44	2.35	-4.03
5i	C20H15NO5	349.34	26	19	0.10	4	5	1	97.99	81.67	3.00	-4.53
5j	C22H19NO3	345.39	26	19	0.14	3	3	1	105.45	55.37	3.25	-5.16
5k	C21H19NO3	333.38	25	19	0.19	4	3	1	101.12	55.37	3.21	-4.78
5l	C21H17NO3	331.36	25	19	0.19	3	3	1	99.01	55.37	2.94	-4.51

5m	C22H19NO3	345.39	26	19	0.23	3	3	1	103.81	55.37	3.17	-4.92
5n	C24H23NO3	373.44	28	19	0.29	3	3	1	112.43	55.37	3.48	-5.74
5o	C25H19NO4	397.42	30	25	0.08	4	4	1	117.68	64.60	3.38	-5.47
5p	C20H17NO3	319.35	24	19	0.15	3	3	1	96.31	55.37	2.88	-4.56
5q	C24H16FNO3	385.39	29	25	0.04	3	4	1	111.15	55.37	3.22	-5.57
5r	C24H16FNO3	385.39	29	25	0.04	3	4	1	111.15	55.37	3.22	-5.57

PROTOX II RESULTS

Table 5: ProTox II results for all designed compounds

Compound no	Predicted LD50 mg/kg	Predicted toxicity class	Hepatotoxicity	carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
5a	600	4	Inactive	active	active	Active	active
5b	600	4	Inactive	active	active	Inactive	active
5c	600	4	Inactive	active	Inactive	Active	active
5d	400	4	Active	Inactive	active	Inactive	Inactive
5e	600	4	Active	active	Inactive	Inactive	Inactive
5f	600	4	Inactive	Inactive	active	Inactive	Inactive
5g	2000	4	Active	Inactive	Inactive	Inactive	Inactive
5h	600	4	Inactive	Inactive	Inactive	Inactive	Inactive
5i	2570	5	Inactive	Inactive	active	Inactive	Inactive
5j	600	3	Inactive	Inactive	active	Active	Inactive
5k	600	4	Inactive	Inactive	Inactive	Inactive	Inactive
5l	600	4	Inactive	active	Inactive	active	active
5m	600	4	Inactive	Inactive	Inactive	Inactive	active
5n	600	4	Inactive	Inactive	Inactive	Inactive	Inactive
5o	2570	5	Inactive	active	active	active	Inactive
5p	600	4	Inactive	Inactive	Inactive	active	active
5q	600	4	Inactive	Inactive	Inactive	Inactive	Inactive
5r	600	4	Inactive	Inactive	Inactive	Inactive	Inactive

Table 6: Swissadme & PreADME results for all designed compounds

Compound no	Lipinski	Ghose	Veber	Egan	Muegge	Bio availability score	GI absorption	BBB permeation	P-gp substrate	Log K_p cm/s	Synthetic accessibility score
5a	Yes	Yes	Yes	Yes	Yes	0.55	High	Yes	No	-6.02	2.99
5b	Yes	Yes	Yes	Yes	Yes	0.55	High	Yes	Yes	-5.89	3.08
5c	Yes	Yes	Yes	Yes	Yes	0.55	High	Yes	Yes	-5.35	3.32
5d	Yes	No	Yes	No	Yes	0.55	Low	No	No	-6.05	4.84
5e	Yes	Yes	Yes	Yes	Yes	0.55	High	No	No	-5.47	3.29
5f	Yes	Yes	Yes	Yes	Yes	0.55	High	No	No	-6.64	3.32
5g	Yes	Yes	Yes	Yes	Yes	0.55	High	No	No	-5.91	3.61
5h	Yes	Yes	Yes	Yes	Yes	0.55	High	No	No	-6.27	3.07
5i	Yes	Yes	Yes	Yes	Yes	0.55	High	No	No	-5.90	3.32
5j	Yes	Yes	Yes	Yes	Yes	0.55	High	Yes	No	-5.21	3.37
5k	Yes	Yes	Yes	Yes	Yes	0.55	High	Yes	Yes	-5.43	3.26
5l	Yes	Yes	Yes	Yes	Yes	0.55	High	Yes	Yes	-5.78	3.20

5m	Yes	Yes	Yes	Yes	Yes	0.55	High	Yes	Yes	-5.48	3.32
5n	Yes	No	Yes	Yes	No	0.55	High	No	Yes	-4.88	3.52
5o	Yes	Yes	Yes	Yes	Yes	0.55	High	Yes	Yes	-5.55	3.43
5p	Yes	Yes	Yes	Yes	Yes	0.55	High	Yes	Yes	-5.60	3.17
5q	Yes	No	Yes	Yes	Yes	0.55	High	No	Yes	-5.38	3.32
5r	Yes	No	Yes	Yes	Yes	0.55	High	No	Yes	-5.38	3.36

8.2 DOCKING

- Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions.

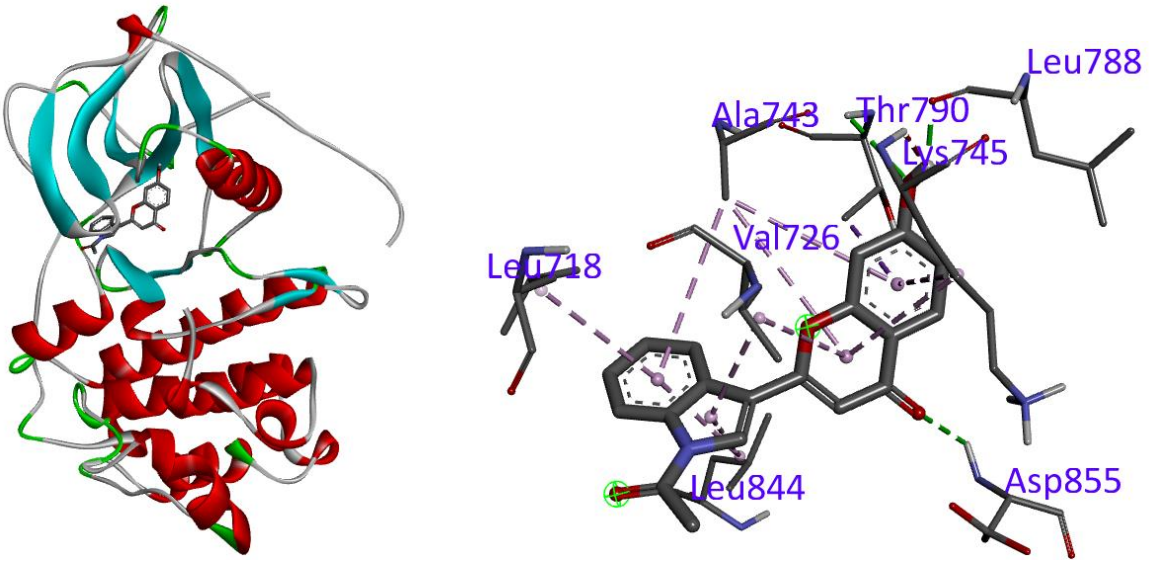
- Docking studies were performed with the active site of Epidermal growth factor receptor (EGFR) Kinase (PDB ID: 2j5f). Protein was downloaded from RCSB with a resolution of 3.00 Å is obtained from the protein data bank. Protein was prepared by removing the ligand groups, nucleic acid groups, heteroatoms, water molecules and then adding polar hydrogens. Prepared protein was saved in .pdb (Protein data bank) format. The structure of the ligand was drawn using ACD/Chemsketch FREEWARE and saved in. mol format. The energy minimization of the ligands was performed using Autodock vina 1.1.2 and converted to. pdbqt format.

- The docking studies for five designed compounds have showed good binding affinities. Compounds **5h, 5k, 5n, 5q, 5r** exhibited binding energies of **-8.9, -8.6, -9.0, -9.1** and **-9.2 (kcal/mol).**

- Compound **5h** interacted with LYSA745 by forming an H-B interaction, and the benzene ring showed hydrophobic interaction with LEU A 844, ALA A 743, and VLA 726. In the unfavorable Donar - Donar interaction of compound **5k** showed LYS A 745 and hydrophobic interaction with three Pi-sigma bond interacted with LEU A 718, LEU A 844, VAL A 726. Compound **5n** showed three Pi-sigma hydrophobic interaction and one Pi - allkyl showed, LYS A 745, ALA A 743, VAL A 726 and LEU A 844. Hydrophobic interactions with compound **5q** showed two pi - alkyl and one pyrrole interactions with LYS A 745, ALA A 743, and VAL A 726, and the center of the benzene and pyrrole rings showed LEUA 844. The convention hydrogen bond of compound **5r** showed H-B interaction with ALA A 743, LYS A 745, Pi-sigma and Pi-Alkyl showed hydrophobic interaction with LEU A 844 and VAL A 726

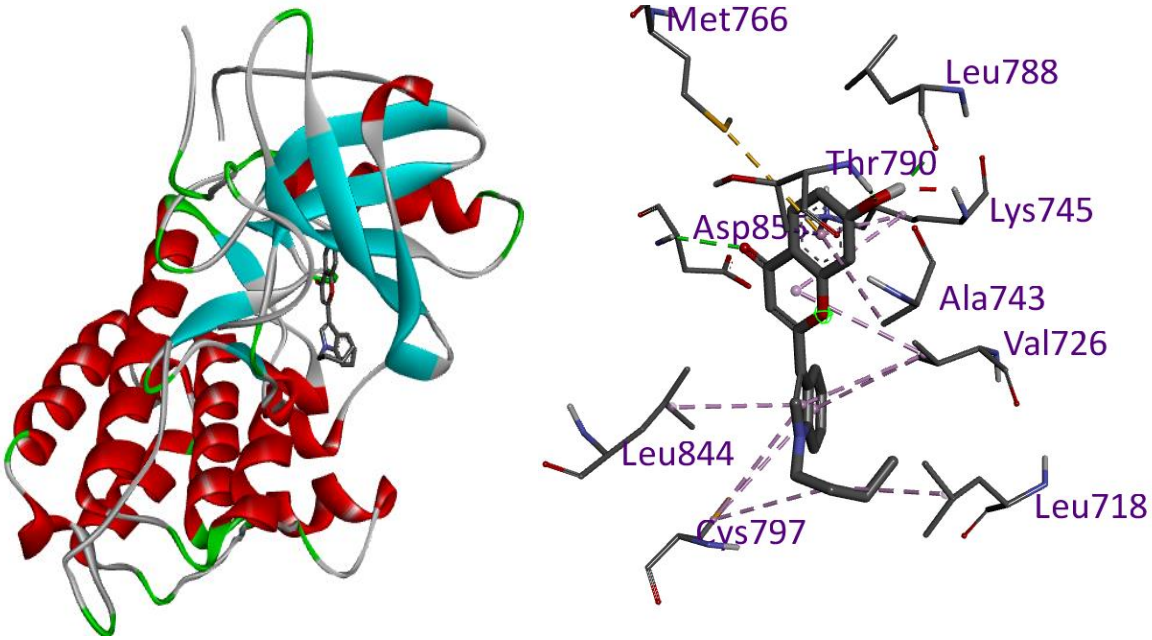
- The benzene ring of the standard compound showed hydrophobic interaction with LYS A 745, 2,3- dihydro 4H-pyran-4-one of two groups showed hydrophobic interaction with ALA A 743, VAL A 726 and Pi-sigma interaction showed LEU A 844. Some of the amino acids which showed interactions with standard drug 6-hydroxy flavone was also similar with desired compounds. These results indicate that ligands can act as anticancer agents against EGFR Kinase.

- The interaction of ligand 5h, 5k, 5n, 5q, and 5r with protein 2j5f is shown in Figures 7 – 11



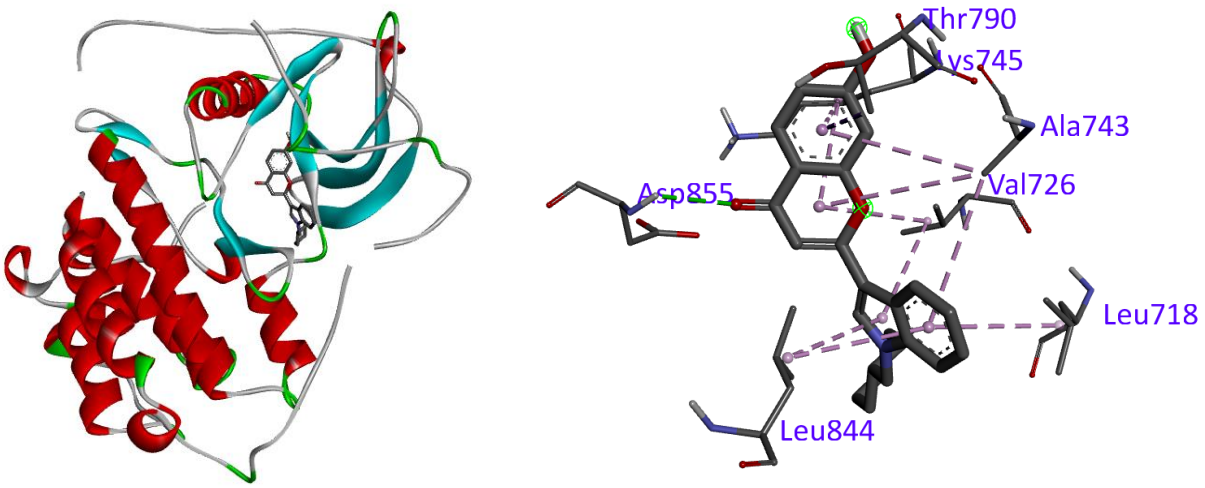
3D **LIGAND INTERACTION**

Figure 7: 3D & Ligand molecular interaction visualizations of ligand 5h with the active site of 2j5f



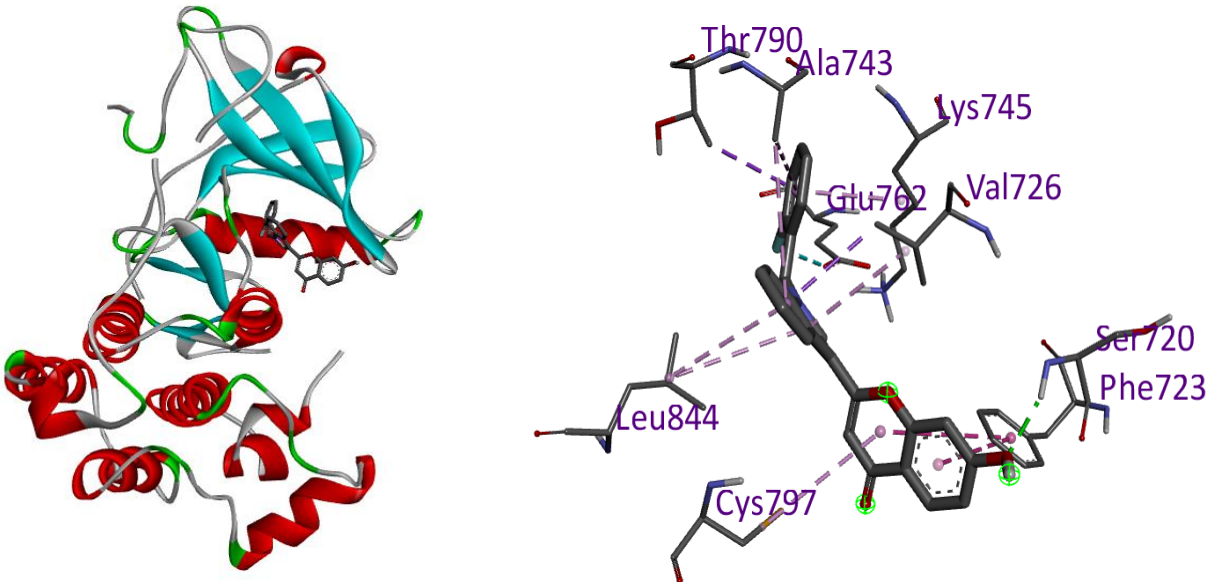
3D **Ligand interaction**

Figure 8: 3D & Ligand molecular interaction visualizations of ligand 5K with the active site of 2j5f



3D **Ligand interaction**

Figure 9: 3D & Ligand molecular interaction visualizations of ligand 5n with the active site of 2j5f



3D **Ligand interaction**

Figure 10: 3D & Ligand molecular interaction visualizations of ligand 5q with the active site of 2j5f

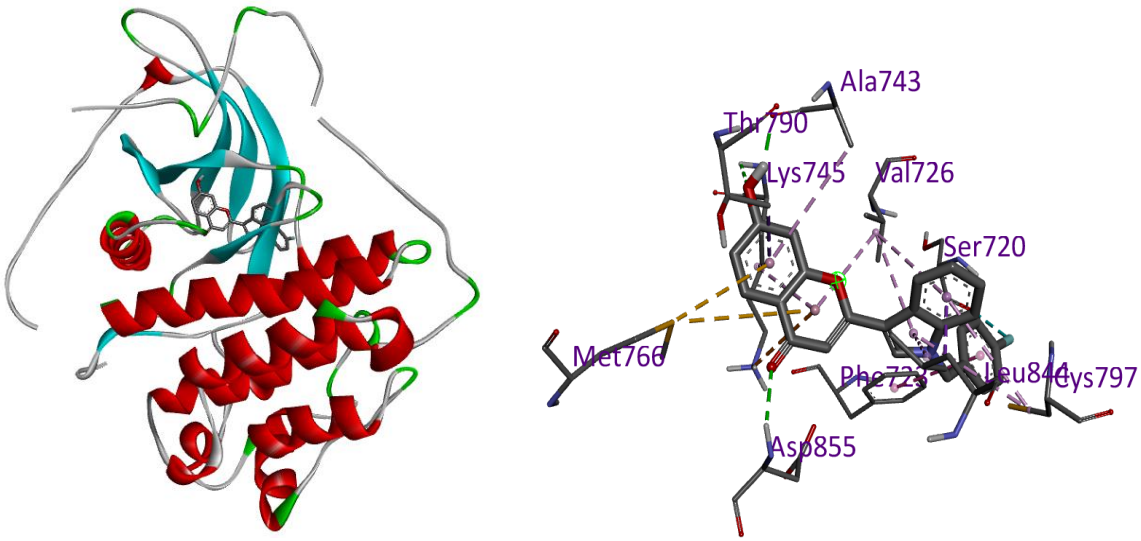


Figure 11: 3D & Ligand molecular interaction visualizations of ligand 5r with the active site of 2j5f

Table 7: Docking interaction of ligands

S.No.	COMPOUND	BINDING AFFINITY (kcal/mol)	Amino acid interaction with Distance
1	5h	-8.9	VAL726 – 4.83 ALA743 – 5.22 LYS745 – 4.19 LEU844 – 4.91

2	5k	-8.6	LEU844 – 5.07 VAL726 – 4.57 ALA743 – 5.16 LYS745 – 4.83
3	5n	-9.0	VAL726 – 4.88 LEU844 – 4.74 ALA743 - 5.41 LYS745 – 5.16
4	5q	-9.1	VAL 726 – 4.83 ALA743 – 5.22 LYS745 – 4.19 LEU844 – 4.91
5	5r	-9.2	LEU844 – 5.23 VAL726 - 5.11 ALA743 – 5.19 LYS745 – 4.00
6	Standard (6-hydroxy flavone)	-8.5	VAL 726 – 4.54 ALA743 – 4.57 LEU844 – 4.77 LYS745 – 4.43

From the docking score, docked five compounds which showed good binding affinity were selected for synthesis.

8.3. SYNTHESIS

The synthetic route of title compounds is outlined in scheme 1. Indole-3-carboxaldehyde is an important heterocycle ring present in indole alkaloids. Many synthetic methods were reported for the synthesis. Among them the synthesis of substituted indole-3-carboxaldehyde flavone from the indoyl chalcones in presence of DMSO/I_2. During the reaction, the indole-3-carboxaldehyde with indole-3-carboxaldehyde substitution gave the better yield. All the compounds were characterized by IR, MS, and elemental analyses.

The yield of the synthesized compounds ranges from 78-85 %. All the compounds were characterized by IR, MS, and elemental analyses. The IR spectra of synthesized compounds showed absorption bands due to stretching vibrations of phenyl-OH, C=O and aromatic-OH at 3418-3445 cm^{-1}, 1614-1656 cm-1 and 2812-2979 cm-1 respectively. The mass spectrum showed molecular ion peak which was in agreement with molecular mass of compound while the base peak was observed at 211 (100%).

8.4. Biological Studies

The synthesized compounds have been subjected to cytotoxic activity on through MTT assay using cisplatin as positive control for MCF-7 cell lines.

Among tested compounds, compound **5h** with acetyl substitution and compound **5k** with 1-chlorobutane substitution with IC_{50} = 9.30 ± 0.625 and 17.56 ± 3.15 showed biological activity.

Compound **5h** with acetyl substitution with IC_{50} = 9.30 ± 0.625 showed potent activity compared to standard cisplatin against MCF-7 cells. The anticancer activity results are given in Table 8. The results of in vitro anticancer activity were in agreement with Insilco docking studies.

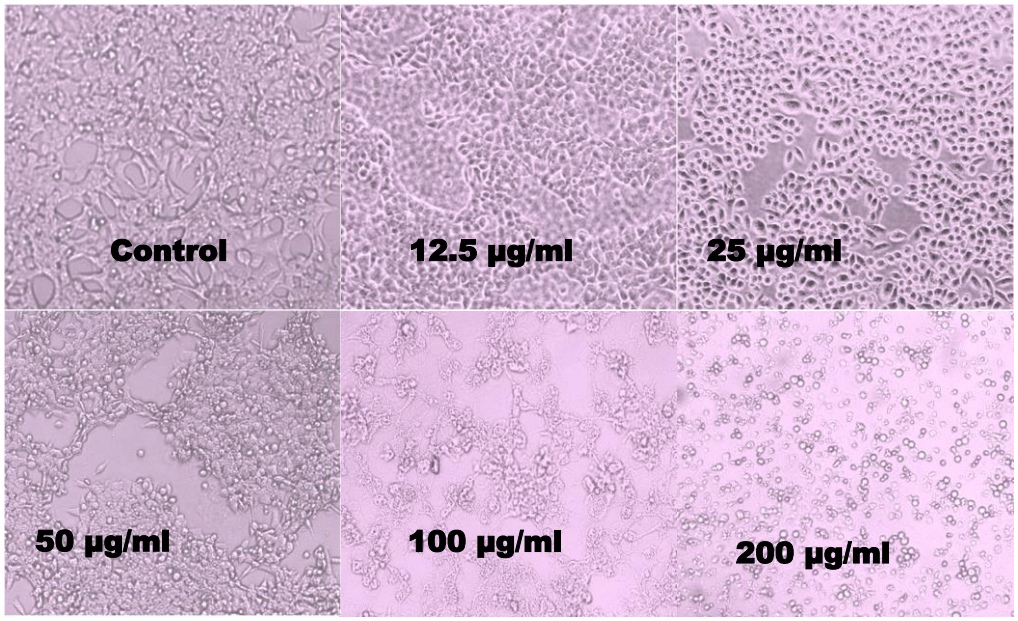


Figure 12: IC_{50} values of synthesized compound 5h

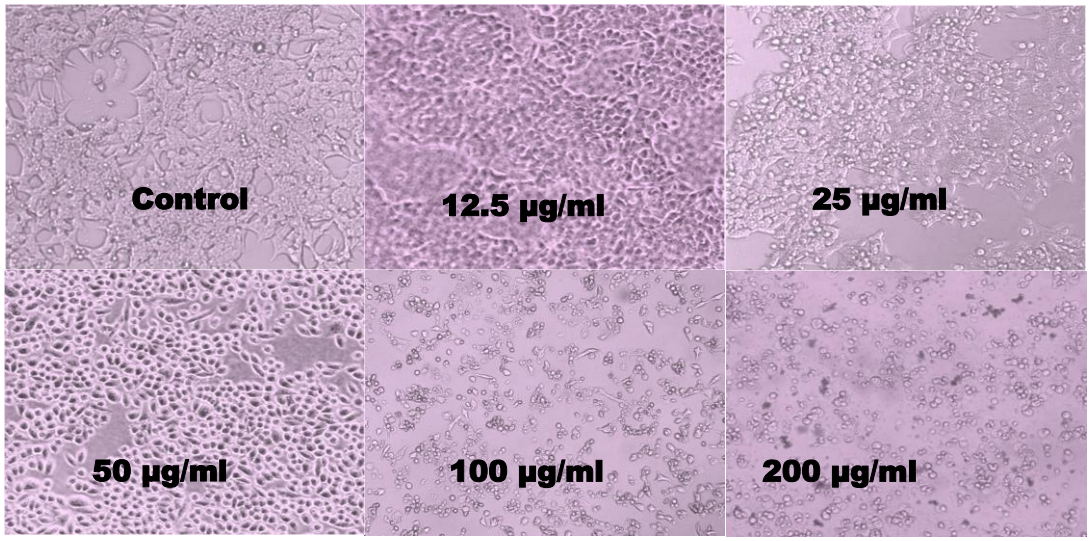


Figure 13: IC_{50} values of synthesized compound 5k

Cisplatin

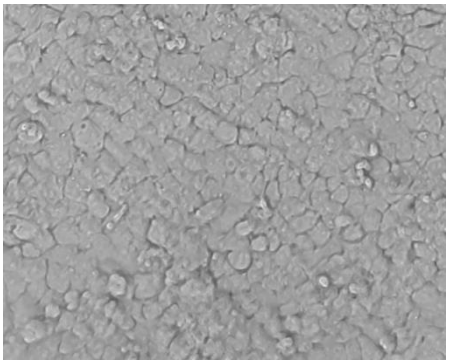


Figure 14: IC_{50} values of standard compound

SI. NO	COMPOUND	IC50 (µM) MCF-7 CELL LINE Mean ± SD
1	**5h**	9.30 ± 0.625
2	**5k**	17.56 ± 3.15
3	**Cisplatin**	8 ± 0.30

Table 8: Anticancer activity against MCF-7 cell lines

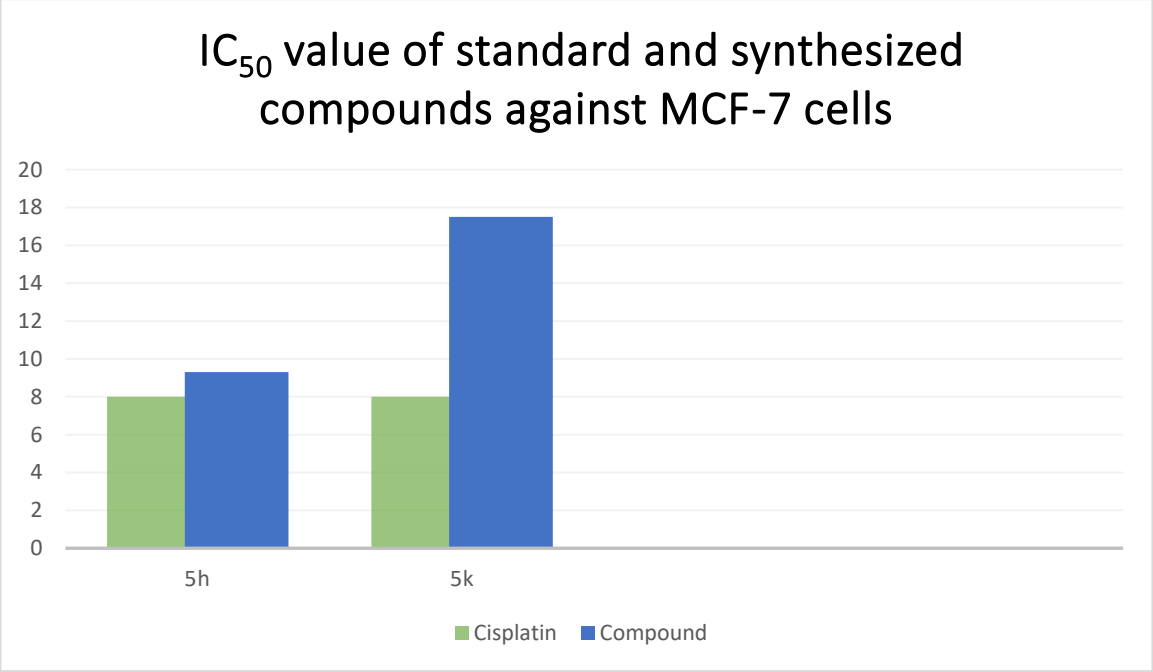


Figure 15: IC_{50} value of standard and synthesized compounds against MCF-7 cells

SUMMARY AND CONCLUSION

IX SUMMARY AND CONCLUSION:

- The present study involves the synthesis, characterization and biological evaluation of Claisen Schmidt condensation of indole-3-carboxaldehyde flavone were synthesized based on Magdy A H Zahran *et al* method.
- All the synthesized compounds of present study were characterized and confirmed by IR, and Mass spectral data. It showed characteristic absorption bands and characteristic peaks.
- The newly synthesized substituted indole-3-carboxaldehyde flavone derivatives were subjected to preliminary Insilco pharmacological screening.
- From the docking results, compounds with good docking score have been synthesized, characterized and subjected to anticancer activity. The results of anticancer study explain that the synthesized active compound could serve as intermediate for generating good biological agents.
- Anti-cancer studies were performed for two synthesized compounds (5h and 5k) against EGFR Kinase. The half maximal inhibitory concentration (IC_{50}) of synthesized compounds against breast cancer cell lines (MCF-7) were determined.
- Anti-cancer activity of synthesized compounds was tested against MCF-7 cell lines. The compounds 5h exhibited good anticancer activity with the IC_{50} value ranging in 9.30 ± 0.625 when compared to standard cisplatin was found to 8 ± 0.30.
- Among the series the compounds **5h** showed potent anti-cancer activity.
- As a result, these compounds can exhibit very promising anticancer potential among the series. These could be some of the most promising targets for breast cancer cells.

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