4'-fluoro-5,7-dihydroxyflavone – Piperazine Hybrids as VEGFR-2 Inhibitors: Design, In-silico Study, Synthesis, And Anticancer Activity

DEPARTMENT OF PHARMACEITICAL CHEMISTRY

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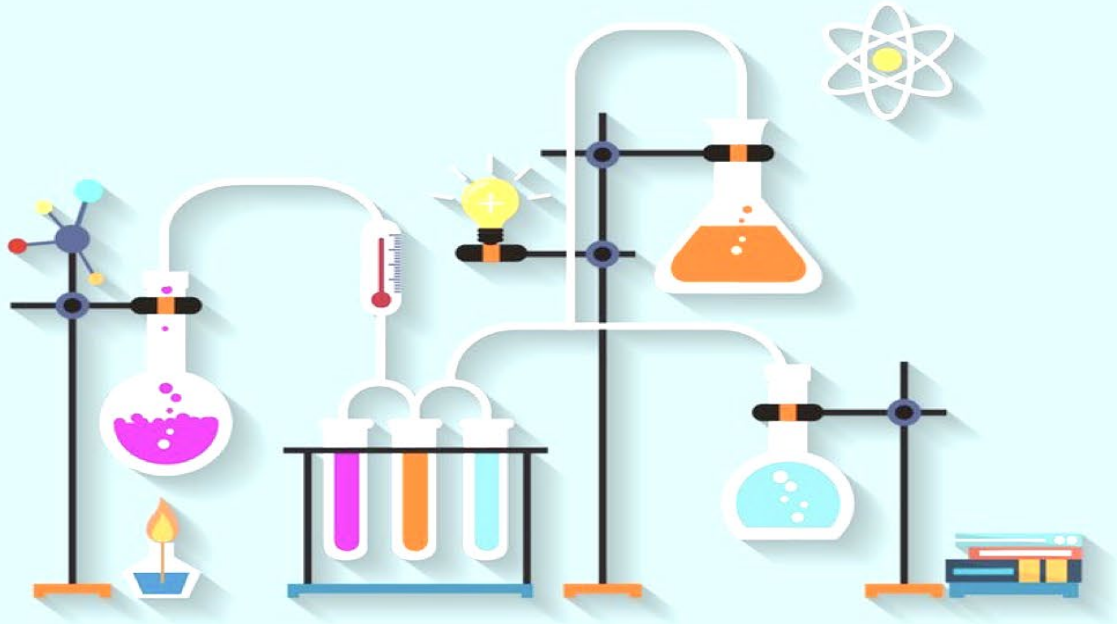

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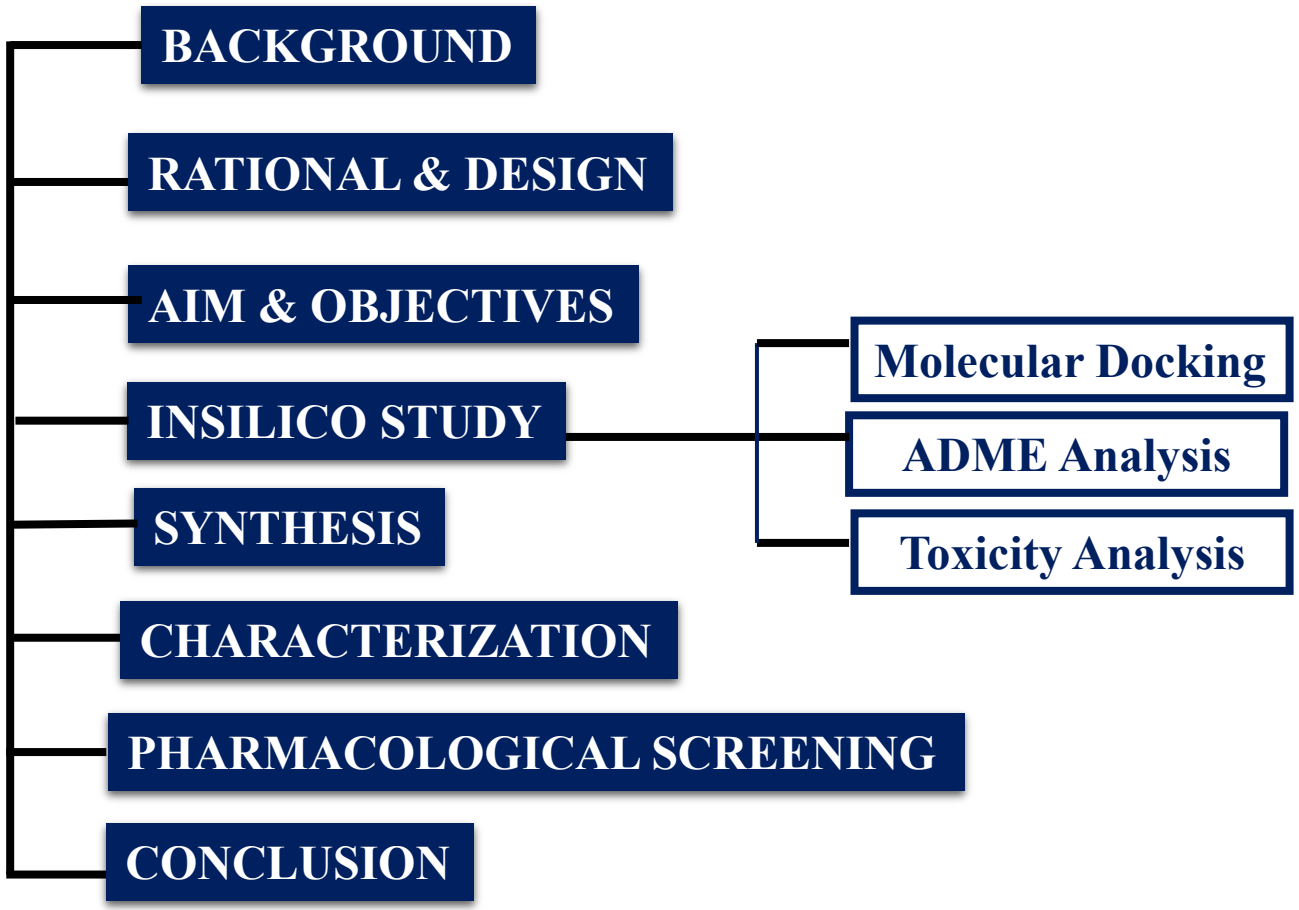
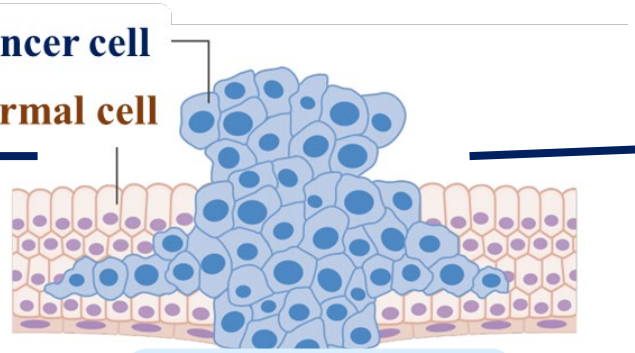
CONTENT


BACKGROUND
RATIONAL & DESIGN
AIM & OBJECTIVES
INSILICO STUDY
Molecular Docking
ADME Analysis
Toxicity Analysis
SYNTHESIS
CHARACTERIZATION
PHARMACOLOGICAL SCREENING
CONCLUSION

BACKGROUND

Cancer



Uncontrolled cell division

Prevalent cause of mortality in world at present

Despite advancements in diagnosis, treatment, and prevention

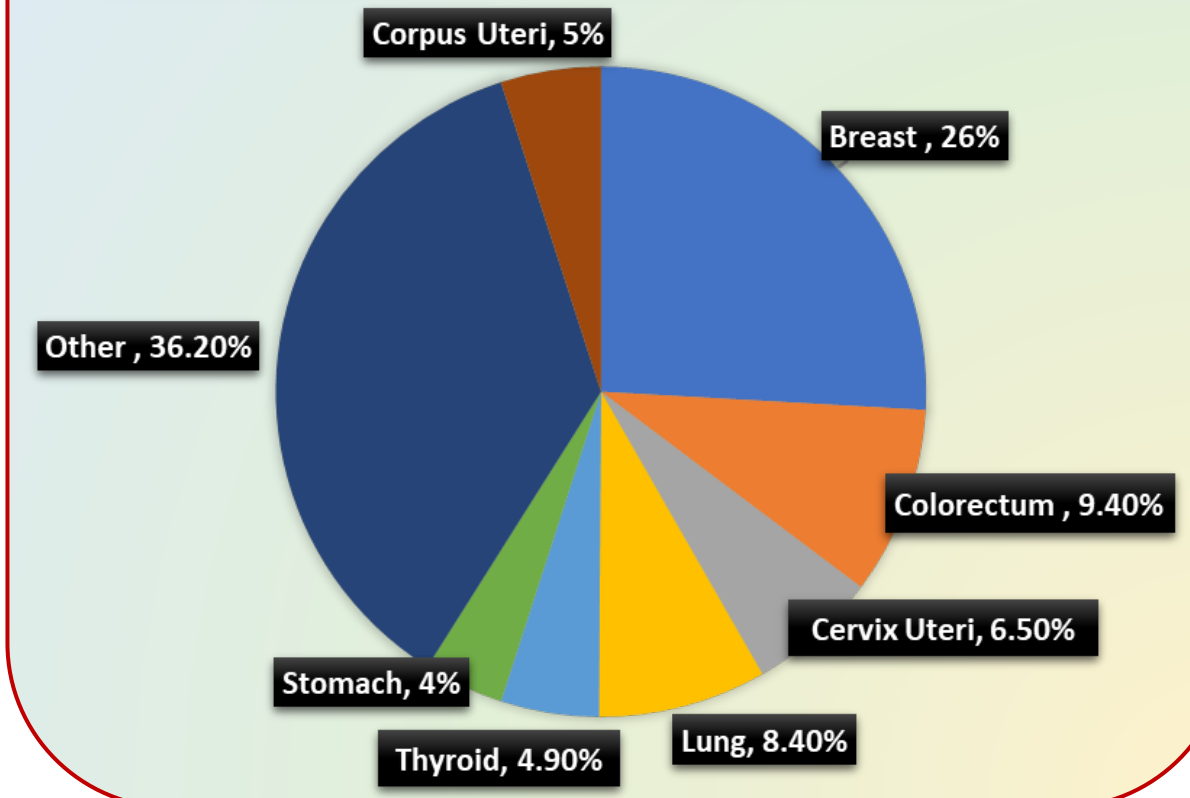
↓

Global burden of cancer death

↓

drastically ↑↑↑↑

World Health Organisation: The number of new cancer cases worldwide in 2020 is estimated.



Development of new anticancer agents

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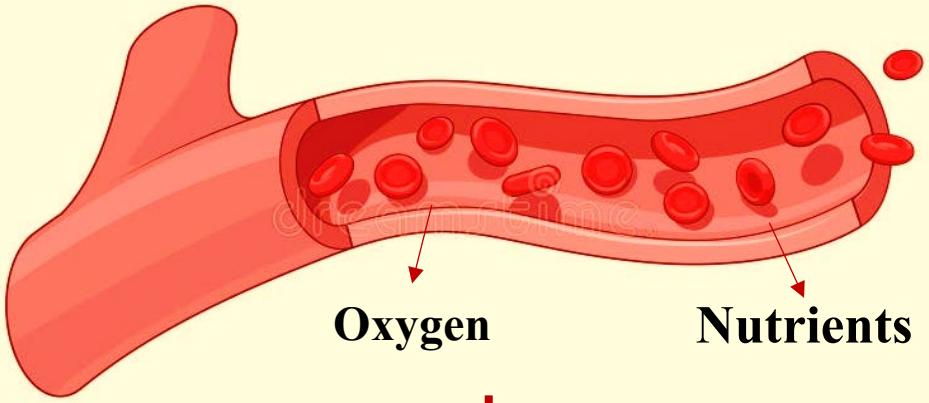
Maximum Effectiveness
&
Minimal Toxicity

↓

Vital trend in anticancer Drug Development and Research

ANGIOGENESIS



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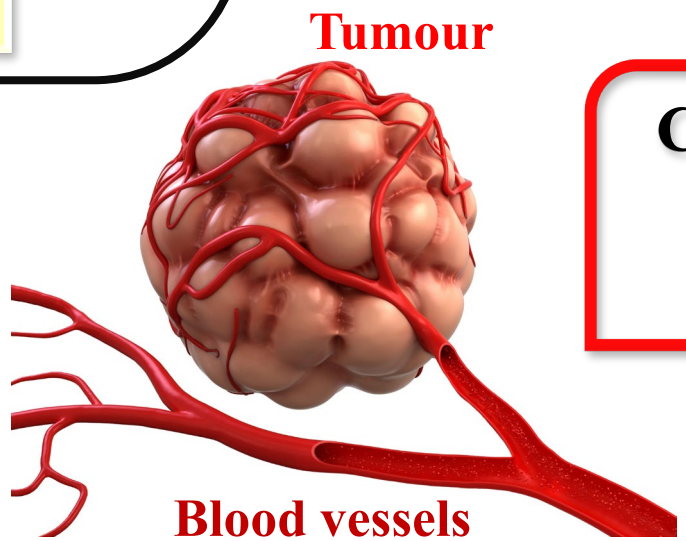
Essential Factor

↓

Cancer Growth
&
Proliferation



Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2)

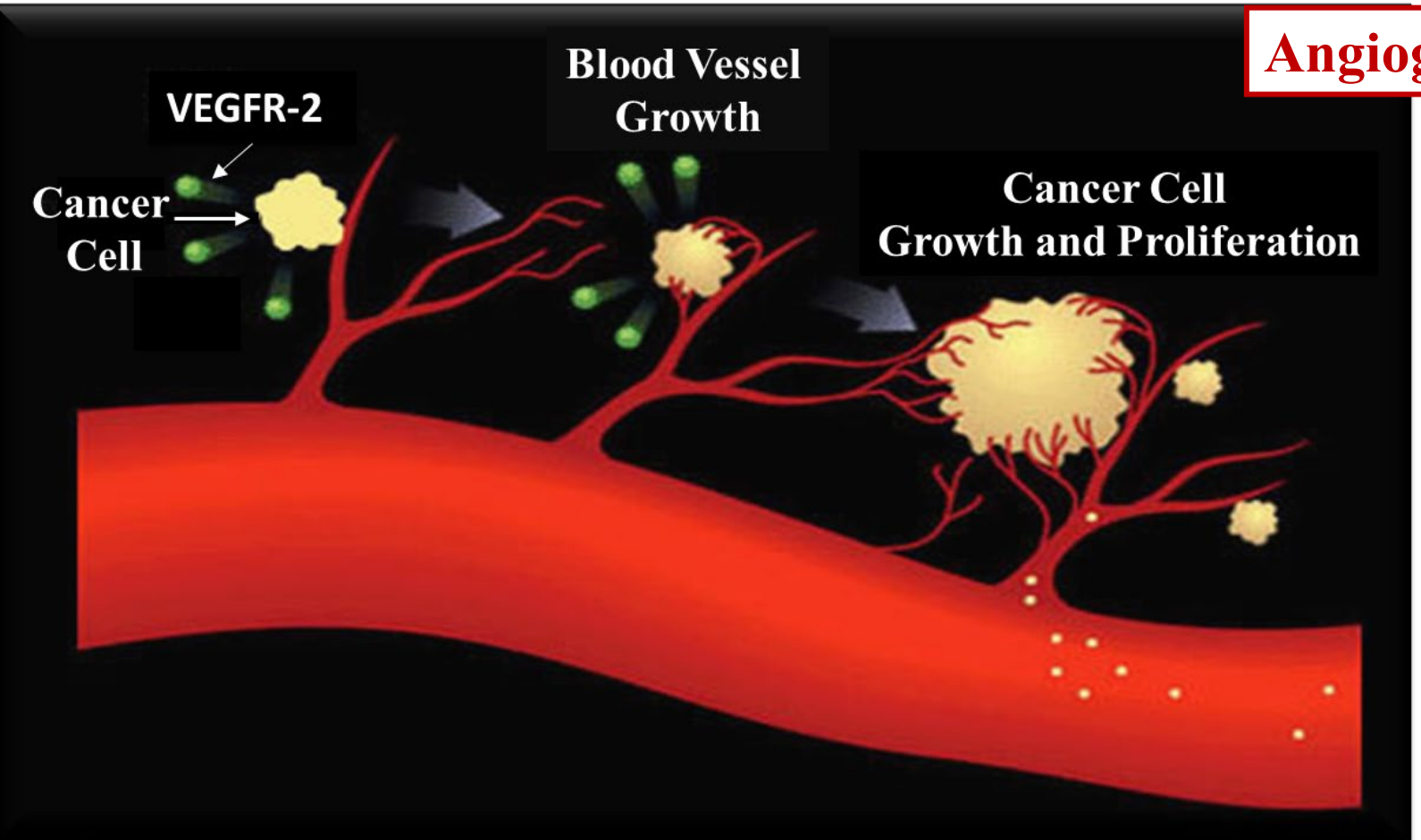
VEGF – exert angiogenesis effect via binding with **VEGFR-2**

↓

Play major role

↓

Angiogenesis



VEGFER-2 pathway

↓

Competent Target

↓

Vital Selectivity for Cancer Cells

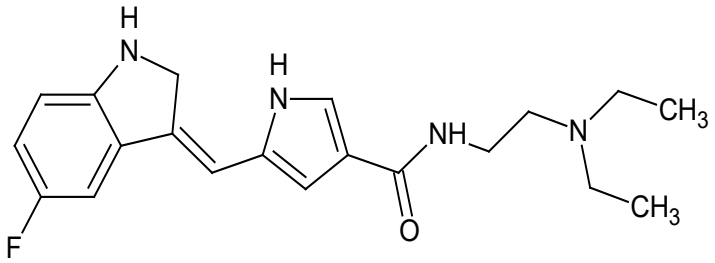
✖

By inhibiting VEGFR-2

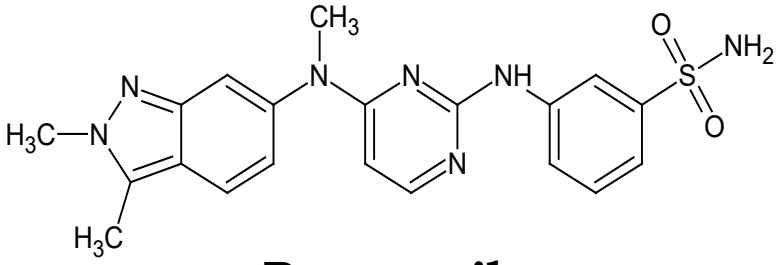
↓

Discovery of New Anticancer Drug

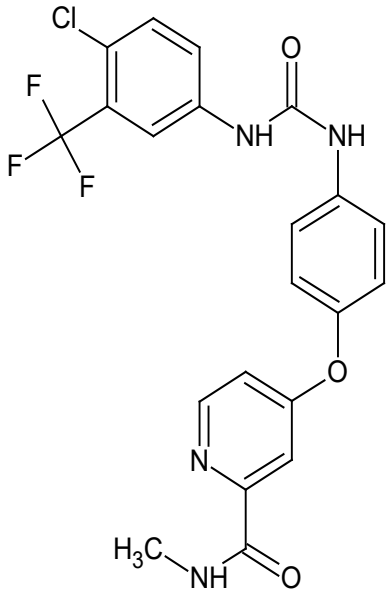
WHY NEW VEGFR-2 INHIBITORS?



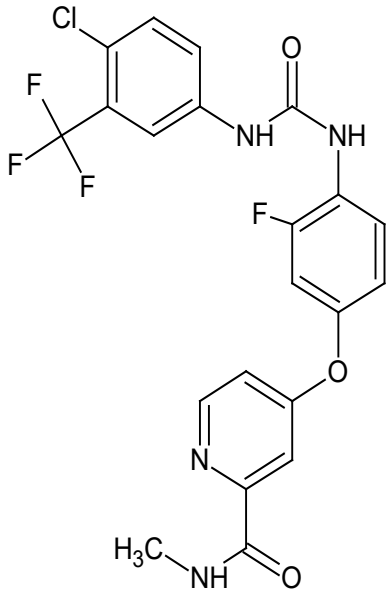
Sunitinib



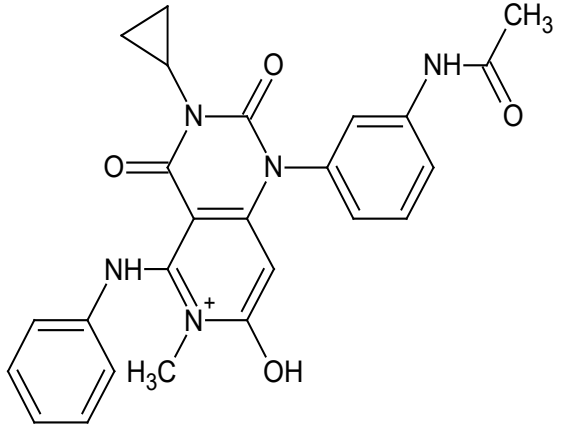
Pazopanib



Sorafenib



Regorafenib



Trametinib

- ❑ **Drug resistance**
- ❑ **Unwanted side effects (Hypertension)**
- ❑ **Decreasing efficacy**

DRAWBACKS

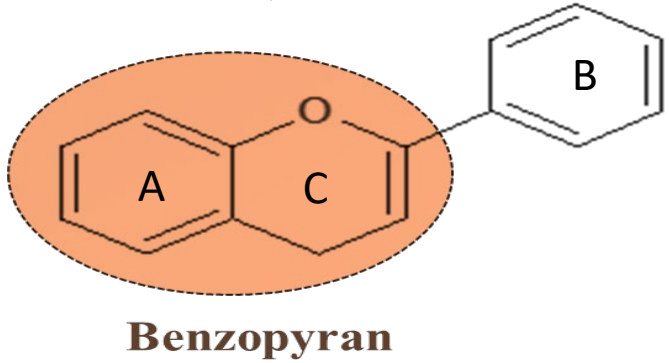
FDA APPROVED VEGFR-2 Inhibitors

FLAVONOIDS

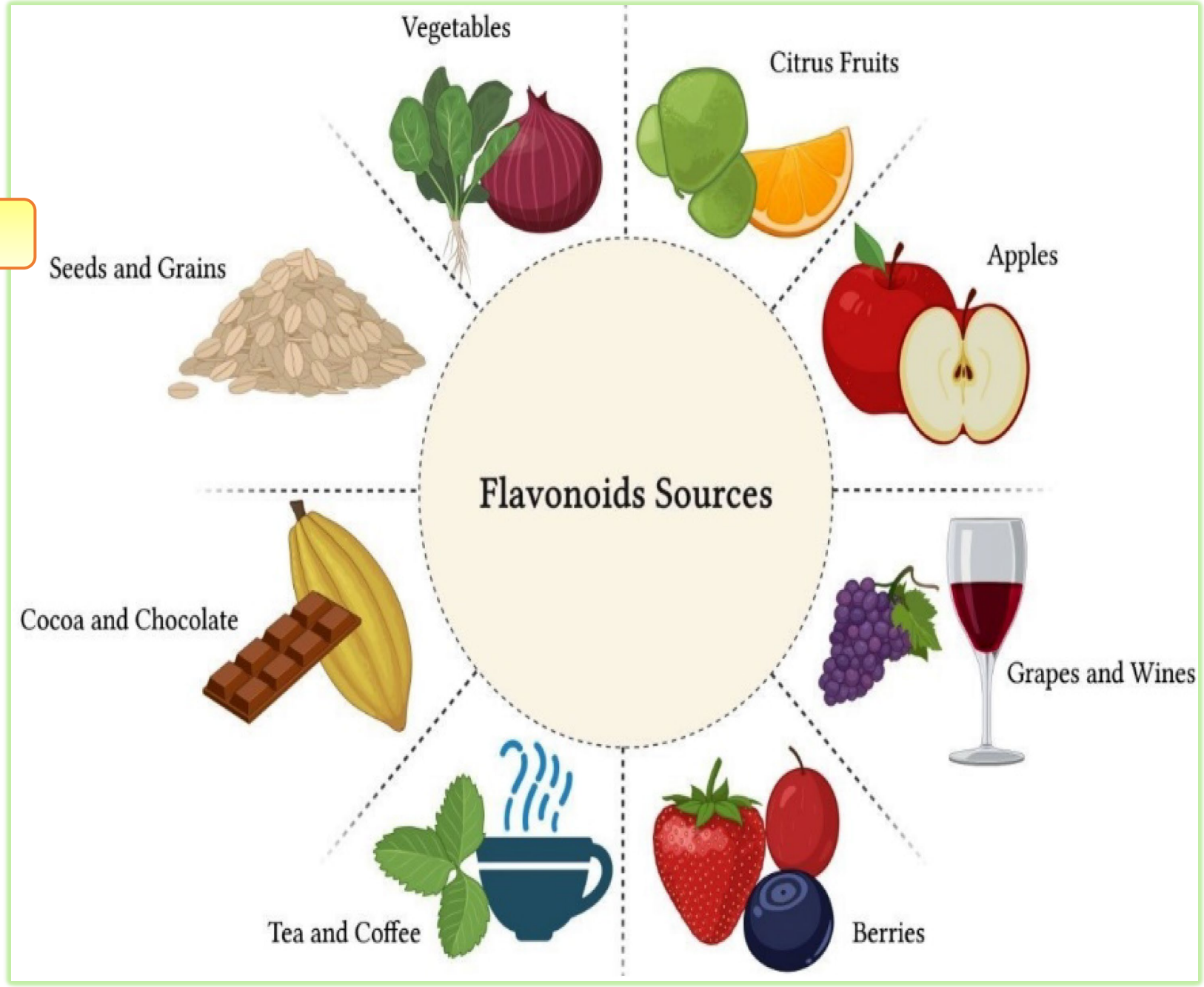
Natural Bioactive Compound

Secondary Metabolite

Consists of **benzopyrane ring** bearing **Phenolic or Polyphenolic groups**

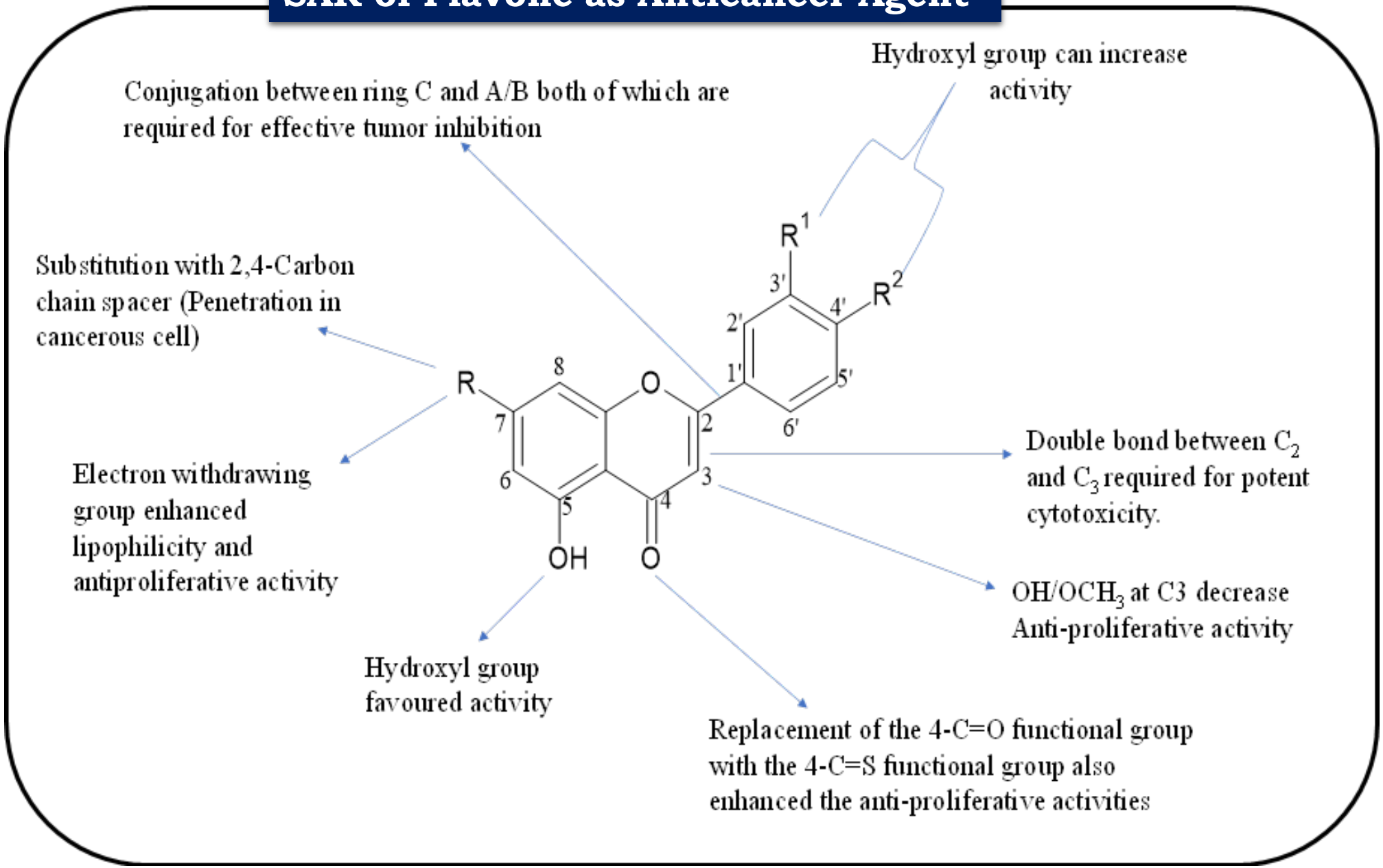


Benzopyran



RATIONAL & DESIGN

SAR of Flavone as Anticancer Agent



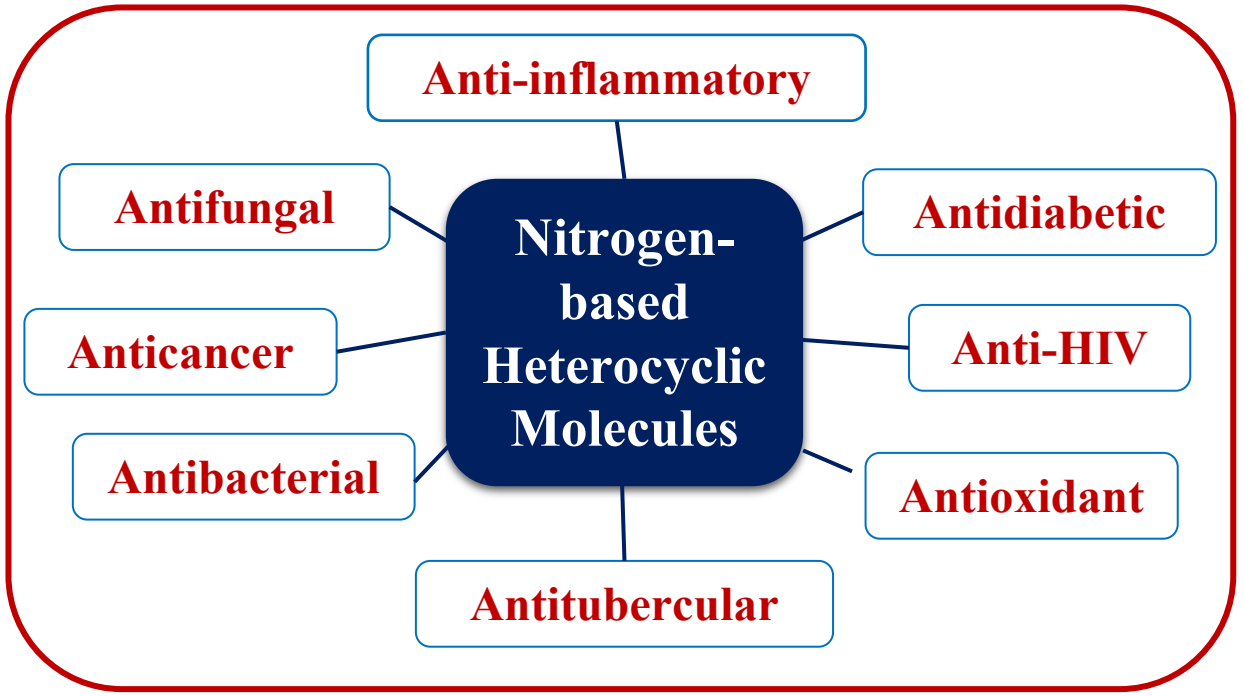
SAR analysis of VEGFR-2 inhibitors

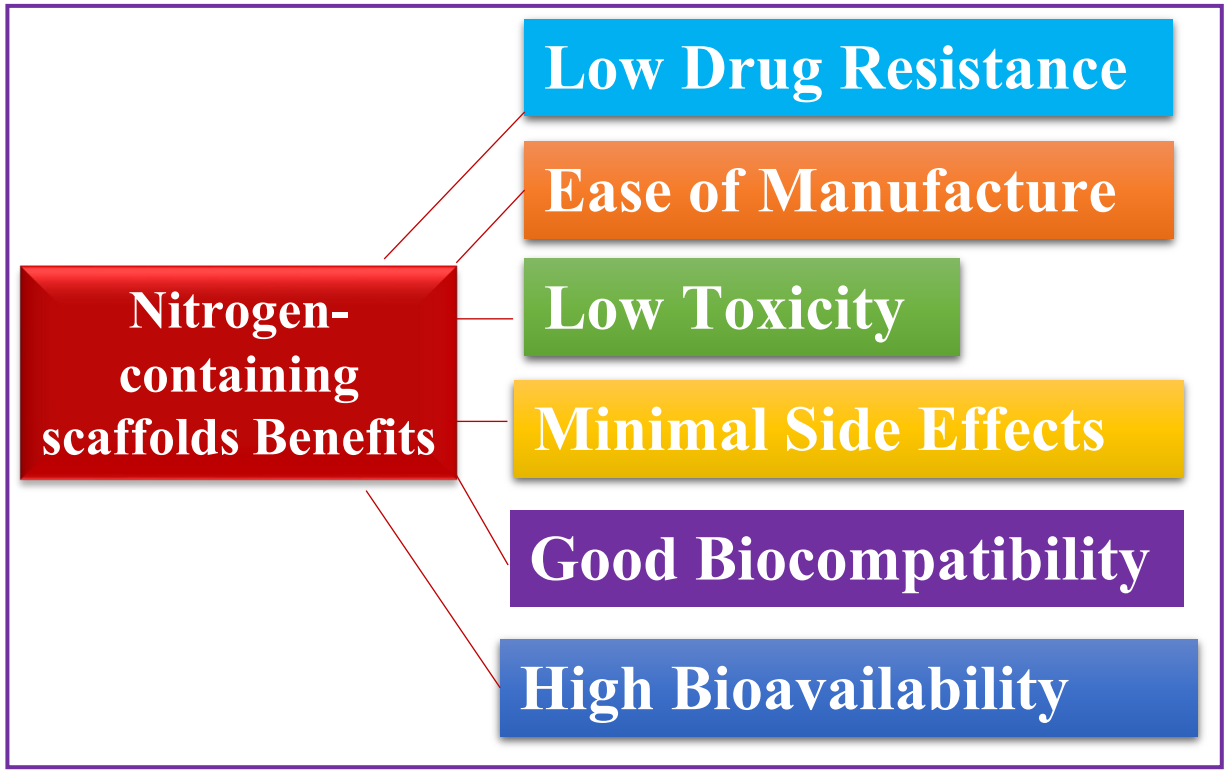
VEGFR-2 Inhibitors

FOUR basic characteristics - common with their core structures

- **Hetero aromatic ring, or head, occupies the catalytic ATP binding domain and engages in hydrogen bonding interactions with the hinge region's Cys919 amino acid residue.** — **Head Hing Bonding**
- **A segment known as a "linker" sits on the gatekeeper residues and joins the ATP-binding and DFG domains.** — **2-5 Carbon Linker**
- **Amino acids (Glu885 and Asp1046) engage with a hydrogen-bonding moiety (pharmacophore) through H-bonds in the protein's DFG motif.** — **Hydrogen Binding Moiety**
- **Terminal hydrophobic (tail) molecule binds to it via various hydrophobic interactions.** — **Tail Hydrophobic moiety**

Importance of N-Heterocycles





Several nitrogen-containing moieties (Ex. Benzotriazole, Pyrazole, Morpholine, Benzothiazole, Pyrimidine, Nicotinamide, Piperazine derivatives, etc) with different scaffolds have shown strong anti-cancer and anti-angiogenesis properties, as they target several receptors such as fibroblast and vascular endothelial growth factor (VEGF), tumor growth factor (TGF), and other kinases required for cancer growth and progression

AIM & OBJECTIVES

AIM:

Design and Synthesis of New, Potent, and Safe 4'-fluoro-5,7-dihydroxyflavone – Piperazine Hybrids as VEGFR-2 Inhibitor.

OBJECTIVES:

1. **To conduct Insilco Drug Design and docking study of 4'-fluoro-5,7-dihydroxyflavone – Piperazine Hybrids on relevant receptors.**
2. **To Synthesis of theses derivatives.**
3. **Characterization of synthesized Derivatives.**
4. **Pharmacological Evaluation of synthesized Derivatives as effective Anti-cancer agents.**

EXPERIMENTAL PROTOCOL

1. INSILICO STUDY

1. Molecular Docking

By utilizing Molsoft ICM pro X64 software

To determine docking score of designed derivatives with biological target VEGFR-2 using PDB ID: 4ASD

⬇

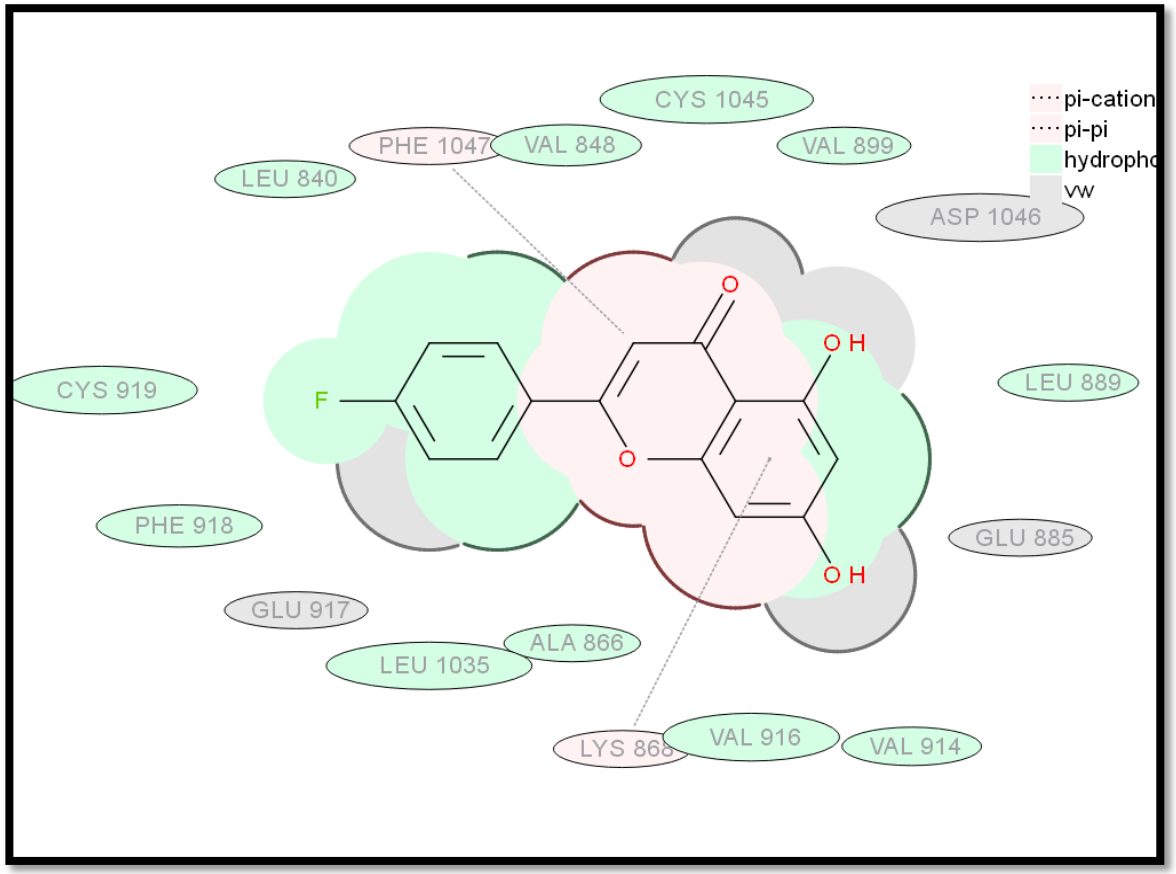
4ASD - CRYSTAL STRUCTURE OF VEGFR2 (JUXTA MEMBRANE AND KINASE DOMAINS) IN COMPLEX WITH SORAFENIB (BAY 43-9006)

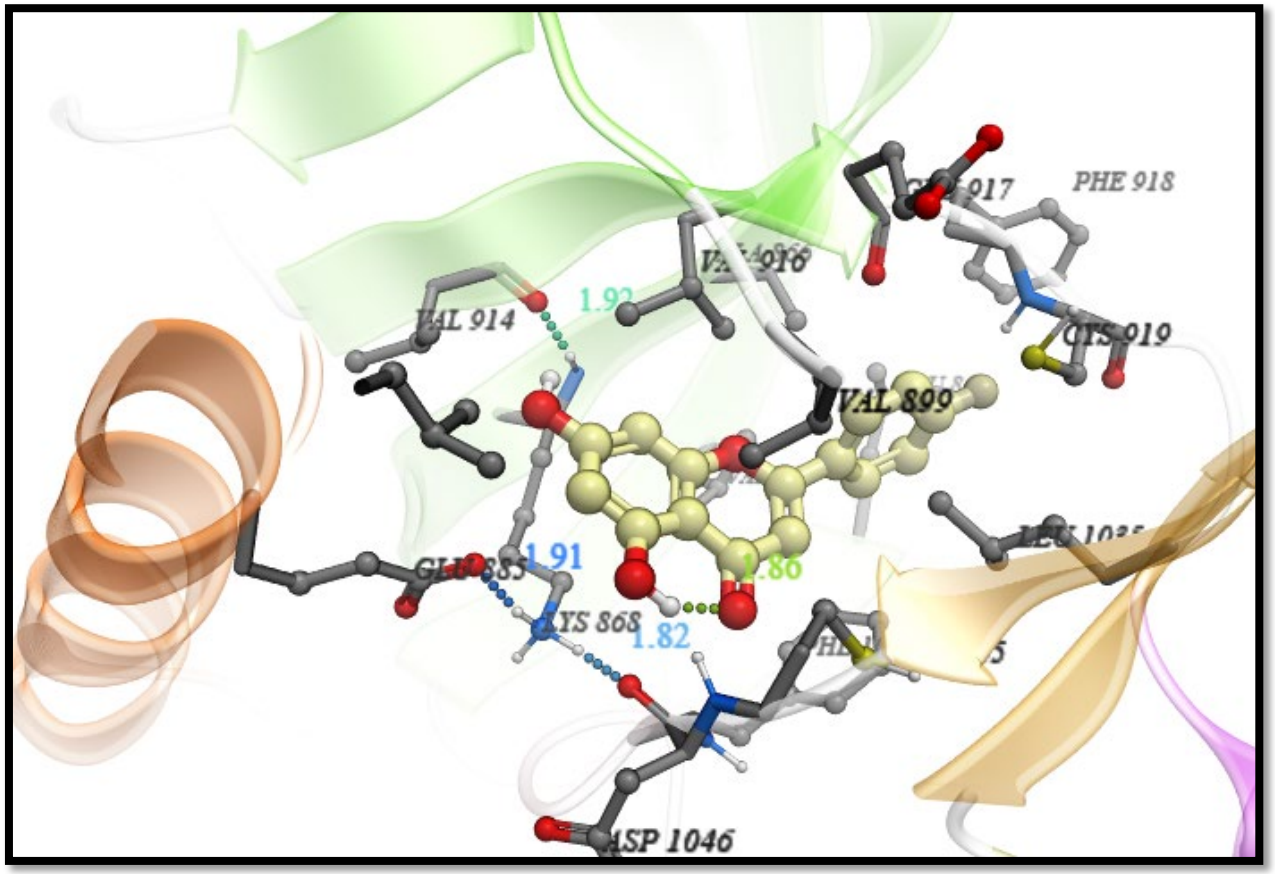
RMSD: 0.91

Compounds	Docking Score
S	-16.4
S1	-23.1
S2	-22.7
S3	-27.5
S4	-26.0
S5	-25.8
S6	-23.8
S7	-29.4
S8	-21.2
S9	-24.4
S10	-29.2

S

Docking Score: -16.4



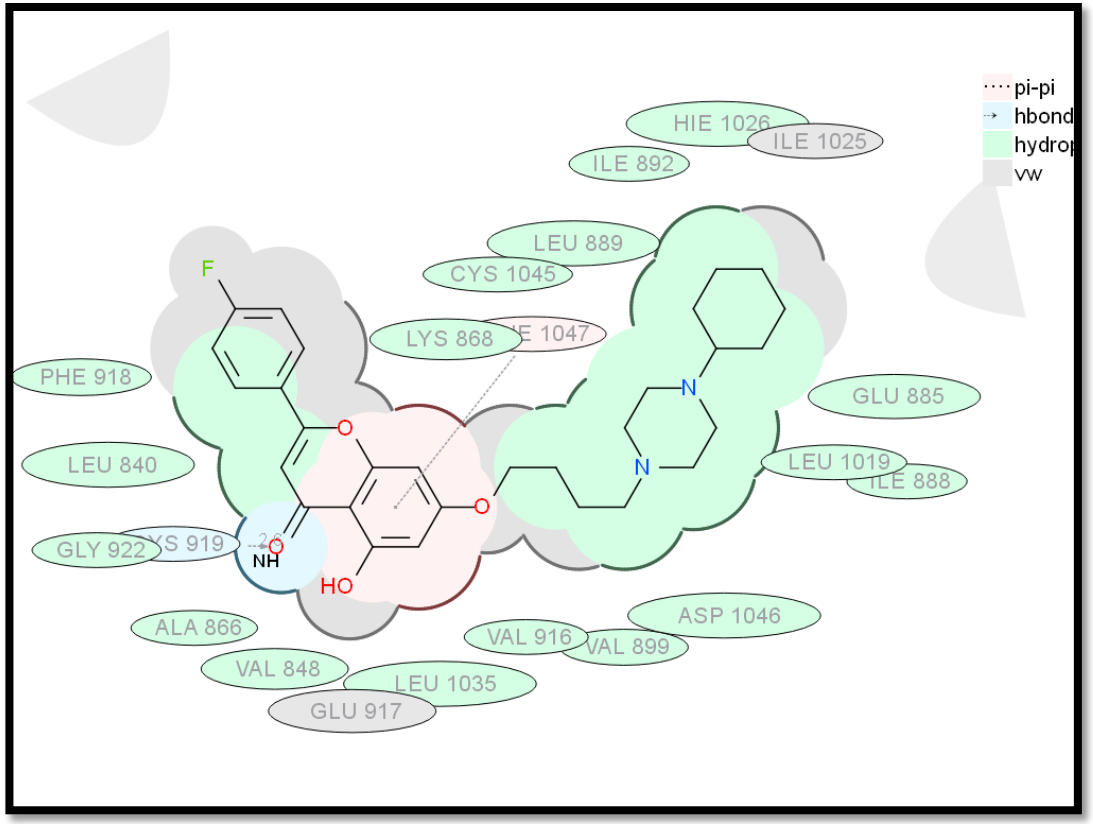


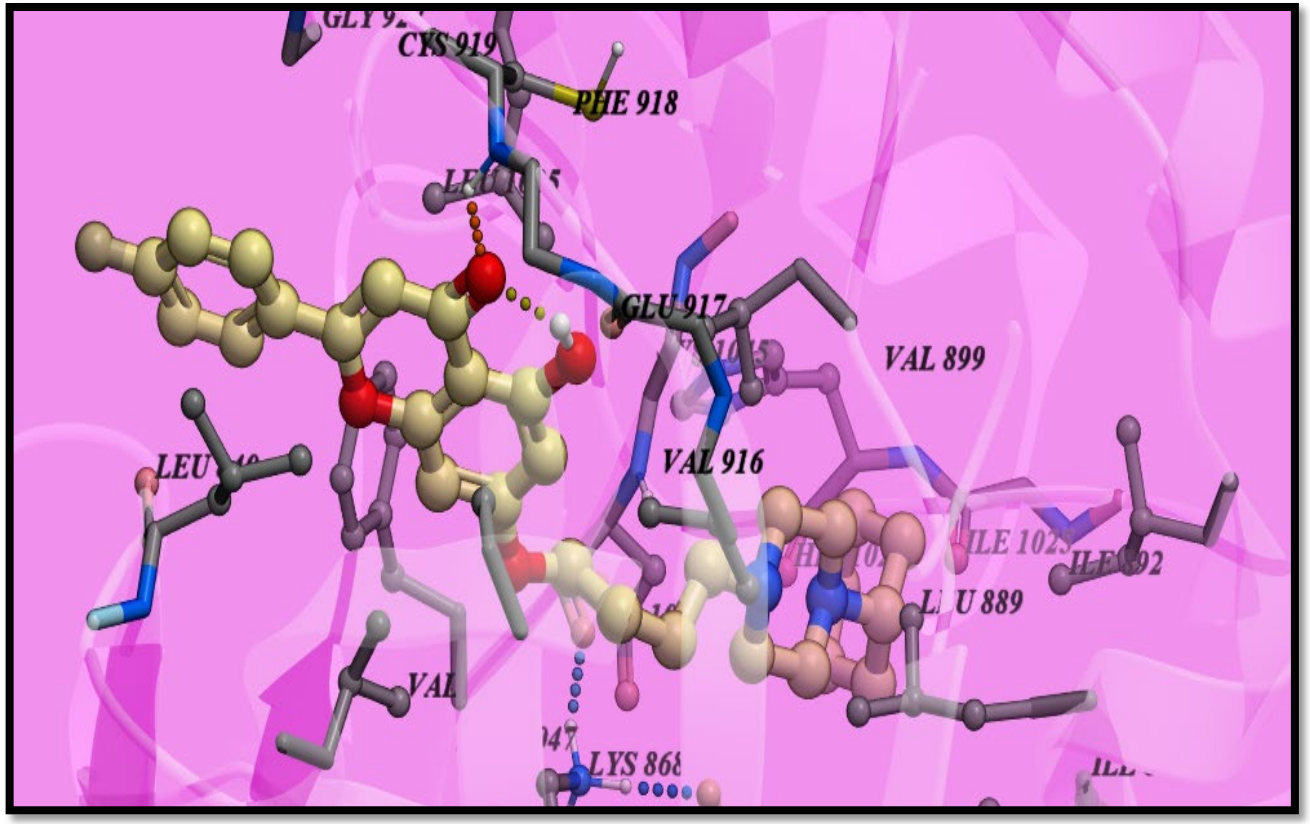
2D

3D

S5

Docking Score: -24.4



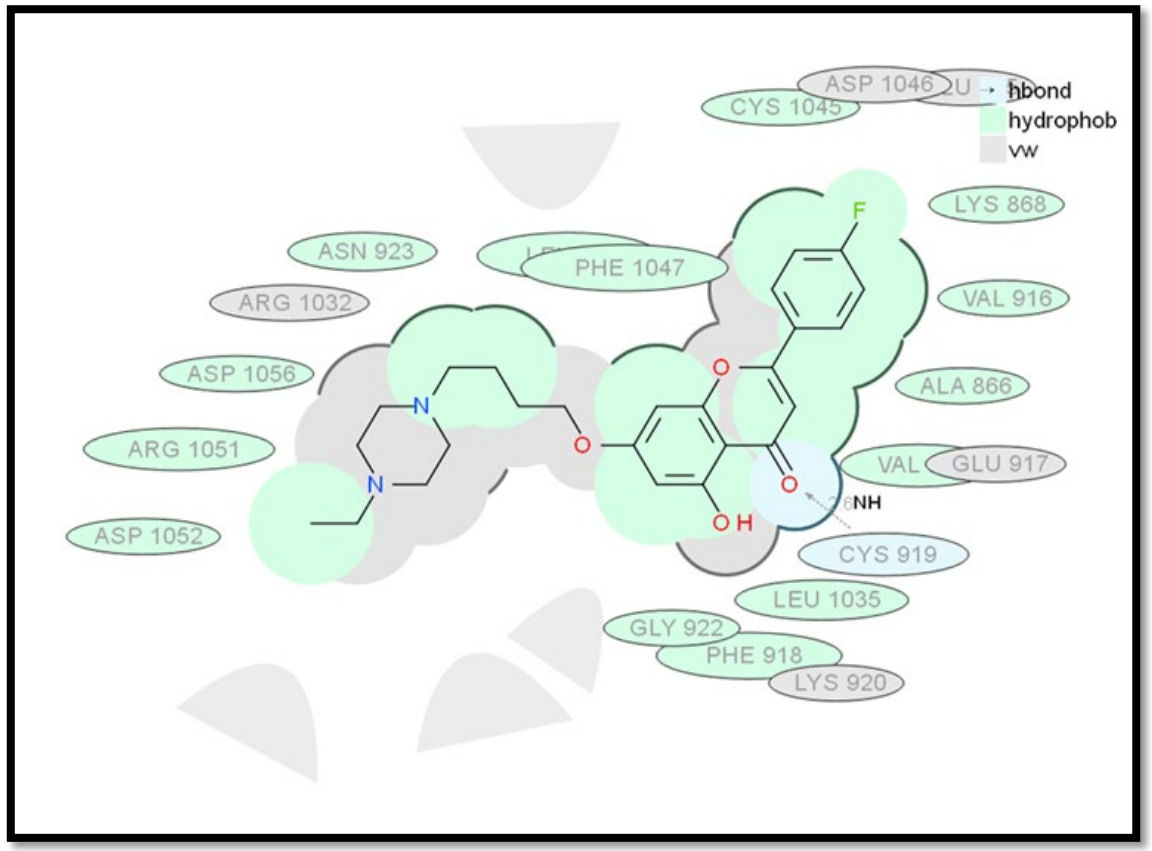


2D

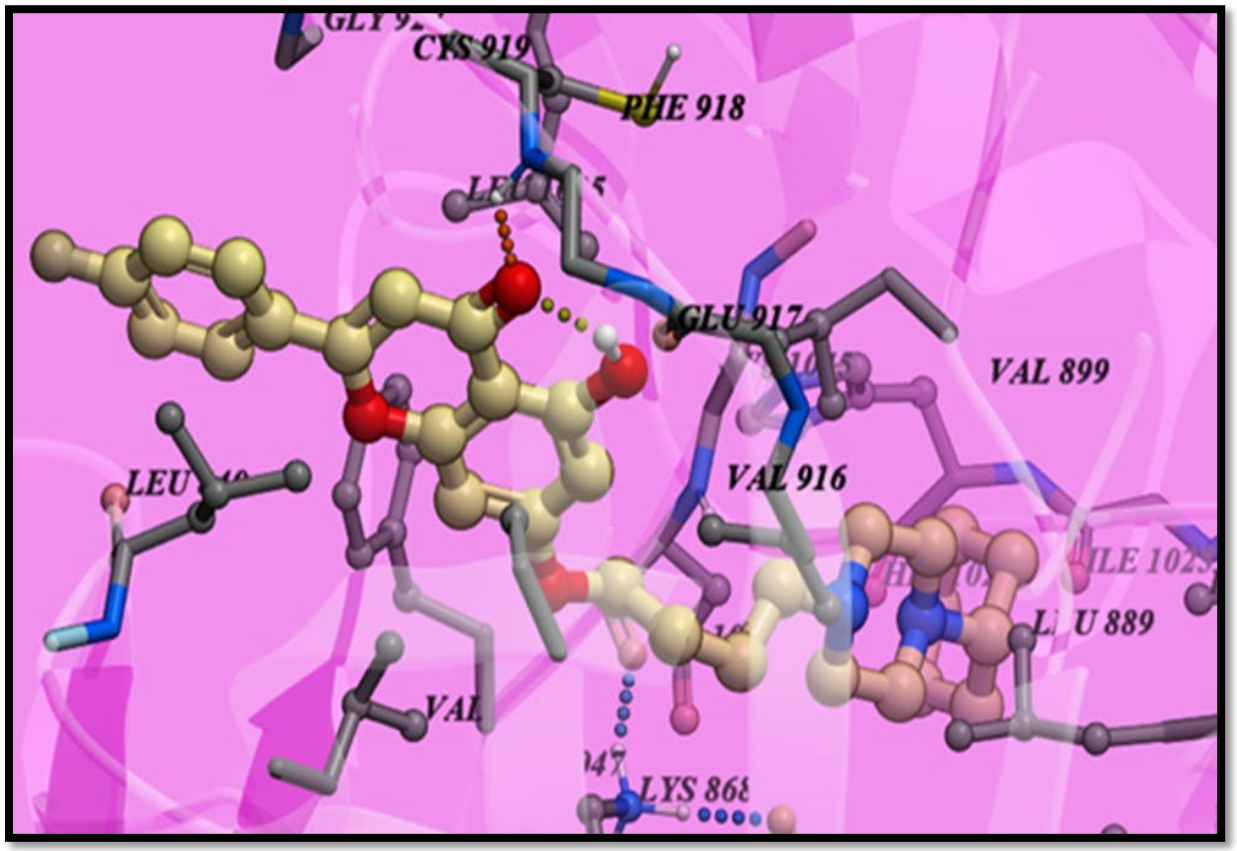
3D

S7

Docking Score: -29.2



2D



3D

2. ADME Analysis

The pharmacokinetic characteristics of the designed compounds were analyzed by using Molsoft ICM Pro X64.

Compounds	Lipinski Rule of 5				
	Log P	Mol. Wt. (g/mol)	HBD	HBA	Lipinski's Rule Violation
S	2.38	272.23	2	5	0
S1	4.13	410.38	2	6	0
S2	2.83	485.38	2	7	0
S3	3.86	410.48	1	6	0
S4	4.52	440.51	1	7	0
S5	5.05	454.53	1	7	0
S6	1.05	616.36	1	7	1
S7	4.6	489.62	1	7	0
S8	4.47	456.51	2	8	0
S9	4.51	455.52	2	8	0
S10	4.89	488.55	2	7	0

All designed derivatives follows Lipinski Rule of Five except S6

3. Toxicity Analysis

Toxicity profile of the designed compounds was estimated using Protox II software.

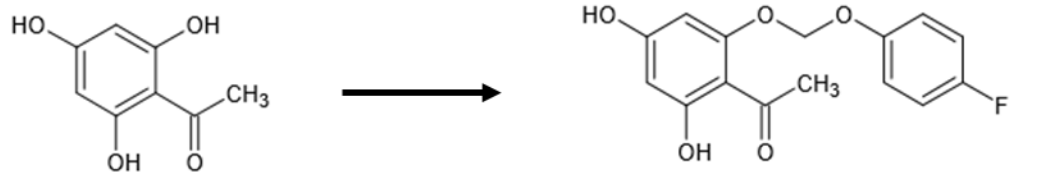
Compounds	Toxicity Class
S	CLASS IV
S1	CLASS IV
S2	CLASS IV
S3	CLASS IV
S4	CLASS IV
S5	CLASS IV
S6	CLASS III
S7	CLASS IV
S8	CLASS IV
S9	CLASS IV
S10	CLASS IV

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

2. SYNTHESIS

Scheme

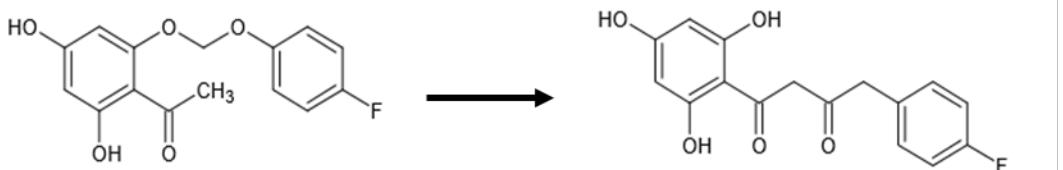
Step 1



1-(2,4,6-trihydroxy phenyl) ethanone

1-{2-[(4-fluorophenoxy)methoxy] -4,6-dihydroxyphenyl} ethanone

Step 2



1-{2-[(4-fluorophenoxy) methoxy] dihydroxyphenyl} ethanone

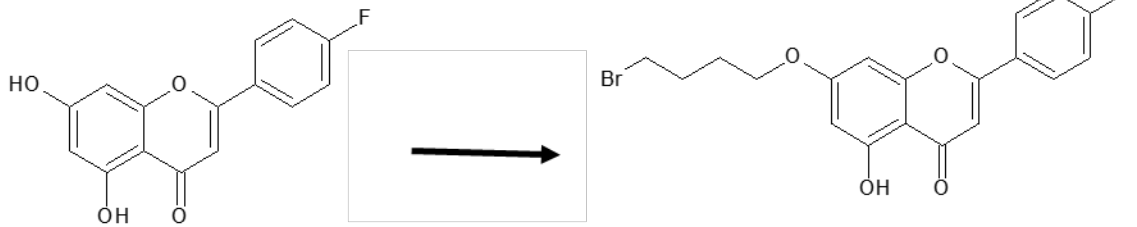
4-(4-flurophenyl)-1-(2,4,6- trihydroxy phenyl) butane-1,3 dione

Step 3

4-(4-fluorophenyl)-1-(2,4,6- trihydroxy phenyl) butane-1,3 dione

2-(4-fluorophenyl)-5,7-dihydroxy -4*H*-chromen-4-one

Step 4



2-(4-fluorophenyl)-5,7-dihydroxy -4*H*-chromen-4-one

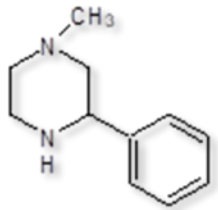
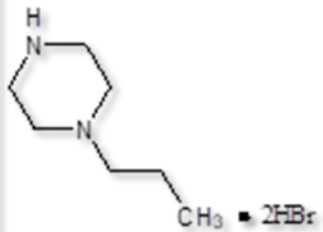
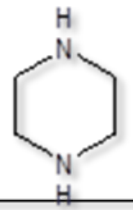
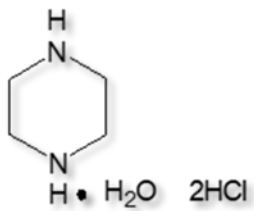
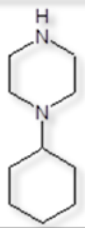
7-(4-bromobutoxy)-2-(4-fluorophenyl)- 5-hydroxy-4*H*-chromen-4-one

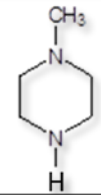
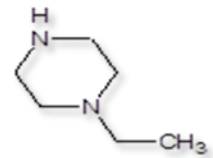
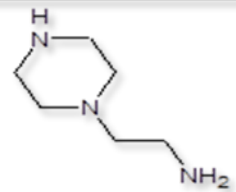
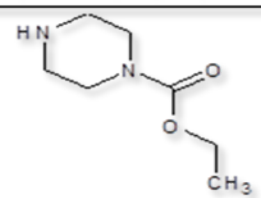
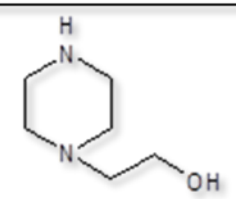
Step 5

7-(4-bromobutoxy)-2-(4-fluorophenyl)- 5-hydroxy-4*H*-chromen-4-one

Substituted piperazine of fluoro analog of Apigenin

Where R- Piperazine Derivatives

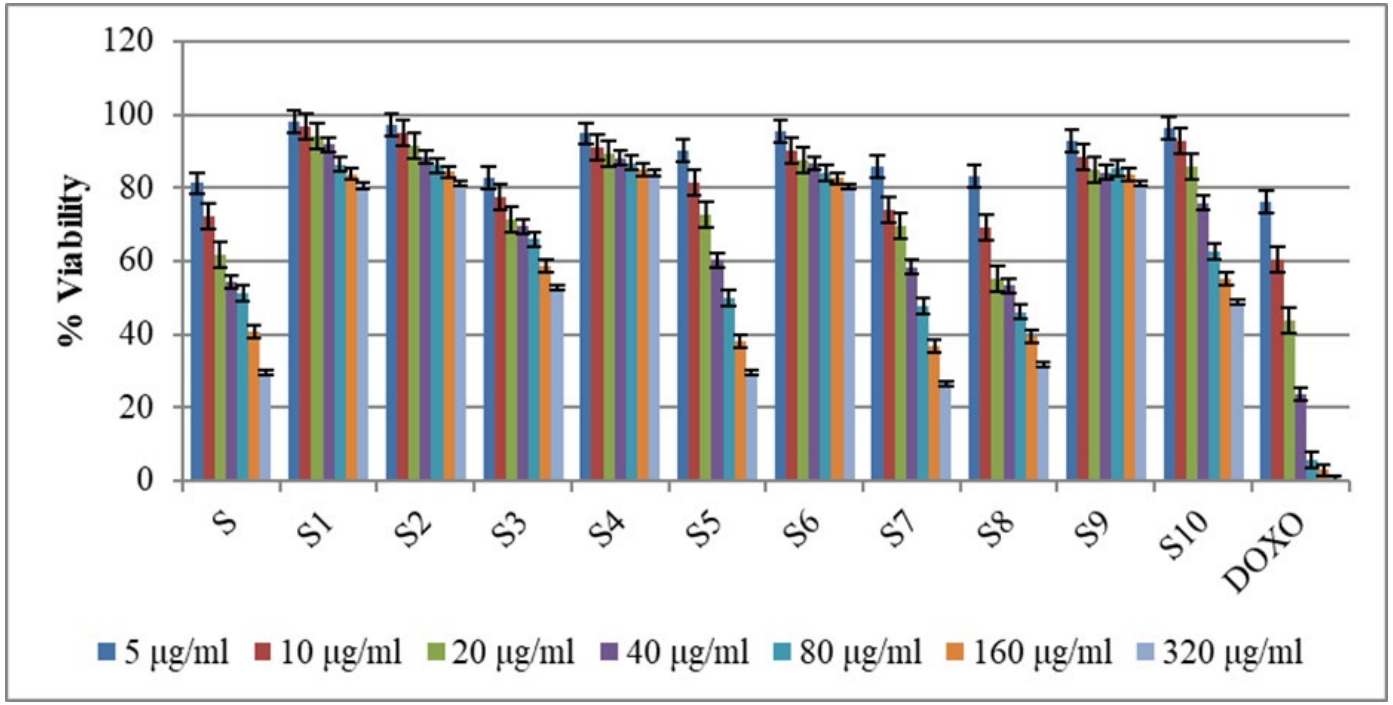
Code	R group	Rf value
S1		0.91
S2		0.68
S3		0.71
S4		0.60
S5		0.72

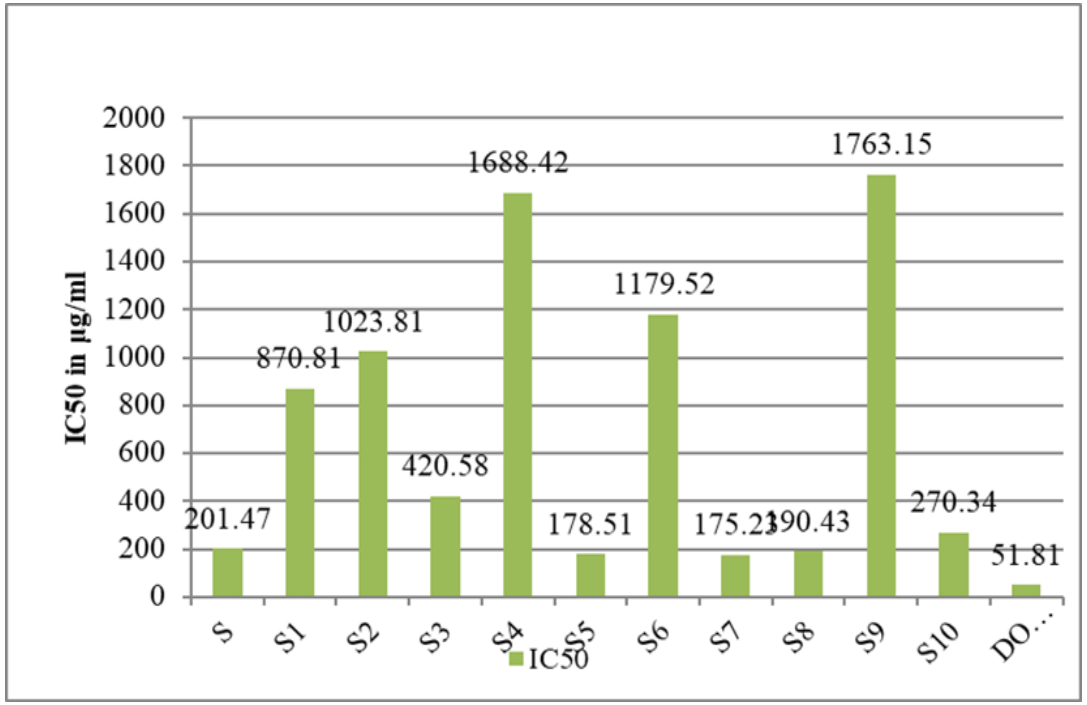
S6		0.61
S7		0.65
S8		0.75
S9		0.80
S10		0.75

3. PHARMACOLOGICAL SCREENING

In Vitro Anti-Proliferative Activities

Performed by using MCF-7 Cell line followed by MTT assay





Potency: S7 > S5 > S8 > S > S10

CONCLUSION

- **Substituted Piperazine Derivatives of 4'-fluro-5,7-dihydroxyflavone was synthesized as a VEGFR-2 inhibitor to improve Therapeutic Activity.**
- **These findings have encouraged us to continue the synthesis and testing of Substituted Piperazine Derivatives of 4'-fluro-5,7-dihydroxyflavone as VEGFR-2 inhibitor**

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THANK YOU