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Design, Synthesis, Anti-inflammatory & Anticonvulsant Activity of Substituted Heterocyclic Compounds

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Authors' contributions

This work was carried out in collaboration among all authors. Author PVA designed the study, wrote the protocol, performed the statistical analysis, and wrote the first draft of the manuscript. Author ARC done the analyses of the study & reviewed manuscript. Author RN done literature review. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To design, synthesize, anti-inflammatory & anticonvulsant activity of substituted coumarin acetohydrazide derivatives.
Study Design: Experimental work related to Anti-inflammatory & Anticonvulsant activity.
Methodology: Coumarin acetohydrazide derivatives were synthesized by reacting substituted isatin with 7-hydroxy-4-methyl-coumarin in glacial acetic acid to determine their anti-inflammatory & anticonvulsant activities. Molecular docking study was performed against the COX enzyme for anti-inflammatory activity & carbonic anhydrase II enzyme for anticonvulsant activity. Anti-inflammatory activity was done by carrageenan induced paw edema method in rats at a dose of 200 mg/kg body weight. Anticonvulsant activity was studied in rats at a dose of 200 mg/kg body weight; in the maximal electroshock (MES) induced seizures model.
Results: (Z)-2-(4,8-dimethyl-2-oxo-2H-chromen-7-yl)oxy)-N-(5-nitro-2-oxoindolin-3-ylidine) aceto-

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hydrazide and (Z)-2-(4-methyl-2-oxo-2H-chromen-7yl) oxy)-N-(1-methyl-2-oxoindolin-3-ylidene) acetohydrazide showed the highest anti-inflammatory activity as compared to Celecoxib after 5 h with 71.94 to 77.96 % inhibition. Most of the compounds displayed anticonvulsant activity in the MES screen at a dose 200 mg/kg. Out of twelve compounds (Z)-N-(5-chloro-2-oxoindolin-3-ylidene)-2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetohydrazide and (Z)-2-((4-methyl-2-oxo-2H-chromen-7yl) oxy)-N-(1-methyl-2-oxoindolin-3-ylidene) acetohydrazide shown most significant activity with remarkable protection (67%) against MES induced convulsions. The structure-activity relationship concluded valuable pharmacophoric information, that the substitution on isatin ring has a significant effect on preventing inflammation & the seizure formation.

Conclusion: From the molecular docking study & biological activity determines that all synthesized coumarin acetohydrazide derivatives shows anti-inflammatory activity as compared to Celecoxib & anticonvulsant activity as compared to Phenytoin.

Keywords: 7-hydroxy-4-methyl-coumarin; isatine; acetohydrazide; anti-inflammatory activity; anti-anticonvulsant activity.

1. INTRODUCTION

Coumarin, a member of benzopyrone system either isolated from natural source like plant or synthesized chemically in the laboratory. There are several heterocyclic compounds which are having broad spectrum of biological activity among those coumarin has huge biological potency. Coumarin shows broad spectrum of activities such as Anti-oxidant [1], Anti-coagulant [2], Anti-microbial [3], Anti-inflammatory [4], Anti-viral [5], Anti-tuberculosis [6], Anti-HIV [7], Anti-cancer [8], Anti-histaminic [9], Anticonvulsant [10], Anti-depressant [11] etc. Inflammation is spontaneous response to any tissue injury [12,13]. Current therapy to treat inflammation is use of steroidal & non-steroidal drugs [14]. The use of these anti-inflammatory drugs is reported to cause some adverse effects like throat swelling, heartburn, indigestion, stomach ulcer, dizziness, allergic reactions like rashes etc. Therefore it is necessary to design &develop new anti-inflammatory compounds with less toxicity & side effects [15]. Coumarins& its related compounds will be a field of interest in the development of anti-inflammatory agents. Epilepsy is a central nervous system disorder and leads to seizures, abnormal behavior & sometimes loss of awareness [16]. For effective treatment one can use anti-epileptic (anticonvulsant) drugs, vagus nerve stimulator drugs, ketogenic diet containing high fat, low carbohydrate containing diet & brain surgery. First line treatment involves anti-seizure medication dose [17,18]. Drugs like levetiracetam (Keppra), lamotrigine (Lamictal), topiramate (Topamax), valproic acid (Depakote), carbamazepine (Tegretol), ethosuximide (Zarontin) available in market [19]. But these drugs may cause side effects like skin rashes, poor coordination, tiredness, fatigue, dizziness, short memory, confusion etc [20]. These undesirable side effects necessities need of development of new anticonvulsant derivatives [21]. After review of literature, Coumarin has shown potential in different biological activities and can open new arena for the development of new substituted derivatives. Docking studies are useful to evaluate the potential of these molecules against specific diseases. In the present work coumarin derivatives molecular studies performed against target molecules like COX-1 enzyme for anti-inflammatory activity (PDB: 3PGH) &carbonic anhydrase II enzyme for anticonvulsant activity TE1 (PDB: 3F8E)

2. MATERIALS AND METHODS

Chemicals & reagents procured from Sigma Aldrich & Merck. Characterization & identification of synthesized coumarin derivatives were carried out by physical, chemical & spectral analysis. Melting point determined on Veego melting point apparatus (VMP PM, 32/1105) & was uncorrected. TLC was carried out by using (G-60 mesh) silica gel. R_f value calculation done for synthesized compounds using n-Hexane: ethyl acetate (8:2).IR spectra were carried out on Fourier Transform Infrared Spectrophotometer by using KBr as a standard (JASCO FTIR).^{1}H NMR spectra recorded on BRUKER spectrometer in $CDCl_3$ using Tetramethylsilan (TMS) as an internal standard. GC-MS was carried out by using BRUKER Compass Data Analysis 4.2.

2.1 Molecular Docking Studies

For anti-inflammatory activity COX-I enzyme coupled with flurbiprofen (PDB: 3PGH).For anticonvulsant activity, human carbon anhydrase II (HCA II) TE1-(2Z)-3-{2-hydroxy-5-[(1S)-1-hydroxy-3-methylbutyl]-4-methoxyphenyl} prop-2-enoic acid (PDB: 3F8E) target enzyme was selected.

2.2 Synthesis of Coumarin Acetohydrazide Derivatives

Coumarin Acetohydrazide derivatives were prepared by reacting substituted resorcinol (I) with ethyl acetoacetate (II) which gives substituted 7-hydroxy Coumarin (III) through Pechmann Condensation reaction. Further compound (III) undergoes esterification reaction to form ethyl 2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetate(IV).Then it was reacted with hydrazine hydrate to afford 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetohydrazide (V).To this substituted isatin (0.002mole) was added in appropriate quantity of glacial acetic acid and refluxed for 8 hours at 70-80°c. Reaction frequently monitored by TLC. Then product was poured off into ice cold water and precipitated solid collected, recrystallized from DMF & Coumarin Acetohydrazide (VI) derivatives were collected (Scheme I) & confirmed by FT-IR, NMR & GC-MS. Different derivatives of coumarin acetohydrazide were synthesized by using substituted 7-hydroxy coumarin & isatin, named as PPI, P5C, P5N, P1M etc (Table 1).

PPI
(Z)-2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-(2-oxoindolin-3-ylidene) acetohydrazide

$C_{20}H_{15}O_5N$,Yield-85%,R_f=0.92, mp-269-271°C, IR(v KBr, cm-1): 3352.64(N-H str. Secondary amides), 3061.44(Aromatic CH-str.), 2912.95 (C-H str. in methylene group), 1650.77 (C=O str.in ketone),1614.13(C=O str.in amide), 1570(C=C str. aromatic), 1393.82(C-H str.in methylene group), 1078.01(C-O str.in C-O-C),3252.36(N-N str.),832.13(Aromatic C-H),1379.21(C-N amide),2233.16(C=N); 1H NMR (400 MHz CDCl3)shows δ ppm value at- 4.78 δ (d ,2H); 7.0δ (q,1H);6.98-7.63δ (t,3H); 9.8 δ (s,1H); 7.0-7.7 δ (q,4H);6.24 δ (d,1H);1.71 δ (t,3H); GC-MS (70Ev) m/z (%):378.10 (M+), 274,230,174.

P5C
(Z)-N-(5-chloro-2-oxoindolin-3-ylidene)-2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetohydrazide

$C_{20}H_{14}O_5N_3Cl$,Yield-80%, R_f=0.89, mp-278-288°C, IR(v KBr, cm-1):3329.5(N-H str. Secondary amides), 3084(Aromatic CH-str.), 2911(C-H str. in methylene group), 1650.77 (C=O str.in ketone),1616.06(C=O str.in amide), 1570(C=C str. aromatic), 1394.28(C-H str.in methylene group), 1077.05(C-O str.in C-O-C), 3220.54(N-N str.),834.06(Aromatic C-H),1379.82(C-N amide),2249(C=N); 1H NMR (400 MHz CDCl3) shows δ ppm value at-3.34δ (s,2H); 7.0δ (q,1H);6.91δ (d,1H);6.96 δ (d,1H);11.13 δ (d,1H); 7.4-7.6 δ (q,4H);6.25(1H,d);2.4 (t,3H); GC-MS (70Ev) m/z (%):412 (M+), 274,231,197.

P5N
(Z)-2-((4-methyl-2-oxo-2H-chromen-7yl) oxy)-N-(5-nitro-2-oxoindolin-3-ylidene) acetohydrazide

C20H14O7N4,Yield-85%, R_f=0.90, mp-280-284°C, IR(v KBr, cm-1):3446(N-H str. Secondary amides), 3087(Aromatic CH-str.), 2917 (C-H str. in methylene group), 1650(C=O str.in ketone),1621(C=O str.in amide), 1568(C=C str. aromatic), 1394(C-H str.in methylene group), 1080(C-O str.in C-O-C),3235 (N-N str.),830(Aromatic C-H),1378(C-N amide),2248 (C=N); 1H NMR (400 MHz CDCl3) shows δ ppm value at- 3.34 δ (s,2H); 7.0δ (q,1H);11.91 δ (d,1H);8.3δ (q,1H);7.7 δ (d,1H); 6.25 δ (d,1H);2.4 δ(q,3H).

P1M
(Z)-2-((4-methyl-2-oxo-2H-chromen-7yl) oxy)-N-(1-methyl-2-oxoindolin-3-ylidene) acetohydrazide

C21H17O5N3,Yield-90%, R_f =0.94, mp-264-267°C, IR(v KBr, cm-1):3341(N-H str. Secondary amides), 3088(Aromatic CH-str.), 2915(C-H str. in methylene group), 1650.77 (C=O str.in ketone),1618(C=O str.in amide), 1573(C=C str. aromatic), 1388(C-H str.in methylene group), 1086(C-O str.in C-O-C), 3237(N-N str.),833(Aromatic C-H),1383(C-N amide),2214(C=N); 1H NMR (400 MHz CDCl3) shows δ ppm value at- 4.83 δ (s,2H,); 7.0δ (q,1H);6.9δ (d,1H);6.8δ (d, 1H);2.42 δ (t,1H); 7.4-7.8 δ (q,4H);6.2 δ (d,1H,CH);1.57 δ (s,3H); GC-MS (70Ev) m/z (%):392 (M+), 274,231,197.

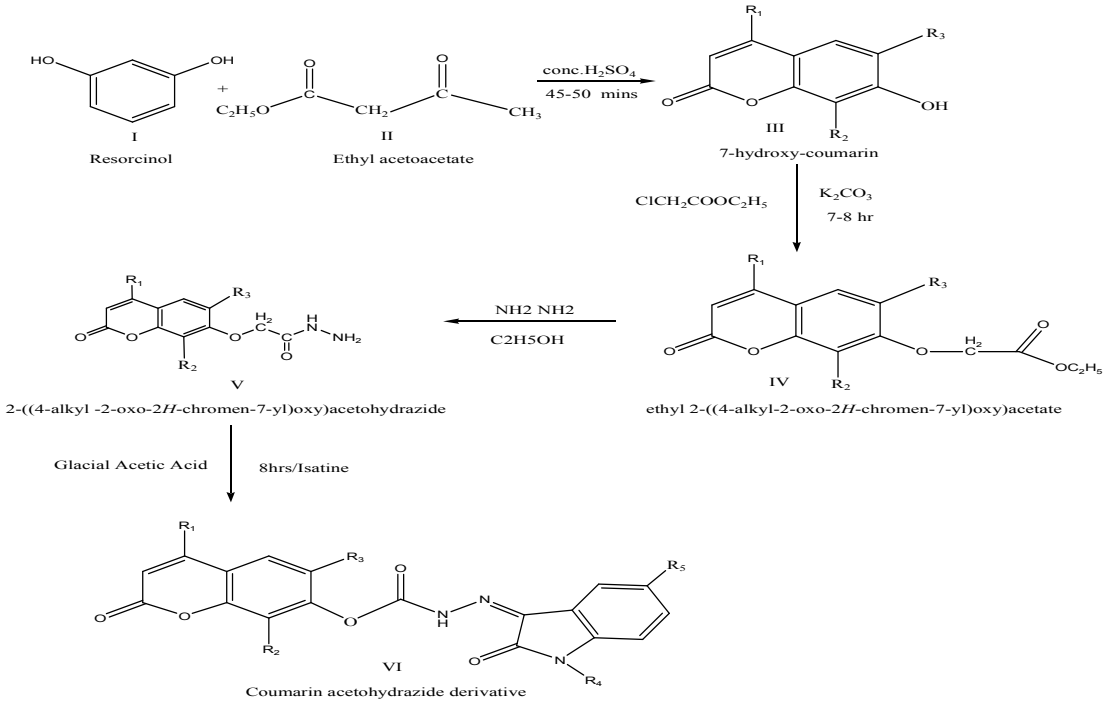

Fig. (A). General Scheme for Synthesis of coumarin acetohydrazide derivatives

Table 1. Physical data of Synthesized Coumarin Derivatives

Product code	R_1	R_2	R_3	R_4	R_5	Molecular Formula	Molecular Weight	% yield	M.P. (^{0}C)	R_f value
PPI	CH_3	H	H	H	H	$C_{20}H_{15}O_5N_3$	377.35	85	269-271	0.92
P5C	CH_3	H	H	H	Cl	$C_{20}H_{14}O_5N_3Cl$	411.80	80	278-288	0.89
P5N	CH_3	H	H	H	NO_2	$C_{20}H_{14}O_7N_4$	422.35	85	280-284	0.90
P1M	CH_3	H	H	CH_3	H	$C_{21}H_{17}O_5N_3$	391.38	90	264-267	0.94
MPI	CH_3	CH_3	H	H	H	$C_{21}H_{17}O_5N_3$	391.38	75	261-270	0.88
M5C	CH_3	CH_3	H	H	Cl	$C_{21}H_{16}O_5N_3Cl$	425.82	85	278-280	0.90
M5N	CH_3	CH_3	H	H	NO_2	$C_{21}H_{16}O_7N_4$	436.37	75	271-284	0.71
M1M	CH_3	CH_3	H	CH_3	H	$C_{22}H_{19}O_5N_3$	405.40	80	295-297	0.76
CPI	CH_3	H	Cl	H	H	$C_{20}H_{14}O_5N_3Cl$	411.80	75	192-202	0.70
C5C	CH_3	H	Cl	H	Cl	$C_{20}H_{13}O_5N_3Cl_2$	446.24	75	182-186	0.84
C5N	CH_3	H	Cl	H	NO_2	$C_{20}H_{13}O_7N_4Cl$	456.79	80	192-196	0.93
C1M	CH_3	H	Cl	CH_3	H	$C_{21}H_{16}O_5N_3Cl$	425.82	75	181-184	0.86

MPI
(Z)-2-((4, 8-dimethyl-2-oxo-2H-chromen-7-yl) oxy)-N-(2-oxoindolin-3-ylidene) acetohydrazide

C21H17O5N3,Yield-75%, R_f =0.88, mp-261-270°C, IR(v KBr, cm-1):3369(N-H str. Secondary amides), 3090(Aromatic CH-str.), 2917(C-H str. in methylene group), 1637 (C=O str.in ketone),1622(C=O str.in amide), 1383(C=C str. aromatic), 1051(C-H str.in methylene group), 1582(C-O str.in C-O-C), 3236(N-N str.),858(Aromatic C-H),1399(C-N amide),2240(C=N); 1H NMR (400 MHz CDCl3) shows δ ppm value at- 4.97 δ (s,2H); 7.1δ (q,1H);6.9δ (d,1H);2.4 δ (t,3H); 7.1-7.6 δ (q,4H);

6.24 δ (d,1H);1.69 δ (s,3H); GC-MS (70Ev) m/z (%):392 (M+), 277,231,197.

M5C
(Z)-N-(5-chloro-2-oxoindolin-3-ylidene)-2-((4, 8-dimethyl-2-oxo-2H-chromen-7-yl) oxy) acetohydrazide

C21H16O5N3Cl,Yield-85%, R_f=0.90, mp-278-280°C, IR(v KBr, cm-1):3356(N-H str. Secondary amides), 3089(Aromatic CH-str.), 2916(C-H str. in methylene group), 1687 (C=O str.in ketone),1635(C=O str.in amide), 1580(C=C str. aromatic), 1380(C-H str.in methylene group), 1078(C-O str.in C-O-C), 3244(N-N str.),835(Aromatic C-H),1330(C-N amide),2255(C=N); 1H NMR (400 MHz CDCl3) shows δ ppm value at- 4.73 δ (s,2H); 7.2δ (s,1H);6.6δ (d,1H),2.38 δ (t,3H); 7.36 δ (q,4H); 6.23 δ (d,1H), 1.81 δ (s,3H).

M5N
(Z)-2-((4, 8-dimethyl-2-oxo-2H-chromen-7-yl) oxy)-N-(5-nitro-2-oxoindolin-3-ylidine) acetohydrazide

C21H16O7N4,Yield-75%, R_f =0.71, mp-271-284°C, IR(v KBr, cm-1): 3356(N-H str. Secondary amides), 3092(Aromatic CH-str.), 2916(C-H str. in methylene group), 1643 (C=O str.in ketone),1625(C=O str.in amide), 1580(C=C str. aromatic), 1375(C-H str.in methylene group), 1085(C-O str.in C-O-C), 3260(N-N str.),829(Aromatic C-H),1392(C-N amide),2330(C=N); 1H NMR (400 MHz CDCl3) shows δ ppm value at- 4.7 δ (s,2H); 7.0δ (s,1H);6.6δ (d,1H);7.3 δ (d,1H);2.34 δ (t,3H); 7.5 δ (q,4H);6.17 δ (d,1H);1.72 δ (s ,3H).

M1M
(Z)-2-((4, 8-dimethyl-2-oxo-2H-chromen-7-yl) oxy)-n-(1-methyl-2-oxoindolin-3-ylidine) acetohydrazide

C22H19O5N3,Yield-80%, R_f =0.76, mp-295-297°C, IR(v KBr, cm-1):3340(N-H str. Secondary amides), 3053(Aromatic CH-str.), 2916(C-H str. in methylene group), 1687 (C=O str.in ketone),1637(C=O str.in amide), 1584(C=C str. aromatic), 1374(C-H str.in methylene group), 1061(C-O str.in C-O-C), 3272(N-N str.),835(Aromatic C-H),1374(C-N amide),2264(C=N); 1H NMR (400 MHz CDCl3) shows δ ppm value at- 4.73 δ (s,2H); 7.0 δ (s,1H);6.84-6.89 δ (q,4H);7.1δ (d,1H);2.41 δ (t,3H); 7.41-7.46 δ (q,4H);1.57 δ (s ,3H),13.88δ (s,1 H).

CPI
(Z)-2-((6-chloro-4-methyl-2-oxo-2H-chromen-7-yl) oxy)-N-(2-oxoindolin-3-ylidene) acetohydrazide

C20H14O5N3Cl, Yield-75%, R_f =0.70, mp-192-202°C, IR(v KBr, cm-1):3358(N-H str. Secondary amides), 3045(Aromatic CH-str.), 2918(C-H str. in methylene group), 1685 (C=O str.in ketone),1634(C=O str.in amide), 1594(C=C str. aromatic), 1365(C-H str.in methylene group), 1064(C-O str.in C-O-C), 3276(N-N str.),838(Aromatic C-H),1370(C-N amide),2265(C=N),1H NMR (400 MHz CDCl3) shows δ ppm value at- 4.87 δ (s, 2H); 7.0δ (s,1H);6.4δ (1H,d);7.5 δ (1H,d); 7.7 δ (4H,q);6.2 δ (1H,CH,d);1.8 δ (3H, s); 8.0 δ (s,1H).

C5C
(Z)-N-(5-chloro-2-oxoindolin-ylidene)-2-((6-chloro-4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetohydrazide

C20H13O5N3Cl2,Yield-75%, R_f =0.84, mp-182-186°C, IR (v KBr, cm-1): 3342(N-H str. Secondary amides), 3065(Aromatic CH-str.), 2917(C-H str. in methylene group), 1649 (C=O str.in ketone),1639(C=O str.in amide), 1573(C=C str. aromatic), 1390(C-H str.in methylene group), 1089(C-O str.in C-O-C), 3263(N-N str.),839(Aromatic C-H),1377(C-N amide),2215(C=N);1H NMR (400 MHz CDCl3) shows δ ppm value at- 4.7 δ (s,2H); 7.2 δ (s, 1H); 6.7 δ (d,1H);7.53 δ (d,1H);7.5 δ (q,3H,);6.20 δ (d,1H); 1.68 δ (s,3H).

C5N
(Z)-2-((6-chloro-4-methyl-2-oxo-2H-chromen-7-yl) oxy)-N-(5-nitro-2-oxoindolin-3-ylidene) acetohydrazide

C20H13O7N4Cl,Yield-80%, R_f =0.93, mp-192-196°C, IR (v KBr, cm-1): 3329(N H str. Secondary amides), 3084(Aromatic CH-str.), 2911(C-H str. in methylene group), 1650 (C=O str.in ketone),1616(C=O str.in amide), 1570(C=C str. aromatic), 1394(C-H str.in methylene group), 1077(C-O str.in C-O-C), 3220(N-N str.),834(Aromatic C-H),1379(C-N amide),2249(C=N); 1H NMR (400 MHz CDCl3) shows δ ppm value at- 4.8 δ (s,2H); 7.2δ (s,1H);6.7δ (d,1H);7.5 δ (d,1H); 7.6 δ (q,4H); 6.2 δ (d,1H);1.7 δ (s,3H); 8.0 δ (NH).

C1M
(Z)-2-((6-chloro-4-methyl-2-oxo-2H-chromen-7-yl)oxy)-N-(1-methyl-2-oxoindolin-3-ylidene) acetohydrazide

C21H16O5N3Cl,Yield-75%, R_f=0.86, mp-181-184°C, IR (v KBr, cm-1): 3352(N H str. Secondary amides), 3063(Aromatic CH-str.), 2917(C-H str. in methylene group), 1649 (C=O str.in ketone),1639(C=O str.in amide), 1572(C=C str. aromatic), 1391(C-H str.in methylene group), 1088(C-O str.in C-O-C), 3251(N N str.),839(Aromatic C-H),1391(C-N amide),2208(C=N); 1H NMR (400 MHz CDCl3) shows δ ppm value at- 4.5 δ (s,2H); 7.1δ (s,1H);6.9δ (d,1H);7.4 δ (d,1H);7.1-7.8 δ (q,4H); 6.2 δ (d,1H);1.9 δ (s,3H); 2.4 δ (q,3H).

2.3 Methodology

2.3.1 Acute Toxicity Studies

Prior to the administration of the substance, the animals were fasted overnight. The samples were administered as a single dose to animals by gavage using a stomach tube. The volume of administration was maintained to 1 ml per animal through proper dilution of sample. The group details are as follows: Normal, Control, standard, Test compound 1etc. It was observed that dose; 2000mg/kg is non-toxic at acute toxicity study. No mortality was observed within two days of administration of dose 2000 mg/kg & it was totally safe.

2.3.2 Anti-inflammatory activity

Carrageenan-induced paw edema method utilized for evaluation of anti-inflammatory activity of synthesized coumarin derivatives by using Wister albino rats (150–200 g) [22]. Animals were divided into four groups & each group consisting of six rats. Group 1 act as vehicle and Group 2 as control whereas Group 3 was given Celecoxibwith (20mg/kg) (reference standard) and Group 4 as test compounds [23]. To induce inflammation carrageenan in saline 0.1 ml (1% w/v) was injected. After carrageenan injection, edema development volume was measured by using digital plethysmometer (at 0, 1, 2, 3, 4, and 5 h) [24]. (Approval No.IIP/IAEC/03/2018-19) The percent inhibition was calculated by formula Percentage inhibition of paw edema = Vt- Vc/ Vc x 100
Where Vt is Paw volume of test animal at time point
Vc is paw volume of control animal at time point

2.3.3 Anticonvulsant activity

The experimental mice for anticonvulsant activity were divided as Vehicle, Control, standard, Test compound 1 etc. each consisting 6 rats of either sex (equal ratio). Group A –Vehicle, Group B - Control, Group C - Phenytoin (20 mg/kg) suspended in twin 80 [25]. Coumarin Acetohydrazide derivatives dissolved in twin 80 and given by oral gavage (200 mg/kg).Coumarin derivatives were investigated for anticonvulsant activity by MES-induced seizures model. Phenytoin and all coumarin derivatives at dose (200mg/kg) were given to various groups prior to 30 min before the administration of MES (20 mg/kg) [26]. After MES injection, behavioral changes of each mice was observed [27] (Approval No.IIP/IAEC/03/2018-19).In this data the percentage inhibition of flexion and extension were calculated as compared to control group by formula: Percentage inhibition of flexion/extension= No. of animals showing flexion in control –No. of animals showing flexion in test/ No. of animals showing flexion in control x 100.

2.4 Statistical Analysis

For both anti-inflammatory & anticonvulsant activity data is expressed as mean ± SEM. Graph Pad Prism 9.0 software was used for Statistical analysis. Data of groups were analyzed by one way ANOVA (Analysis of Variance) followed by Dunnet test.

3. RESULTS AND DISCUSSION

3.1 Molecular Docking

3.1.1 Anti-inflammatory activity

All computational work done on Pentium (R) Dual-Core CPUE5500@ 2.80 HGz with memory (RAM) =2.00 GB and docking was done by using V Life software.3D ultra 8.0 software was used to construct 3D structures of all compounds and then optimized geometry of compounds by energy minimization with Merck Molecular Force Field (MMFF), Convergence criteria (rms gradient: 0.01), systemic method (1000Kcal/mol) was selected for conformation generation and save generate conformer as a MDL Mol Files (*mol).For anti-inflammatory activity & Anticonvulsant activity two different target enzymes were selected. COX-1complexed with flurbiprofen (PDB: 3PGH).For anticonvulsant activity, carbonic anhydrase II TE1-(2Z)-3-{2-hydroxy-5-[(1S)-1-hydroxy-3-

methylbutyl]-4-methoxyphenyl}prop-2-enoic acid (3F8E)target enzyme was selected & retrieved from the Protein Data Bank. Results of interaction of coumarin derivatives with COX-1 are shown in (Table 2).

In docking studies all the synthesized coumarin derivatives exhibited good binding affinity towards the target protein flurbiprofen with binding energy ranging from -9.107 to -7.0 kJ mol^{-1} and compound M5N was having a minimum binding energy (-9.107kJ/mol) and highest binding affinity. Carbonyl oxygen of the amide and nitrogen of the amide are actively involved in electrostatic interactions with (flurbiprofen).In M5N (Fig. 1 & Fig. 2) the carbonyl oxygen of amide group elicit hydrogen bonding with Tyr 355(interatomic distance 6.24A^0).Coumarin oxygen shows hydrogen bonding with Arg 120 (interatomic distance 4.51A^0).

For P1M (Fig. 3& Fig. 4) carbonyl oxygen of amide group exhibit hydrogen bonding with Arg 120 (inter atomic distance 4.61 A^0). In Coumarin acetohydrazide derivative C1M, nitrogen of Isatin ring exhibit hydrogen bonding with Arg 120(inter atomic distance 3.78A^0). In case of PPI, nitrogen of Isatin ring exhibit hydrogen bonding with Arg 120 (inter atomic distance 3.97 & 4.73A^0). In MPI, nitrogen of Isatin ring exhibit hydrogen bonding with Arg 120((inter atomic distance 5.21 & 4.58 A^0).In all derivatives, benzene rings & methyl groups are in close proximity with the hydrophobic region of receptor and are involved in hydrophobic interactions with (flurbiprofen).

Table 2. Docking Score for Anti-Inflammatory Activity

Compound	Binding Energy	Residues Involved	H-bond distance (A^0)
Flurbiprofen	-9.868	Arg120	5.33
		Tyr355	5.72
M5N	-9.107	Arg120	4.51
		Tyr355	6.24
P1M	-9.079	Arg120	4.61,4.03
C1M	-9.039	Arg120	3.78,3.89
PPI	-8.999	Arg120	3.97,4.73
MPI	-8.781	Arg120	5.21,4.58
M5C	-8.659	Arg120	5.07,4.41
C5N	-8.379	Arg120	5.33
		Tyr355	5.72
P5C	-8.113	Arg120	4.98
P5N	-8.098	Arg120	5.05
		Tyr355	6.24
M1M	-8.009	Ser530	3.96,4.31
CPI	-7.693	Arg120	5.31
		Tyr355	5.72
C5C	-7.096	Arg120	5.29
		Tyr355	5.96

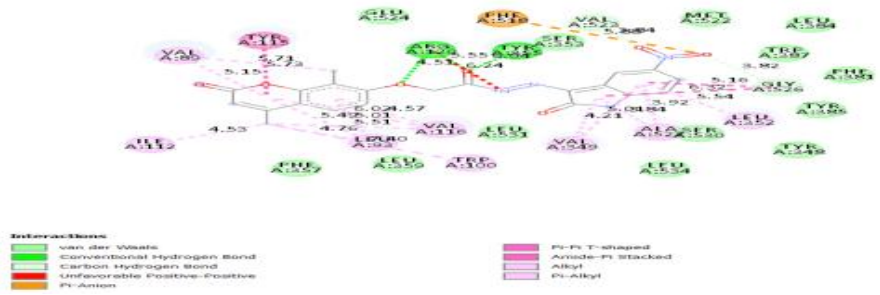

Fig. 1. 2D Interactions of Compound M5N with flurbiprofen (3PGH) active site

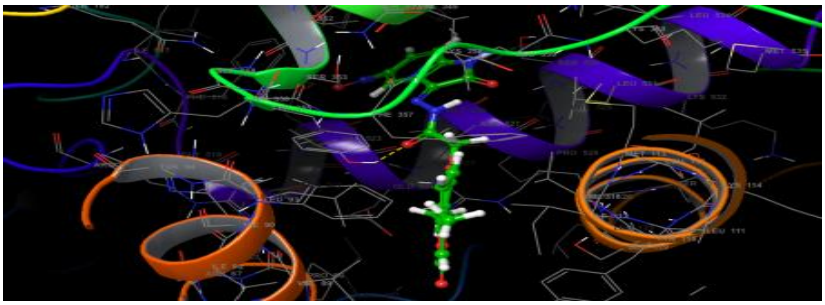

Fig. 2. 3D representation of M5N with flurbiprofen (PDB: 3PGH) active site

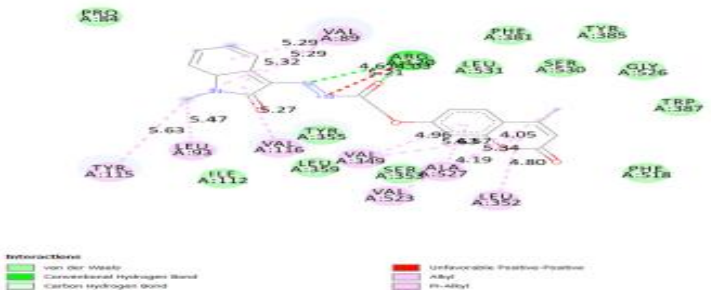

Fig. 3. 2D Interactions of Compound P1M with flurbiprofen (3PGH) active site

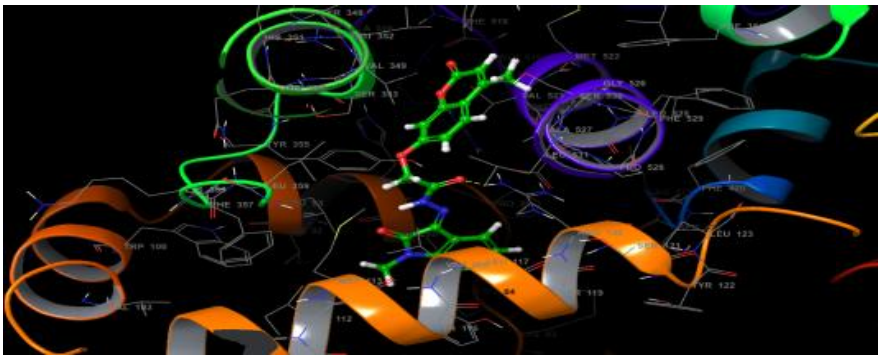

Fig. 4. 3D representation of P1M with flurbiprofen (PDB: 3PGH) active site

Compound M5N, P1M& C1M found to show significant binding interactions whereas remaining coumarin derivatives PPI, MPI, M5C, C5N, P5C, P5N, M1M, CPI, C5C has shown good results in docking studies. By observing interactions between derivatives and protein molecule we can conclude that functional groups like NO_2, Cl, CH_3, oxygen in amide group has played an important role in interactions. Substitutions of this group on coumarin ring may be results in active molecule and therefore decided to synthesize Coumarin acetohydrazide derivatives.

3.1.2 Anticonvulsant activity

In docking studies all the synthesized coumarin derivatives exhibited good binding affinity towards the target protein carbonic anhydrase II (*HCA II*) with binding energy ranging from -5.31 to -3.64 kJ $mol^{-1}$and M5N was having a minimum binding energy (-5.31kJ/mol) and highest binding affinity. All derivatives interact with carbonic anhydrase II (*HCA II*) through hydrogen and hydrophobic interactions. The obtained docking scores of results of synthesized coumarin derivatives are presented in (Table 3).

Table 3. Docking score of Anticonvulsant Activity

Compound	Binding Energy	Residues Involved	H-bond distance (A^0)
TE1	-6.972	Asn62	5.39
		Asn67	5.16
M5N	-5.31	Asn62	5.38
		Asn67	4.99
		Ile91	4.77
P1M	-5.142	Asn62	5.37
		Asn67	4.83
		His64	5.27
		Gln92	4.96
M5C	-5.069	Asn62	5.39
		Asn67	5.16
		His64	5.53
M1M	-5.026	Asn62	5.39
		Asn67	5.16
		His64	5.53
C5C	-4.922	Asn62	5.73
		Asn67	5.14
		His64	5.27
		Asp72	4.09
C1M	-4.486	Asn62	5.6
		Asn67	5.40
		His64	5.16
		Gln92	6.09
C5N	-4.476	Asn62	5.56,5.2
		Asn67	5.14
		His64	5.04
		Gln92	4.34
CPI	-4.12	Asn62	4.08
		Asn67	5.38,5.8
		His64	5.14
PPI	-4.016	Asn62	5.43
		Asn67	4.58
		Gln92	5.02,5.6
			4.17,4.63
		Thr200	
MPI	-3.902	Gln92	6.03
P5C	-3.825	Thr200	4.49
P5N	-3.64	Thr200	4.53

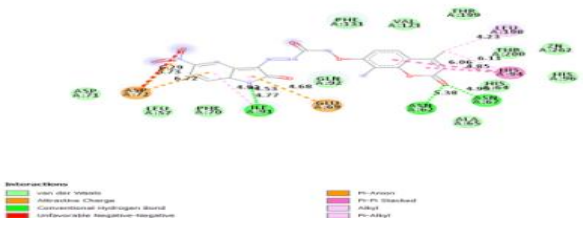

Fig. 5. 2D Interactions of Compound M5N with TE1 (3F8E) active site

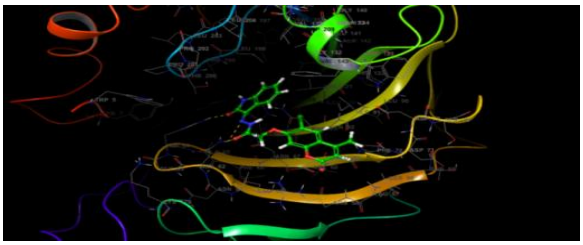

Fig. 6. 3D representation of M5N with TE1 (3F8E) active site

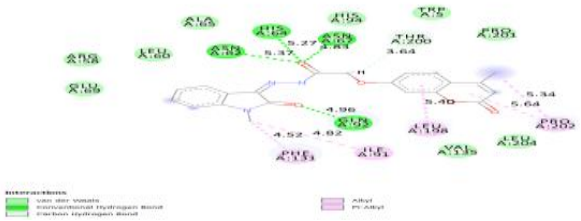

Fig. 7. 2D Interactions of Compound P1Mwith TE1 (3F8E) active site

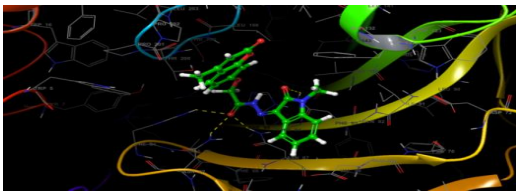

Fig. 8. 3D representation of P1M with TE1 (3F8E) active site

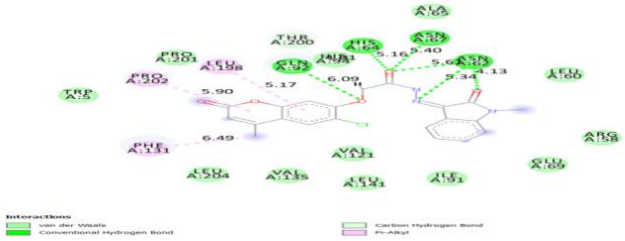

Fig. 9.2D Interactions of Compound C1M with TE1 (3F8E) active site

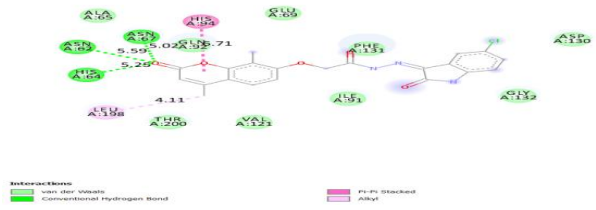

Fig. 10. 2D Interactions of Compound M5C with TE1 (3F8E) active site

For M5N (Fig. 5 & Fig. 6) the carbonyl oxygen of coumarin ring elicit hydrogen bonding with Asn62 (interatomic distance 5.38 A^0) & Asn67(interatomic distance 4.99A^0) while amide nitrogen shows hydrogen bonding with Ile91(interatomic distance 4.77A^0).In case of

P1M (Fig. 7 & Fig. 8) carbonyl oxygen of amide group exhibit hydrogen bonding with Asn67 (interatomic distance 4.83 A^0) His64 (interatomic distance 5.27) Asn62 (interatomic distance 5.37A^0) while Carbonyl oxygen of Isatin ring exhibit hydrogen bonding with Gln92 (interatomic distance4.96A^0).

In C1M (Fig. 9), carbonyl oxygen of amide group exhibit hydrogen bonding with Asn67 (interatomic distance 5.40A^0) His64 (interatomic distance 5.16A^0) Asn62 (interatomic distance 5.6A^0).In M5C (Fig.10), carbonyl oxygen of coumarin ring exhibit hydrogen bonding with Asn67 (interatomic distance 5.16A^0) His64 (interatomic distance 5.53A^0) Asn62 (interatomic distance 5.39A^0).

For M1M, carbonyl oxygen of coumarin ring exhibit hydrogen bonding with Asn67 (interatomic distance 5.16 A^0) His64 (interatomic distance5.53A^0) Asn62 (interatomic distance5.39A^0).

Remaining all coumarin derivatives C5C, C5N, CPI, PPI, MPI, P5C &P5N were found to show significant results in docking studies by observing interactions between derivatives and protein molecule we can conclude that functional groups like NO_2, Cl, CH_3, carbonyl oxygen of amide group, carbonyl oxygen of coumarin has played an important role in interactions. Substitutions of these groups on coumarin ring may results in active molecule and therefore decided to synthesize Coumarin Acetohydrazide derivatives. Mechanism of Coumarin acetohydrazide derivatives by above scheme was already reported [28]. Total 12 compounds synthesized & structures of the compounds have been confirmed on the basis of spectral analysis & further results of anti-inflammatory & anticonvulsant activity mentioned below.

3.1.3 Anti-inflammatory activity by carrageenan-induced paw edema method

Carrageenan-induced rat paw edema method was used for evaluation of anti-inflammatory activity & compound M5N, P1M and C1M were shown significant anti-inflammatory activity. Docking studies also shows similar results. Anti-inflammatory activity was evaluated at different time intervals (0, 1, 2, 3, 4, and 5 hr) as shown in Fig. 11 & Fig.12. Out of Twelve compounds M5N have shown very good result. Synthesized coumarin compounds show anti-inflammatory activity in the range of 58-77 %. Coumarin derivatives containing substitutions like Chlorine able to show moderate anti-inflammatory activity. Particularly substitution with NO_2 & CH_3 increases the anti-inflammatory activity.

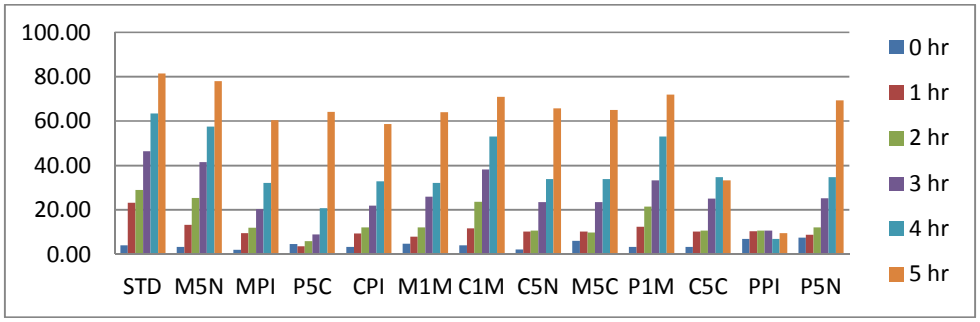

Fig. 11. Graph of Anti-inflammatory activity of CoumarinAcetohydrazide derivatives

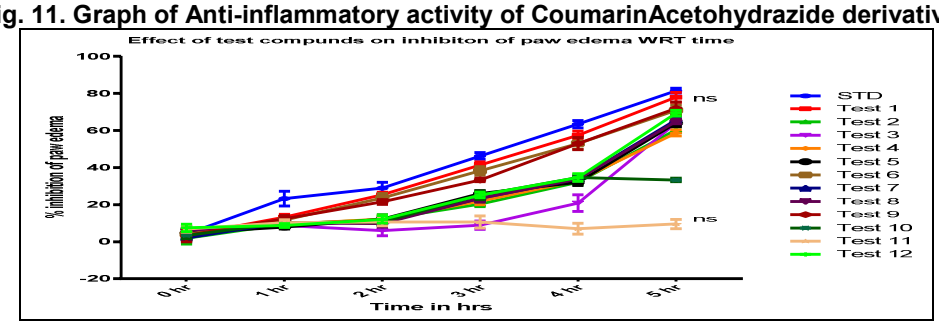

Fig. 12. Percentage inhibition in paw edema produced between standard and test groups compared with respect to time

At 5 hr it was found that standard Celecoxbi treated group shown percentage inhibition 81.52±1.34. Test no.M5N, C1M, P1M, P5N shown maximum inhibition 69.32±1.50 to 77.96±2.67 compared with that of the standard. Test no MPI, P5C, CPI, M1M, C5N, M5C shown moderate inhibition ranging from 58.74±1.68 to 65.71±1.50. Whereas test no C5C, PPI shown lesser inhibition 9.56±2.51 to33.32±0.90.

Statistical Analysis of Percentage inhibition of paw edema & Percentage Inhibition of Tonic flexion and extension shown by test substances mentioned in Table 4 & Table 5 respectively.

3.1.4 Anticonvulsant activityby Maximal electro shock-induced convulsions model

3.1.4.1 Percentage of inhibition of Tonic flexion

Anticonvulsant activity performed by Maximal electro shock-induced convulsions method. All synthesized coumarin compounds shown significant anticonvulsant activity. Percentage inhibition of Statistical Analysis of Tonic flexion& Tonic Extension was calculated and mentioned in Table 5.

Table 4. Statistical Analysis of Percentage inhibition of paw edema by test substances

Time in Hrs / Treatments	0	1	2	3	4	5
Standard	4.05±2.69	23.25±3.91	28.92±3.21	46.31±1.88	63.44±1.98	81.52±1.34
M5N	3.33±2.76	13.23±1.54	25.34±1.35	41.47±1.61****	57.42±2.26	77.96±2.67
MPI	1.94±3.20	9.53±0.61****	12.04±2.61****	20.28±1.35****	32.13±1.32****	60.44±1.55****
P5C	4.75±2.27	3.56±1.81****	5.94±2.66*****	8.85±2.27*****	20.84±4.50****	64.05±1.49****
CPI	3.30±2.72	9.43 ±2.00****	12.15±1.76****	21.90 ±1.98****	32.96±1.34****	58.74±1.68****
M1M	4.75±1.63	7.98±1.21****	12.19±1.28****	25.99±1.54****	32.09±1.75****	63.96±1.85****
C1M	4.06± 1.81*	11.78±0.94	23.65± 2.35	38.21± 2.32	53.04 ±3.00	70.94± 2.81*
C5N	2.05±1.80	10.29±0.90 ****	10.63±1.35****	23.49±1.72****	33.89±0.86****	65.71±1.50****
M5C	6.08±2.26	10.22±1.41 ****	9.80±1.98*****	23.49±2.15	33.85±1.35****	64.88±1.23****
P1M	3.33± 3.33	12.40±1.69 *	21.47 ± 1.35	33.37± 1.02***	53.02± 3.31	71.94 ± 3.44
C5C	3.39±1.70	10.23±1.29 ****	10.60±1.71****	25.08±2.38****	34.72±1.34****	33.32±0.90****
PPI	6.81± 1.64	10.36±1.55 **	10.70± 2.20****	10.67± 3.28****	6.98± 2.95****	9.56± 2.51****
P5N	7.50±1.95	8.74±1.00****	12.12±2.03****	25.20±1.48****	34.63±2.07****	69.32±1.50****

**The data is expressed as mean ± SEM. The data for percentage inhibition of paw volume (n=6) is analyzed by one way- ANOVA (Analysis of Variance) followed by Dunnet test. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, NS: non significant*

Table 5. Percentage Inhibition of Tonic flexion and extension

Time in Hrs / Treatments	Inhibition of Tonic flexion	Inhibition of Tonic Extension	Quantal protection	% of Protection
Standard	71.57±0.07	83.42±0.93	6/6	100
M5N	64.14±0.10****	10.96±0.09****	3/6	50
P5N	62.21±0.10****	4.43±0.16****	4/6	67
C5N	72.65±0.09****	12.43±0.15****	4/6	67
CPI	78.00±0.09****	14.29±0.12****	4/6	67
M1M	84.7±20.14****	27.14±0.22****	4/6	67
C1M	81.74±0.05****	19.10±0.16****	4/6	67
P1M	86.64±0.17****	47.71±0.10****	4/6	67
M5C	84.68±0.07****	33.05±0.10****	4/6	67
P5C	74.72±0.19****	52.58±0.58****	4/6	67
C5C	85.46±0.37****	15.62±0.12****	4/6	67
PPI	85.10±0.05****	21.21±0.28****	4/6	67
MPI	79.23±0.07****	43.83±0.04****	3/6	50

**The data is expressed as mean ± SEM. The data for percentage inhibition of flexion and extension (n=6) is analyzed by one way- ANOVA (Analysis of Variance) followed by Dunnet test. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, NS: non significant*

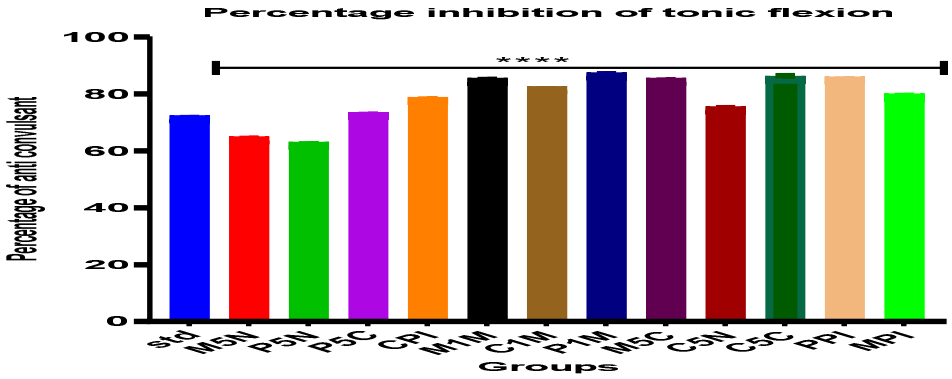

Fig. 13. Percentage inhibition of Tonic Flexion

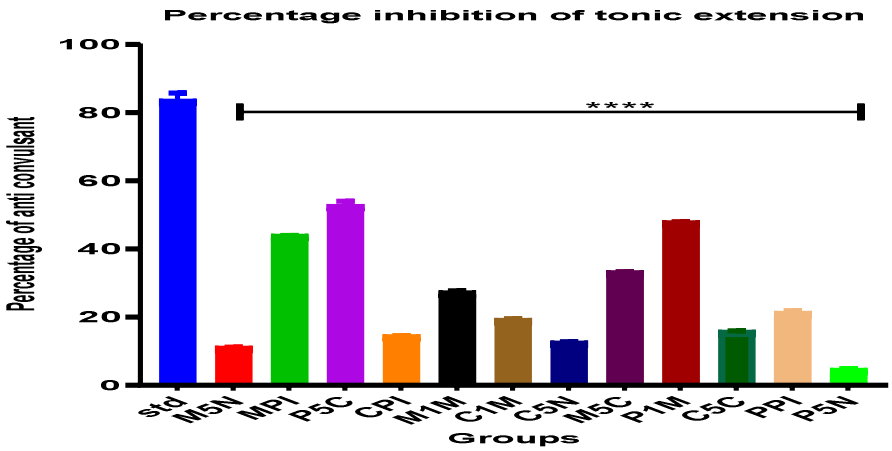

Fig.14. Percentage inhibition of Tonic Extension

In Tonic Flexion, (Fig. 13) M1M, C1M, M5C, C5C, P1M & PPI shown maximum inhibition ranging from 81.74to 86.64 % whereas P5C, CPI & MPI shown moderate inhibition74.72to 79.23%.M5N, P5N & C5N shown lowest inhibition 62.21to 72.65%.

3.1.4.2 Percentage of inhibition of tonic extension

In Tonic Extension, (Fig. 14) P5C, MPI & P1M shown maximum inhibition ranging from 43.83 to 52.58% whereas CPI, C1M, M1M, M5C, PPI &C5C shown moderate inhibition& M5N, C5N, & P5N shown lowest inhibition4.43 to 12.43%.

From result of docking studies, compound P5C & P1M compounds were shown good interaction with TE 1 protein molecule with minimum binding energy as compared with standard. All Coumarin acetohydrazide derivatives had shown Anticonvulsant effect on mice at their selected doses. Out of twelve compounds, P5C have shown very good result in Tonic Extension.For anticonvulsant activity attachment of substituted isatin ring at C-7 position of Coumarin ring is important. Maximal electoral shock induced model used correlates with Grand Mal type of seizures in humans.

4. CONCLUSION

To know the appropriate binding interaction between the newly synthesized compounds with COX & carbonic anhydrase II enzyme, molecular docking study of synthesized coumarin acetohydrazide derivatives was carried out. Good binding interactions suggested by molecular docking study explain the better biological activity. A series of coumarin acetohydrazide derivatives were synthesized and evaluated by carrageenan-induced hind paw edema method for anti-inflammatory activity. Compound M5N, C1M, P1M, P5N (69.32% to 77.96%) showed maximum inhibition. The coumarin compound MPI, P5C, CPI, M1M, C5N & M5C showed moderate inhibition ranging from 58%- 69 %. The Coumarin compound C5C, PPI (9.56 to 33.32 %) with non-substituted isatin moiety shows minimum inhibition as compared to substituted isatin compounds. In particular compound M5N, NO_2 substituted isatin proved to be the most active among these substituted compounds. Further determination of mode of binding and structure optimization of coumarin acetohydrazide derivatives will be determined in future. Coumarin derivatives showed significant anticonvulsant activity in MES model. This pharmacological activity might be due to the electron withdrawing group & isatin ring attachment at C-7 position together shown a significant role in anticonvulsant activity.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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