In-Silico Studies, Synthesis, Characterization, Biological Evaluation Of Few Novel Schiff Bases Of Coumarin Derivatives.

MD Ameer[1], Rakesh Panda[2], Dr. Kalpana Devi[3], Dr Md Salahuddin[4], Ayesha Nida[5].

[1]PG Student, Department of Pharmaceutical Chemistry, Al-Ameen College of Pharmacy, Bengaluru Karnataka-560027.

ABSTRACT

A series of coumarin derivatives were synthesized and characterized using IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. The synthesis involved three steps: condensation of resorcinol with ethyl acetoacetate to yield 7-hydroxy-3-methyl coumarin, confirmed by an IR band at 3489.41 cm^{-1}; Pechmann condensation with substituted benzaldehydes forming coumarin derivatives, indicated by IR peaks at 1642 cm^{-1} (C=C), 1207 cm^{-1} (C–O), and 1736 cm^{-1} (C=O); and Schiff base formation with primary amines producing MARP-1 to MARP-10, confirmed by C=N stretching at 1600–1700 cm^{-1}.

In-silico studies showed that MARP-3 (Cl), MARP-9 (NO_2), and MARP-10 (2,2-diphenyl) had strong binding affinities toward PDB ID: 8THH, correlating with notable anticonvulsant, antioxidant, and anti-inflammatory activities. MARP-3 and MARP-10 exhibited the best anticonvulsant effects, while MARP-8 showed 90% antioxidant inhibition in the DPPH assay. MARP-2 and MARP-8 displayed strong anti-inflammatory activity, preventing 89.15% and 85.41% of BSA protein denaturation, respectively.

Antimicrobial studies revealed MARP-4, MARP-8, and MARP-10 were active against Staphylococcus aureus and Escherichia coli, with MARP-10 showing the largest inhibition zone. ProTox-II predictions classified most compounds as Class IV, suggesting good safety profiles with no major toxic effects. All compounds followed Lipinski's rule of five, and SwissADME results confirmed favorable absorption and pharmacokinetic profiles.

Overall, the synthesized coumarin derivatives demonstrate significant therapeutic potential supported by both experimental and computational studies.

1. Introduction

Coumarin is a naturally occurring oxygen-containing heterocyclic compound composed of benzene and α-pyrone rings. According to IUPAC nomenclature, it is named 2H-1-benzopyran-2-one and belongs to the lactone class. Coumarins are classified into pyranocoumarins, furanocoumarins, and pyrone-substituted derivatives.

Coumarins, also known as benzopyran-2-ones or chromen-2-ones, are widely distributed in plants, fungi, and bacteria and can be easily modified to yield various biologically active compounds. N-substituted 4-aminocoumarins and their derivatives have gained significant interest due to their promising biological properties. The coumarin framework remains important for its occurrence in natural products, pharmacological activities, and photophysical characteristics.

Numerous natural and synthetic coumarins exhibit antimicrobial, antifungal, cytotoxic, antitumor, anticoagulant, and anti-inflammatory effects. Some natural derivatives, such as novobiocin, warfarin, acenocoumarin, and umbelliferone, are already used clinically. 3-Aminocoumarin derivatives also show diverse actions like CNS depressant, antibacterial, and antiallergic effects. Because of their nucleophilic

and electrophilic behavior, coumarins participate in various reactions, making them versatile building blocks in organic and medicinal chemistry.

Schiff Base Synthesis

Schiff bases are compounds containing the imine (–C=N–) group, first reported by Hugo Schiff in 1864. They are synthesized by the condensation of primary amines or hydrazides with carbonyl compounds, releasing water. Structurally, Schiff bases are nitrogen analogs of carbonyl compounds where C=O is replaced by C=N.

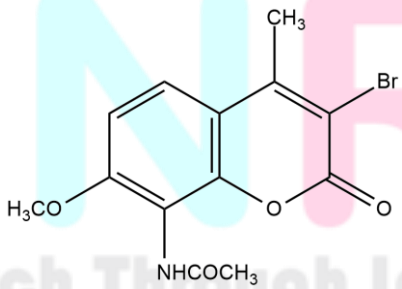
They serve as important intermediates in organic synthesis and exhibit broad biological activities, including anti-inflammatory, antimicrobial, anticancer, and antioxidant properties, making them valuable precursors for pharmacologically active molecules.

2. OBJECTIVES

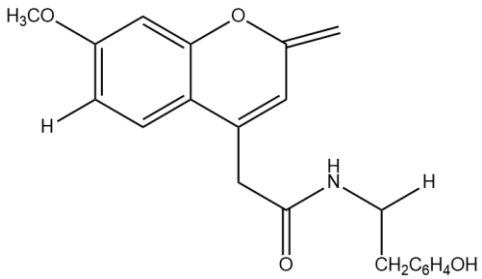
1) Synthesis of some novel coumarin derivatives.
2) Characterization of newly synthesized derivatives using spectral studies.
3) Drug likeliness property of the derivatives by using Swiss ADME and Molinspiration web servers.
4) Online toxicity prediction.
5) Molecular Docking studies by using Autodock software
6) *In vitro* evaluation of the derivatives for their anti-inflammatory and antimicrobial, antioxidant.
7) *In vivo* evaluation of the derivatives for their anti convulsant activity.

Review of Literature

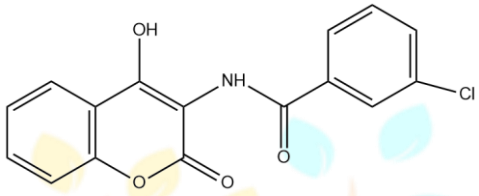
Z M Nofal and co-workers synthesized a number of novel 3-bromo-4-methyl-7-methoxy-8-amino substituted coumarins and 2-substituted 7-bromo-6-methyl-8H-pyrano-benzimidazoles, benzoxazoles and/or benzoxazine-8-ones for the purpose of pharmacological evaluation. The representative compounds showed antitumor activity *in-vitro* on Ehrlich ascites carcinoma in the preliminary testing.



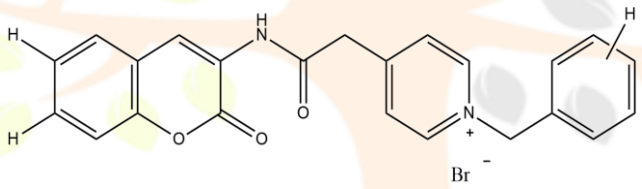
Chandarajoti K and coworkers designed new coumarin derivatives using a 2-(2-oxo-2H-chromen-4-yl)acetic acid scafold conjugated with amino acid esters or tyramine. The anti-tyrosinase and anti-lipid peroxidation activities of the synthesized compounds were investigated. Coumarin derivatives showed strong anti-lipid peroxidation activity. The structures of the synthesized compounds were confrmed by infrared spectroscopy, ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. Few of reported compounds offer potential for development as skin-lightening agents and vitiligo therapy agents, respectively.



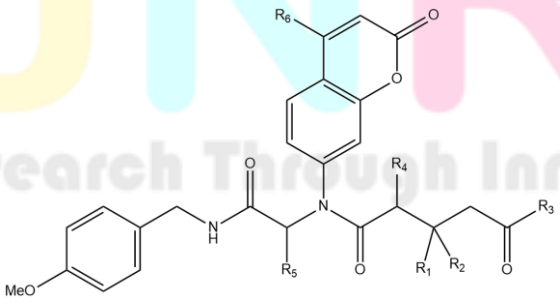
Zhou R and co-workers synthesized new coumarin derivatives and evaluated their biological activities. Their structures were confrmed by NMR data. Ten of the synthesized compounds were investigated for antimetastatic activity against lung carcinoma cells.



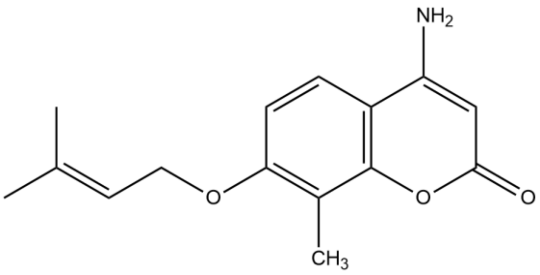
Kowalczyk P and coworkers synthesized a library of novel peptidomimetics via Ugi multicomponent reaction. Most of these peptidomimetics contain differently substituted aminocoumarin 7-amino-4-methylcoumarin and 7-amino-4-(trifluoromethyl) coumarin. ^{1}H- and ^{13}C NMR spectra were recorded in Chloroform-d at Bruker 400 and Varian 500 MHz sepctrometer using TMS. Preliminary cellular studies suggested that the compounds with coumarin derivatives had more potential as antimicrobial agents compared to the compounds without coumarin.



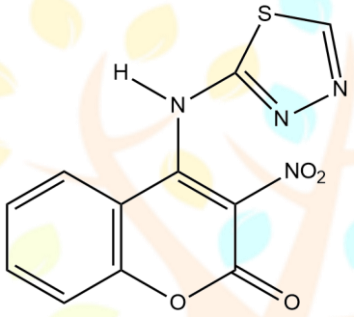
Khunnawutmanotham N and co-workers synthesized a series of 3-amino-6,7-dimethoxycoumarins conjugated with the N-benzylpyridinium moiety through an amide-bond linkage and evaluated for their acetylcholinesterase inhibitory activity. Docking studies revealed that the synthesized compounds inhibit the target enzyme by a dual binding site mechanism.



Xu L and co workers designed and synthesized 4-amino coumarin-based derivatives. The structures had been confirmed by MS, ^{1}H NMR and ^{13}C NMR. Most of the compounds expressed broad-spectrum antifungal activity against four plant pathogenic fungi using the mycelium growth inhibition method. The present work indicated that these derivatives would serve as novel potential fungicides targeting SDH



Novica Ristic and coworkers reported the synthesis, spectral characterization, and evaluation of *in vitro* antioxidant activity of a new coumarin derivative, 4- ((1,3,4-thiadiazol-2-yl)amino)-3-nitro-2H-chromene-2-one. The synthesis of the new product was performed in three reaction steps, with a good overall yield (78%). The structure was corroborated by detailed spectral analysis, including the 1D and 2D NMR experiments. *In vitro* antioxidant activity was evaluated using the DPPH test. The synthesized compound possessed a good free-radical scavenging activity and could serve as a model for the synthesis with promising antioxidant effects.



4.METHODOY

SYNTHETIC SCHEME:

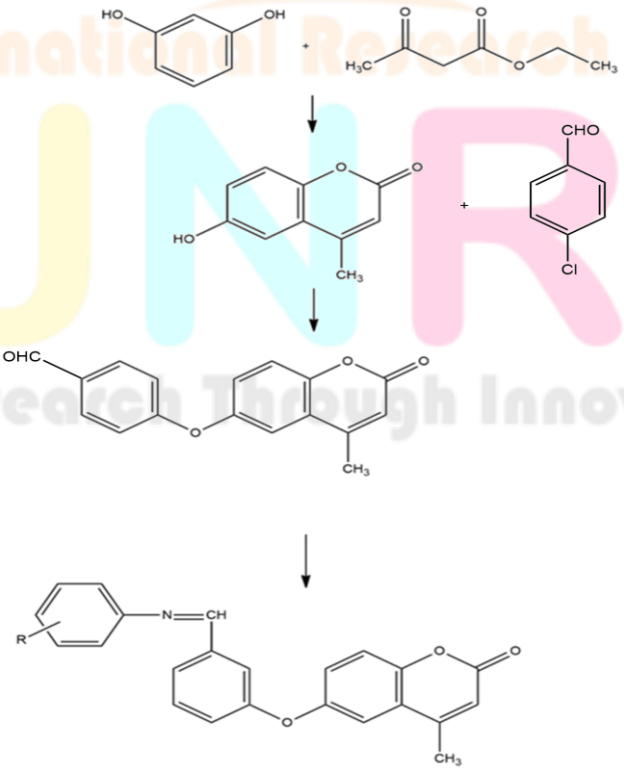
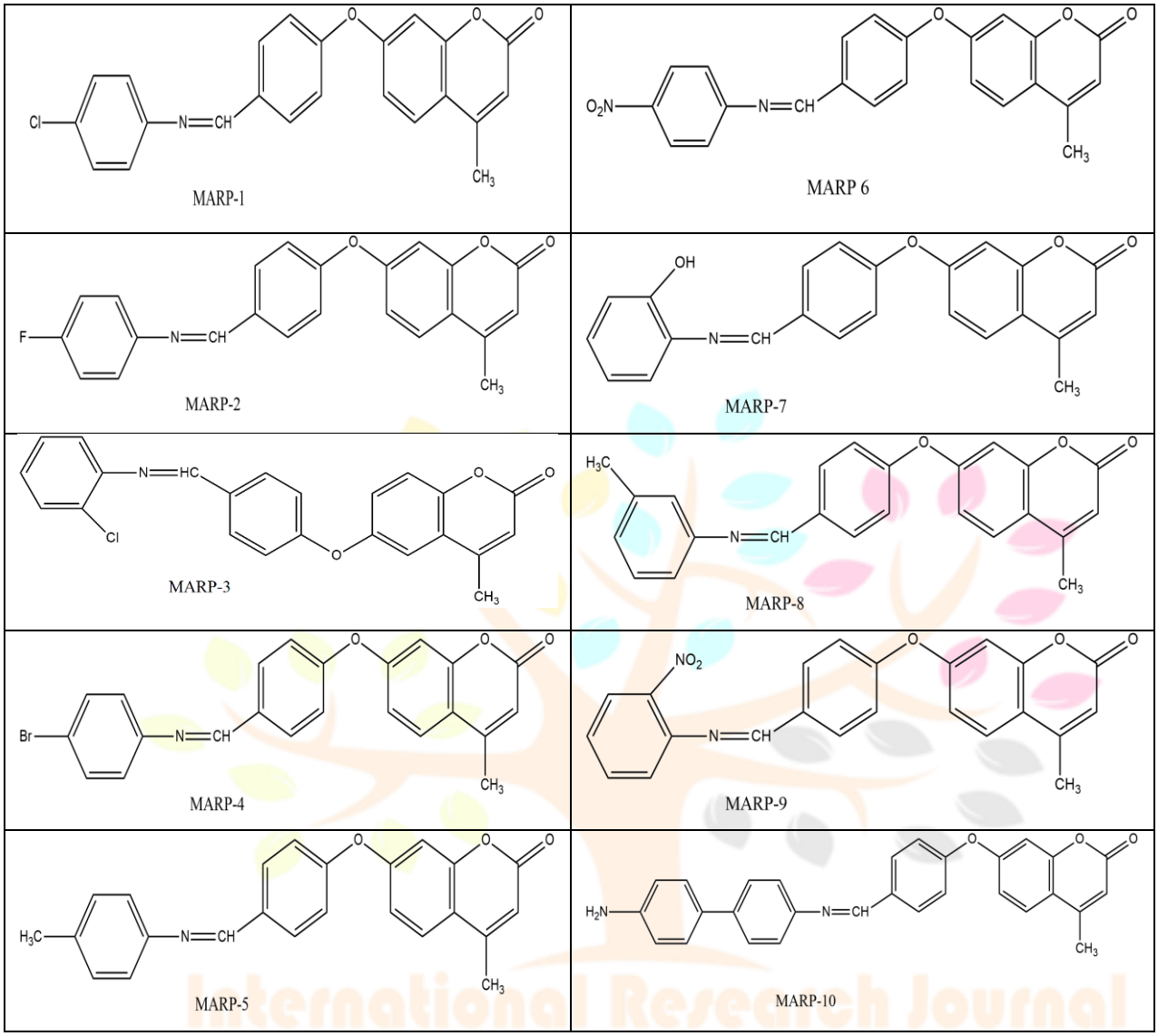


Table 1: List of the 4-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)benzaldehyde (MARP 1-10)

(MARP 1-10 derivatives).



Procedure for the synthesis of novel coumarin :

STEP-1: 1.11gm of resorcinol (0.01mol) was added to 1.27ml (0.01mol) of distilled ethyl-acetoacetate and kept in an ice bath. 10ml of H_2SO_4 was added drop wise to the mixture and stirred continuously in a magnetic stirrer. The completion of the reaction was confirmed by TLC using n-hexane and ethyl acetate (3:2). The reaction mixture was poured slowly into ice-cold water with stirring .The precipitate obtained was filtered washed with cold water and recrystallized with 65% ethanol.

STEP-2: To a solution of compound (2) (2.34g, 0.01 mol) in (30 ml) Ethanol, appropriate aromatic aldehyde (4-Bromo benzaldehyde 2- Bromo benzaldehyde , 2- Chlorobenzaldehyde and 4-Chloro benzaldehyde) (0.02 mol)was added in the presence of (4-5) drops of Piperidine with continuous stirring for 30 min .the mixture was refluxed for 8 hours ,then the reaction mixture was cooled , The product was filtered and recrystalized from Ethanol solvent.

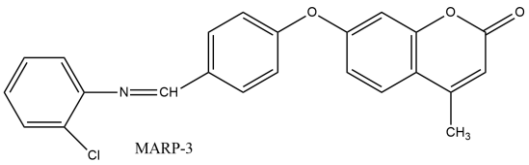
STEP-3: To a solution of (0.01 mole) compound (2) in (25 ml) absolute ethanol, an appropriate aromatic amine, (0.01 mole) of aniline and (0.005 mole) of 4-amino benzoic acid and 4-nitro aniline), was added

with continuous stirring, and then was refluxed for a period of 30 min, the precipitate formed was filtered, dried and recrystallized from ethanol.

MARP-3: 6(4(((2chlorophenyl)imino)methyl)phenoxy)-4-methyl-2H-chromen-2-one

Compound code: MARP-3

Structure:



MARP-3

IUPAC Name: 6-(4-(((2-chlorophenyl)imino)methyl)phenoxy)-4-methyl-2H-chromen-2-one

Physical Properties-

Mol formula & weight: $C_{23}H_{16}ClNO_3$ and 389.83,

Colour: Off Pink,

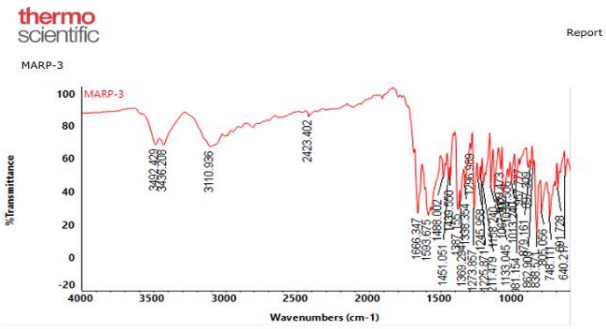
Melting Rang: 111-115^{0}C,

Rf Value: 0.80,

Solubility: Methanol, DMSO,

%Yield : 56%

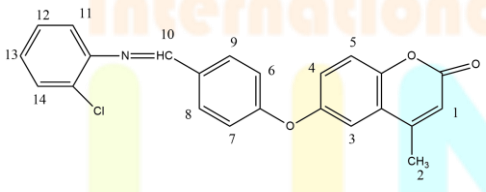
MARP-3

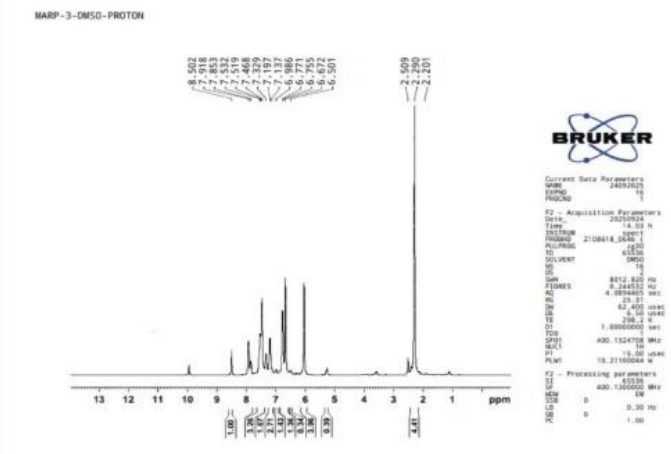


IR cm^{-1}:

3110.9(CH3 str), 1666.6(C=N str), 1488(Ar C=C), 1593.6(C=O), 1273(C-O), 748(C-Cl).

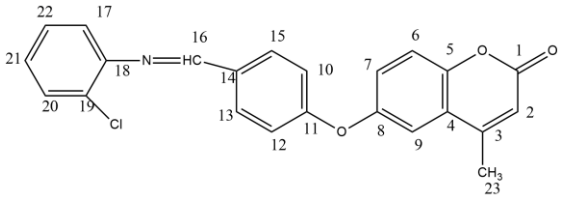
^{1}HNMR:

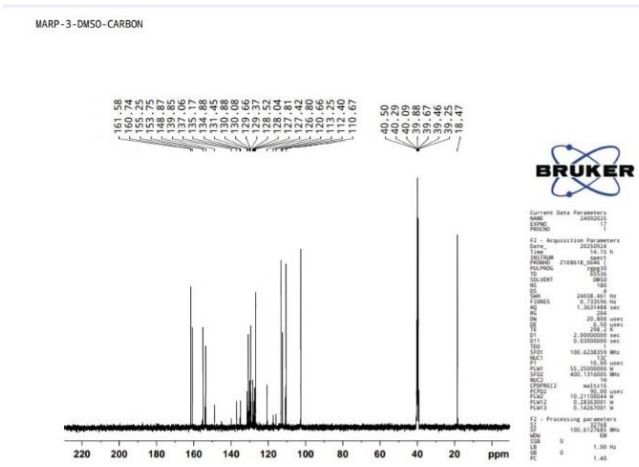




δ: H(10- 8.502), H(2- 2.509), H(1- 6.501), H(14- 7.532), H(13- 7.519), H(12 4- 7.468)

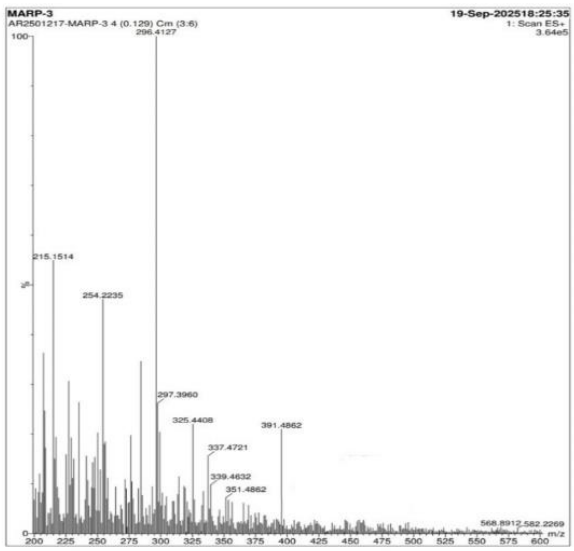
^{13}CNMR:





δ: (C1=161.58), (C23=18.47), (C16=160.74), (C12 C10 =129.37), (C3=148.87)

MASS:

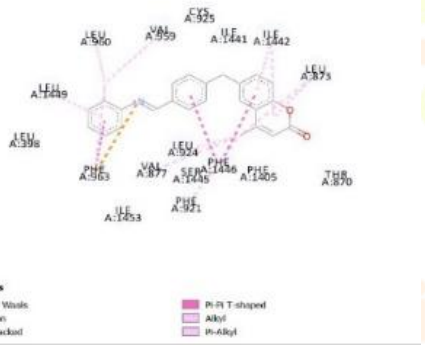


(m/z)=391.48[M+]

DOCKING STUDIES

: Binding energy of synthesized compounds with 8THH (Anti-Convulsant activity):

Ligand	Binding Affinity
MARP-1	-10.2
MARP-2	**-10.9**
MARP-3	**-11.5**
MARP-4	-
MARP-5	-10.4
MARP-6	-10.1
MARP-7	-10.2
MARP-8	-10.3
MARP-9	-10.7
MARP-10	**-11.9**
PHENYTOIN (Standard)	-8.6

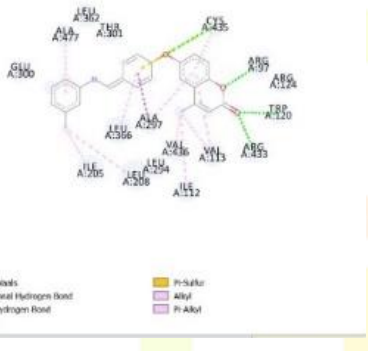


MARP-3

6-(4-(((2-chlorophenyl)imino)methyl)phenoxy)-4-methyl-2H-chromen-2-one

Binding energy of synthesized compounds with 1OG5 (Antioxidant activity):

Ligand	Binding Affinity
MARP-1	**-8.2**
MARP-2	**-8**
MARP-3	-7.6
MARP-4	-4.9
MARP-5	-7.2
MARP-6	-7.5
MARP-7	-7.6
MARP-8	**-8.5**
MARP-9	-7.7
MARP-10	-7.7
Ascorbic acid	-5.4



MARP-8

6-(4-(((2-chlorophenyl)imino)methyl)phenoxy)-4-methyl-2H-chromen-2-one

Binding energy of synthesized compounds with 4CYG (Anti-Inflammatory activity):

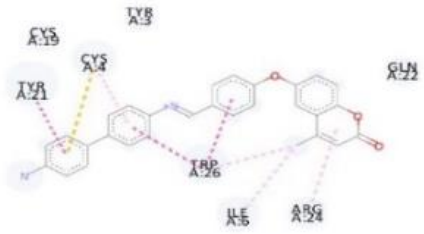
Ligand	Binding Affinity
MARP-1	**-11**
MARP-2	**-10.7**
MARP-3	**-10.9**
MARP-4	**–**
MARP-5	**-10.9**
MARP-6	**-10.8**
MARP-7	**-10.7**
MARP-8	**-11**
MARP-9	**-10.7**
MARP-10	**-10.7**
INDOMETHACIN	**-8.6**

MARP-1

7-(4-(((4-chlorophenyl)imino)methyl)phenoxy)-4-methyl-2H-chromen-2-one

Binding energy of synthesized compounds with 3GNY(Gram positive) 1ZMH(Gram negative) (Anti-Microbial activity):

Ligand	Binding Affinity	
	GRAM POSITVE	GRAM NEGATIVE
MARP-1	**-6.9**	-6.8
MARP-2	-6.8	**-6.9**
MARP-3	**-6.9**	**-6.9**
MARP-4	-	-
MARP-5	-6.6	**-6.9**
MARP-6	-6.6	-6.7
MARP-7	-6.5	-6.6
MARP-8	**-6.9**	**-6.9**
MARP-9	-6.8	**-6.9**
MARP-10	**-7.4**	**-7.1**
STANDARD DRUG	-4.2 (Penicillin)	-4.1 (Cephalosporin)



MARP-10

6-(4-(((4'-amino-[1,1'-biphenyl]-4-yl)imino)methyl)phenoxy)-4-methyl-2H-chromen-2-one

***In-silico* Toxicity studies of synthesized compounds**

Compound code	*Predicted LD50 (mg/kg)*	*Predicted Toxicity Class*	*Hepatotoxicity*	*Carcinogenicity*	*Immunotoxicity*	*Mutagenicity*	*Cytotoxicity*
MARP-1	2850	5	Active	Active	Inactive	Inactive	Inactive
MARP-2	2850	5	Active	Active	Active	Inactive	Inactive
MARP-3	2850	5	Active	Active	Inactive	Inactive	Inactive
MARP-4	1190	4	Active	Inactive	Active	Inactive	Inactive
MARP-5	2850	5	Inactive	Active	Inactive	Inactive	Inactive
MARP-6	2000	4	Active	Inactive	Active	Active	Inactive
MARP-7	1500	4	Active	Active	Inactive	Inactive	Inactive
MARP-8	2850	5	Inactive	Active	Inactive	Inactive	Inactive
MARP-9	2000	4	Active	Active	Active	Active	Inactive
MARP-10	2000	4	Active	Active	Inactive	Active	Inactive

***In-Silico* Physicochemical studies obtained from Molinspiration**

Compound Code	*miLogP*	*TPSA*	*natoms*	*MW (g/mol)*	*nON*	*nOHNH*	*n violation*	*n rotb*	*Volume*
MARP-1	6.33	51.81	28	389.84	4	0	1	4	333.75
MARP-2	5.82	51.81	28	373.38	4	0	1	4	325.14
MARP-3	6.28	51.81	28	389.84	4	0	1	4	333.75
MARP-4	6.46	51.81	28	434.29	4	0	1	4	338.10
MARP-5	6.10	51.81	28	369.42	4	0	1	4	336.77
MARP-6	6.10	51.81	28	369.42	4	0	1	4	336.77
MARP-7	5.39	72.04	28	371.39	5	1	1	4	323.23
MARP-8	6.08	51.81	28	369.42	4	0	1	4	336.77
MARP-9	6.05	51.81	28	369.42	4	0	1	4	336.77
MARP-10	6.52	77.83	34	402.91	5	2	1	5	402.91

Table 35: *In-silico* ADME properties obtained from SwissADME

Compound code	**MARP-1**	**MARP-2**	**MARP-3**	**MARP-4**	**MARP-5**	**MARP-6**	**MARP-7**	**MARP-8**	**MARP-9**	**MARP-10**
Num. heavy atoms	28	28	28	28	28	30	28	28	30	34
Num. Arom. Heavy atoms	22	22	22	22	22	22	22	22	22	28
Num. Rotatable bonds	4	4	4	4	4	6	4	4	5	5
Num. H-bond acceptors	4	4	4	4	4	5	5	4	6	4
Num. of H-bond donors	0	0	0	0	0	0	1	0	0	1
Molar refractivity	112.67	112.67	112.67	112.67	112.63	116.48	109.68	112.67	116.48	137.50
Total Polar Surface Area(Å)	51.80	51.80	51.80	51.80	51.80	97.62	72.03	51.80	97.62	77.82
Log Po/w (ilogp)	4.04	4.04	4.14	4.04	3.99	3.58	3.39	3.86	3.50	4.11
Water solubility	Moderately soluble	Moderately soluble	Moderately soluble	Poorlysoluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble	Poorly soluble
GI absorption	High	High	high	High	High	High	High	High	High	Low

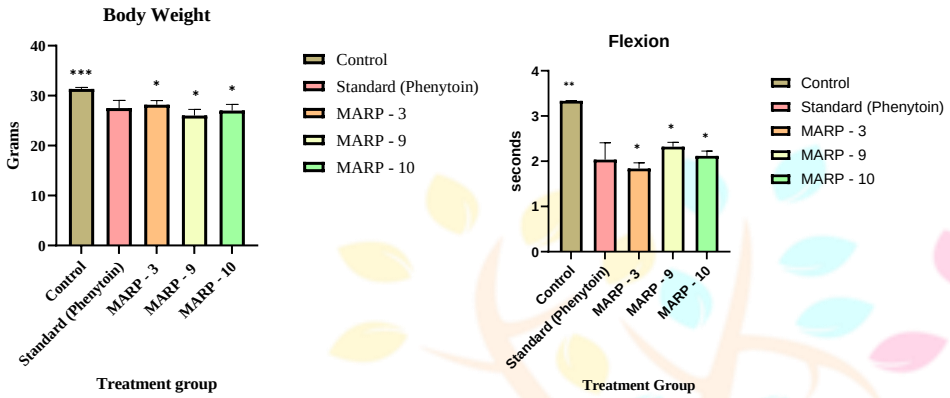
BBB permeant	No	No	No	No	No	No	No	No	No	No
Drug likeness (violation)	Yes; (1)	Yes; (0)	Yes; (1)	Yes; (1)	Yes; (0)	Yes; (0)	Yes; (0)	Yes; (0)	Yes; (0)	Yes; (1)
Lead likeness (violation)	No; 2 violations: MW>350, XLOGP3>3.5	No; 2 violations: MW>350, XLOGP3>3.5	No; 2 violations: MW>350, XLOGP3>3.5	No; 2 violations: MW>350, XLOGP3>3.5	No; 2 violations: MW>350, XLOGP3>3.5	No; 2 violations: MW>350, XLOGP3>3.5	No; 2 violations: MW>350, XLOGP3>3.5	No; 2 violations: MW>350, XLOGP3>3.5	No; 2 violations: MW>350, XLOGP3>3.5	No; 2 violations: MW>350, XLOGP3>3.5

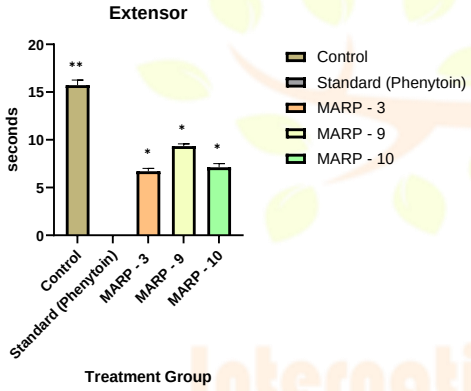
Biological activities

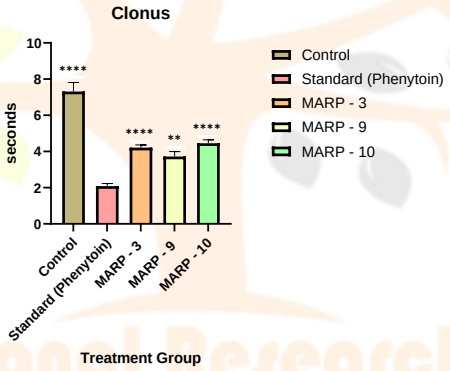
Anti-convulsant activity 4-methyl-7-(4-((p-tolylimino)methyl)phenoxy)-2H-chromen-2-one against MES – induced convulsion.

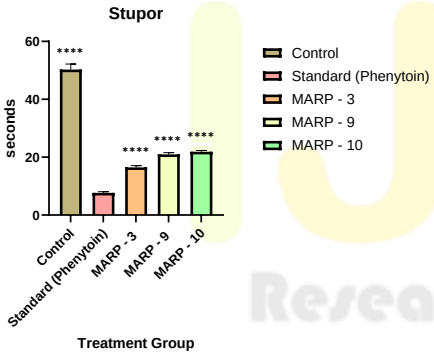
Sl No.	Groups	Body weight	Dose and Route	Flexion (sec)	Extensor (sec)	Clonus (sec)	Stupor (sec)	Recovery (%)
1	**Control**	31.3±0.333	0.5% (CMC) (i.p)	3.33±0.007	15.7±0.539	7.31±0.498	52.6±2.301	0
2	**Standard**	27.5±1.565	25mg Phenytoin (i.p)	2.03±0.374	Absent	2.09±0.137	7.68±0.372	100%
3	**MARP-3**	28.1±1.264	50mg MARP-3 (i.p)	1.8±0.324	6.7±0.303	4.21±0.150	16.49±0.58	66.6%
4	**MARP-9**	26±1.264	50mg MARP-9	2.32±0.127	9.34±0.227	3.72±0.277	20.7±0.580	33.3%

			(i.p)					
5	**MARP -10**	27±1.238	50mg MARP-10 (i.p)	2.11±0.105	7.13±0.376	4.45±0.194	21.8±0.461	100%









: *In-vitro* antioxidant activity of synthesized Coumarin substituted compounds.

SI. No	***Compound code***	***% inhibition***					
		50µg	**100 µg**	**200 µg**	**400 µg**	**600 µg**	**IC_{50}**
1	**MARP-1**	39.7	60.7	67.7	83.1	90	162.9
2	**MARP-2**	15.9	32.57	45.5	66.3	81.4	336.2
3	**MARP-3**	22.5	43	56.7	70.2	82.5	50.45
4	**MARP-4**	34.7	56.3	62.2	78.4	85.2	218
5	***MARP-5***	25.64	39.6	50.3	75.7	83.9	279
6	**MARP-6**	20.6	35.8	44.6	60.7	77.8	337
7	**MARP-7**	21.1	41.9	48.1	68.9	83.8	303.4
8	**MARP-8**	35.8	57	63.4	81.1	88.2	198.7
9	**MARP-9**	33.3	46.9	57.1	77.5	84.2	320.4
10	**MARP-10**	27.9	33.1	43.3	59.1	75.9	302.9
11	**Ascorbic acid**	49.3	62.5	79.1	85.5	97.8	58.7

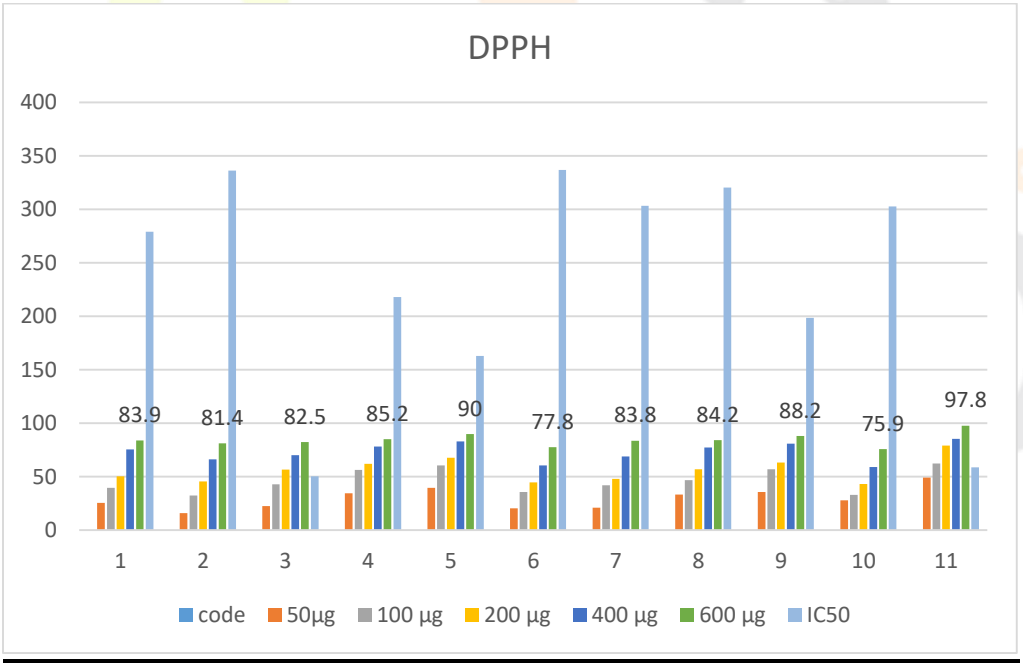
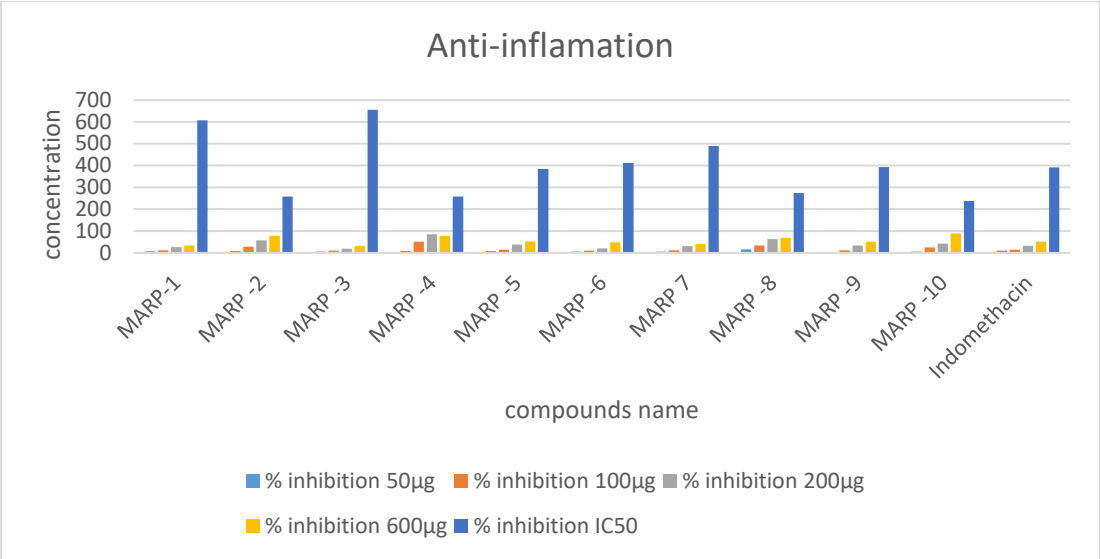


Fig31: Graphical representation of antioxidant activity by DPPH method

DPPH Experimentation

: *In-vitro* anti-inflammatory activity of synthesized coumarin derivatives.

SI. No	***Compound code***	***% inhibition***				
		50µg	***100µg***	***200µg***	***600µg***	***IC_{50}***
1	***MARP-1***	8	27	57	78	258
2	***MARP-2***	7	12	26	34	607.4
3	***MARP-3***	5	10	19	31	655.76
4	***MARP-4***	8	51	84	78	258.10
5	***MARP-5***	8	15	38	52	384.09
6	***MARP-6***	6	10	20	48	411.52
7	***MARP 7***	6	12	31	41	490
8	***MARP-8***	16	33	62	69	273.5
9	***MARP-9***	3	12	33	51	392.7
10	***MARP-10***	5	24	42	89	236.7
11	**Indomethacin**	10	14	32	51	391.4



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