Design, Synthesis, Structural Characterization, and Biological Profiling of new Nitrogen and Oxygen based six-membered Heterocyclic Scaffolds as potent Analgesic activity

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ABSTRACT

A novel series of oxazine derivatives (SMS-1 to SMS-10) were synthesized through a four-step process and characterized using IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. The synthesis involved condensation, acetylation, Claisen–Schmidt, and cyclization reactions, confirmed by characteristic IR peaks at 3426(NH Str), 3252(OH Str), 1652(C=O Str), 3076(CH=CH Str), and 1513(C=N Str) cm^{-1}. In-silico docking studies revealed strong binding affinities of SMS-3(4-F), SMS-9(3-Cl), and SMS-10(3-NO_2) with target proteins (1ZXM, 1HD2, 5HN1, and 3GNY), aligning with their observed biological activities. SMS-1 and SMS-9 exhibited potent analgesic effects, while SMS-9 (3-Cl) showed excellent antioxidant activity (IC_{50} = 248.36 μg/mL). SMS-3(4-F) and SMS-8(tri-methoxy) displayed notable anti-inflammatory potential, and SMS-5, SMS-9, and SMS-1 demonstrated significant antimicrobial activity against *S. aureus* and *E. coli*. Toxicity predictions indicated Class IV safety with no major toxic effects. All compounds complied with Lipinski's rule and exhibited good pharmacokinetic properties, suggesting strong therapeutic potential with multi-target biological efficacy.

Key-words: Oxazine synthesis, *In-vivo* analgesic activity, *In-silico* and *in-vitro* antioxidant and anti-inflammatory activity.

1.INTRODUCTION

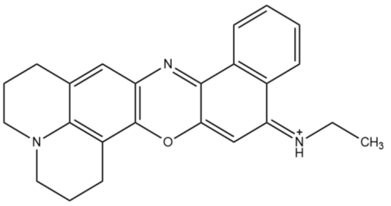
Oxazine derivatives are six-membered heterocyclic compounds containing one oxygen and one nitrogen atom, existing in three isomeric forms: 1,2-, 1,3-, and 1,4-oxazines[1]. These compounds have attracted great interest due to their broad spectrum of biological activities[2], including anti-inflammatory[3], antioxidant[4], antimicrobial[5], analgesic[6], anticancer[7], and antiviral[8] properties. Among them, 1,3-oxazine derivatives are particularly significant for their potent pharmacological effects and role as intermediates in drug synthesis, notably in non-nucleoside reverse transcriptase inhibitors for HIV-1[9]. Chalcones, which possess an α, β-unsaturated carbonyl system, serve as key precursors in oxazine synthesis and exhibit various biological activities[10]. *In-silico* tools such as molecular docking, SwissADME, and ProTox-II play a vital role in predicting drug-likeness[11], pharmacokinetics[12], and toxicity[13], reducing experimental time and cost. Overall, the synthesis and computational evaluation of oxazine derivatives hold great promise for developing novel therapeutic agents with enhanced safety and efficacy.

2. OBJECTIVE OF STUDY

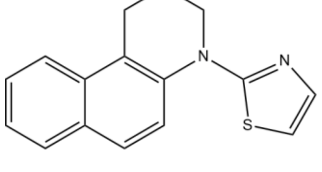
a) Synthesis of some novel oxazine derivatives.
b) Characterization of newly synthesized derivatives using spectral studies.
c) Drug likeliness property of the derivatives by using SwissADME and Molinspiration web servers.
d) Online toxicity prediction using proTOX-II software.
e) Molecular Docking studies by using PyRx software
f) *In-vitro* evaluation of the derivatives for their anti-inflammatory, anti-microbial, and anti-oxidant activity.
g) *In-vivo* evaluation of the derivatives for their analgesic activity.

3. LITERATURE REVIEW

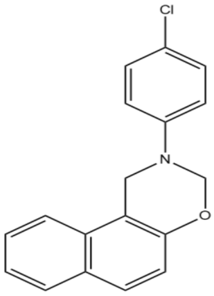
i. Roja Y and co-workers synthesized 1,3-oxazine derivatives in several ways. [1,3]-oxazine derivatives were reported to have antiplatelet aggregation, antitubercular, anti-inflammatory, antidiabetic, hypolipidemic, antimycobacterial, antibacterial, antifungal, anticoagulant, antioxidant and cytotoxic activities. They synthesized oxazine derivatives via Mannich reaction and Betti reaction[14].



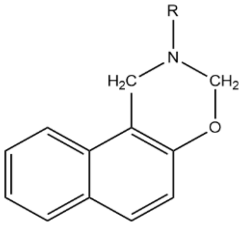
ii. Ajely M A and co-workers reported the synthesis of new oxazine compounds using two routes. The first one includes grinding technique using formaldehyde, aromatic amines and methanolic ammonia. The second route involve the condensation of aryl aldehydes with anthranilic acid. The Schiff bases were cyclized using acetic anhydride into the corresponding oxazine compounds. All the synthesized oxazines were studied using IR, ^{1}H-NMR methods and are discussed[15].



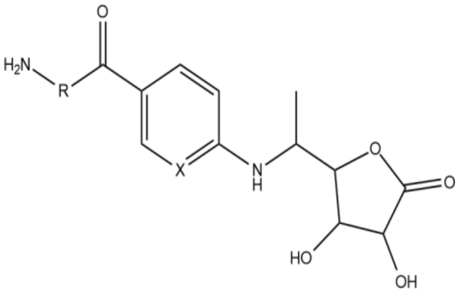
iii. Khollat A and co-workers prepared oxazine by using the magnetic solid acid nanocatalyst based on ferrierite as catalyst using the green synthesis in water at room temperature. The structure of synthesized products was investigated by spectroscopic methods such as FT-IR, ^{1}H NMR and ^{13}C NMR. Docking calculations on the structure of the synthesized compounds proved their medicinal properties against breast cancer cells[16].



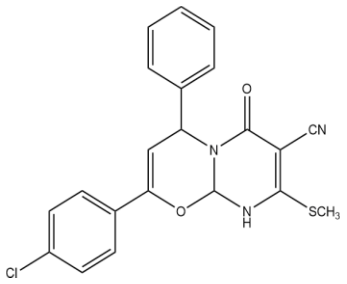
iv. Keihanfar M and co-workers reported an eco-friendly protocol for the synthesis of naphtho oxazines in the presence of FNAOSiPAMP/CuII, as a new natural based green catalyst via the reaction of β-naphthol, primary amines and formaldehyde. ^{1}H NMR and ^{13}C NMR spectra were obtained, Fourier transform infrared (FT-IR) spectra recorded by ATR method for the synthesized compounds[17].



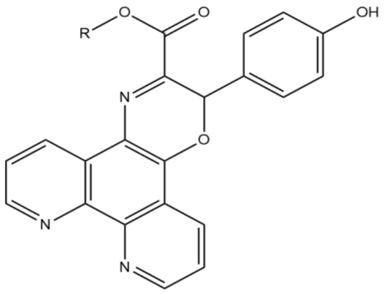
v. Krupkova S and co-workers prepared derivatives of 3-methyl-3,6-dihydro-2H- 1,2-oxazine-6-carboxylic acid by using regioselective hetero Diels-Alder reaction of arylnitroso compounds with sorbic acid. The final compounds obtained as a mixture of stereoisomers were analyzed with use of chiral HPLC and SFC. The synthetic and analytical conditions were thus possible for development of stereoselective synthesis of oxazine as well as ɣ-lactone derivatives and the products of their transformation on solid support[18].



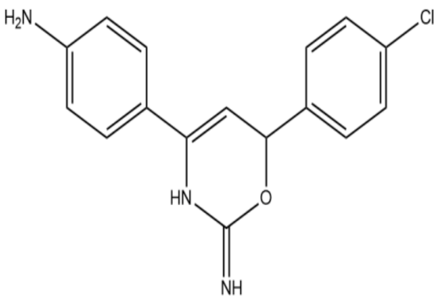
vi. Shivraj B S and co-workers prepared novel oxazine from chalcone and urea. The synthesized compounds were characterized by spectral methods[19].



vii. Evans C and co-workers synthesized and characterized three Ag(I) bis(phenanthroline-oxazine) complexes with varying lipophilicity. NMR studies were also carried out to investigate the fuxional behaviour of the Ag(I) complexes in solution. The biological activity of the silver(I) complexes and the corresponding ligands towards a clinical strain of *Candida albicans* MEN was studied using broth microdilution assays[20].



viii. Sabre H M and co-workers reported the synthesis of novel Oxazine and Thiazine derivatives which were prepared by cyclization of substituted chalcones derivatives in the presence of urea or thiourea using potassium hydroxide as a catalyst. The new synthesized compounds were identified using various physical techniques like ^{1}H-NMR and FT-IR spectra[21].



4. METHODOLOGY

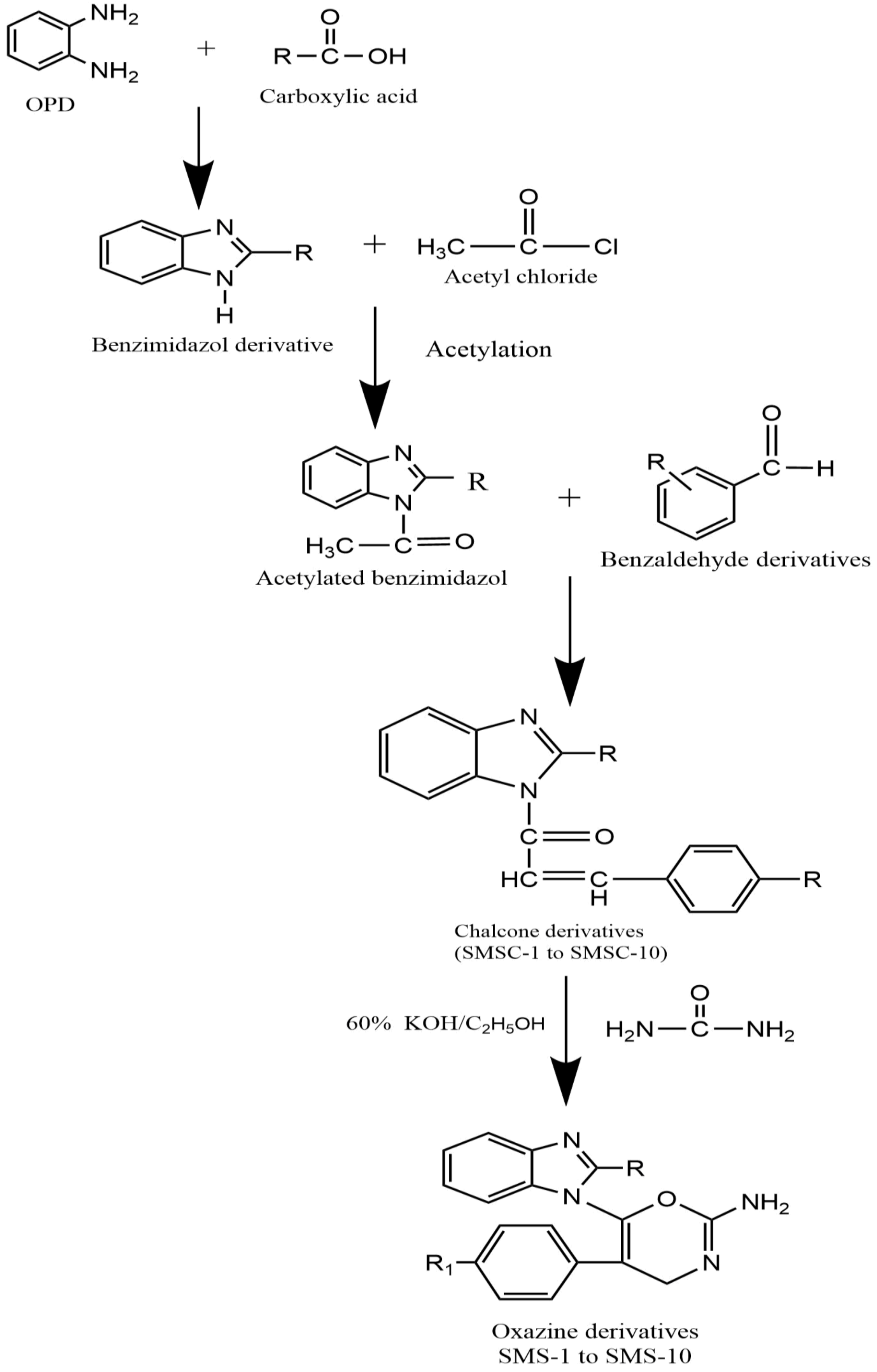
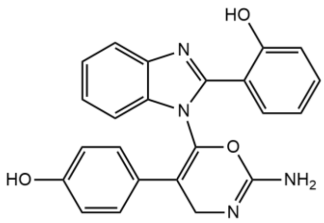
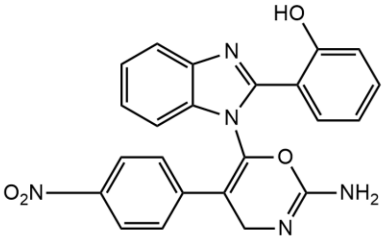
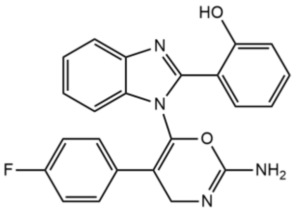
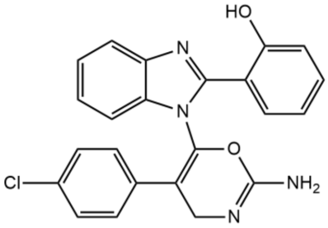
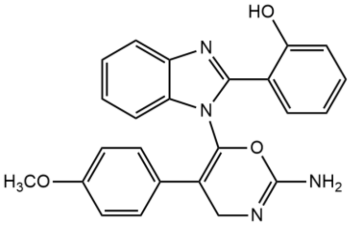
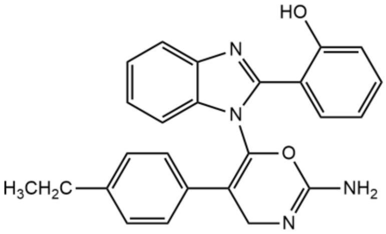
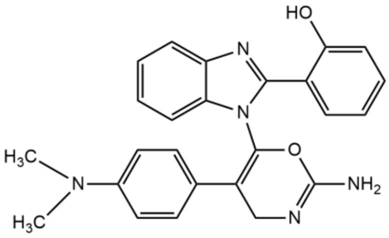
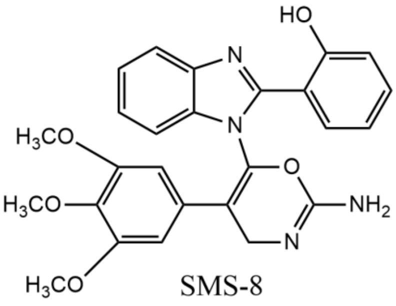
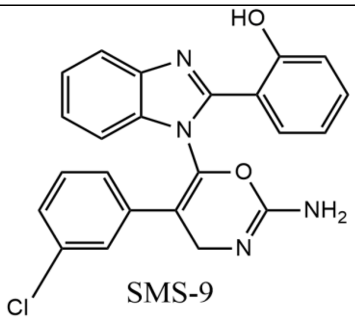
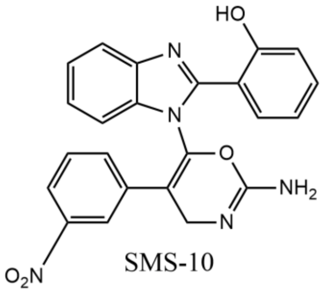


Table-1: Structure of synthesized oxazine derivatives.

 SMS-1	 SMS-2
 SMS-3	 SMS-4
 SMS-5	 SMS-6
 SMS-7	 SMS-8
 SMS-9	 SMS-10

STEP-1: **General method for the synthesis of Benzimidazole phenol:**

Condensation of *o*-phenylenediamine and aromatic acid (salicylic acid, benzoic acid, cinnamic acid, etc). Then the reaction mixture was stirred for 2 hrs at 80 °C–90 °C on Hot plate with the use of ammonium chloride (NH_4Cl) as a catalyst in Ethanol then the product is filtered and dried and recrystallized with ethanol[22].

STEP-2: **General method for the synthesis of Hydroxyphenol Benzimidazole Ethanone:**

Equimolar quantities of benzimidazole 0.01 mol (1.18 g) and 0.01mol (0.79 g) of acetyl chloride were added, mixed and left for 4 h. Concentrated ammonia was added and the product obtained was recrystallized from 20% aqueous ethanol[23]**.**

STEP-3: **General method for the synthesis of Hydroxyphenol Benzimidazole Chalcone derivatives:**

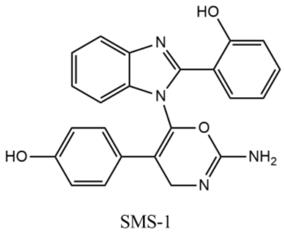
Equimolar quantities of 2-acetyl benzimidazole and ortho- or para-substituted benzaldehyde were dissolved in 20 mL of ethanol and 40% KOH (7.5 mL). The reaction mixture was then allowed to stir for 24 h on a magnetic stirrer. The obtained mixture was poured into crushed ice, and the precipitate was filtered. After drying, the products were recrystallized from methanol. All reactions were monitored by performing thin-layer chromatography using hexane and ethyl acetate (2: 0.5) as the mobile phase[24].

STEP-4: General method for the synthesis of Hydroxyphenyl oxazine Benzimidazole phenol derivatives:

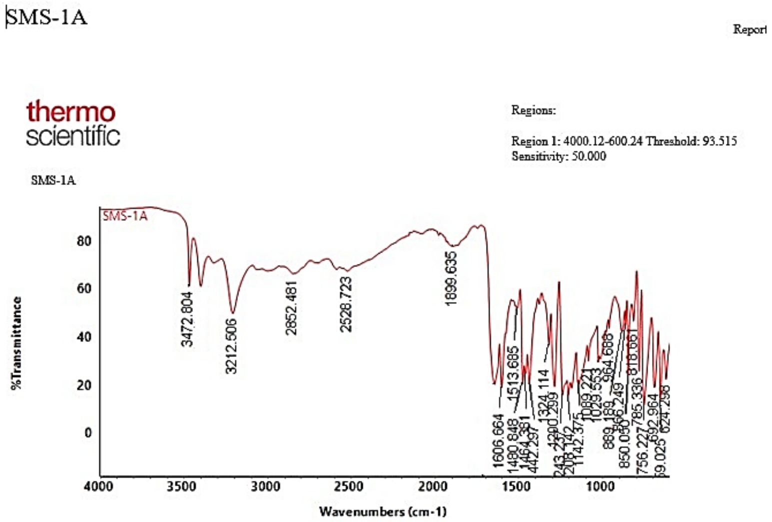
To a mixture of urea (2 g; 0.033 mole) and ethanol (20 ml) was added enough hydrochloric acid to get a clear solution. 0.02 mole of hydroxyphenol benzimidazole chalcone was then added and the mixture refluxed on a steam bath for 8 hours at 100-110^{0}C with constant stirring. Monitor the reaction progress by using TLC ethyl acetate:n-hexane (3:2). The reaction mixture was then concentrated, cooled and made alkaline with ammonium hydroxide. The product obtained was filtered off and recrystallized from the methanol[25].

5. RESULTS

Table-2: Physical properties and Spectral data of compound SMS-1

Compound code	**SMS-1**					
Structure						
IUPAC Name	2-(1-(2-amino-5-(4-hydroxyphenyl)-4H-1,3-oxazin-6-yl)-1H-benzo[d]imidazol-2-yl)phenol					
Physical Properties	Mol formula & weight	Colour	M.P range	Rf Value	Solubility	%Yield
	$C_{23}H_{18}N_4O_3$ 398.42g/mol	off green	160-164^{0}C	0.89	Methanol DMSO	51.56

a) IR (KBr) cm^{-1}


SMS-1A

Report

Regions:

Region 1: 4000.12-600.24 Threshold: 93.515
Sensitivity: 50.000

Fig-1: IR spectrum of compound SMS-1

3472 (NH str), 3212 (OH str), 2852 (Ar C-H str), 1606 (Ar C=C str), 1513 (C=N str), 1200-O-C str).

b) ^{1}H-NMR

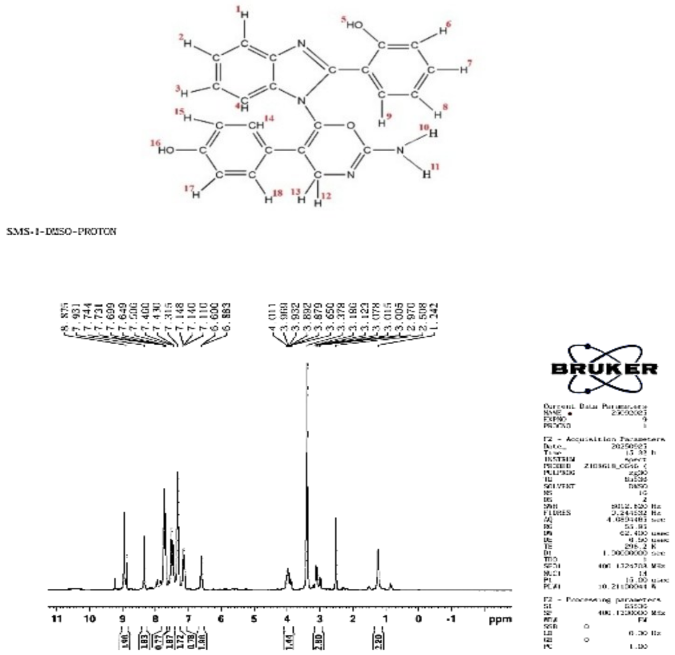


Fig-2: ^{1}H-NMR spectra of SMS-1

δ: 8.85 (2H, H_{10} H_{11}), 7.315 (2H, H_5 H_{16}), 7.148 (2H, H_{12} H_{13}),7.931-7.430 (12H, Ar-CH).

c) ^{13}C-NMR

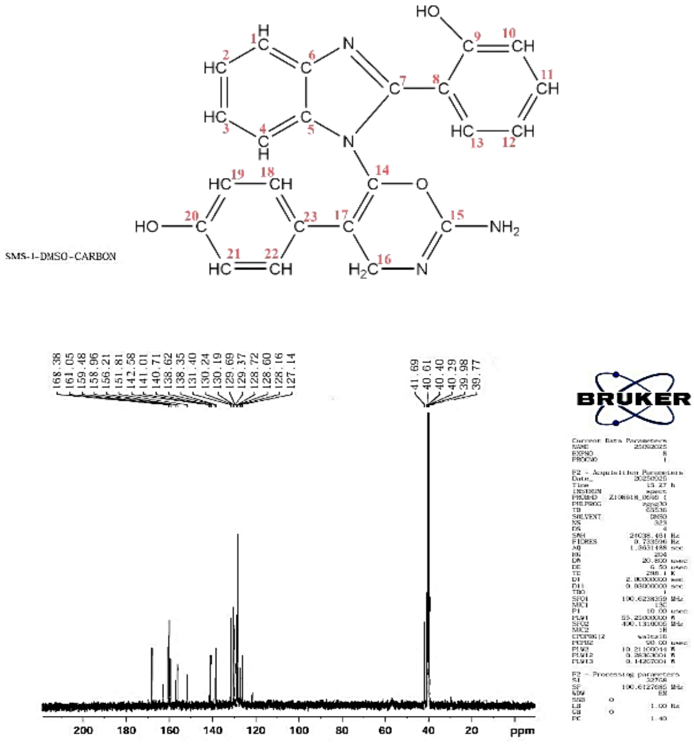
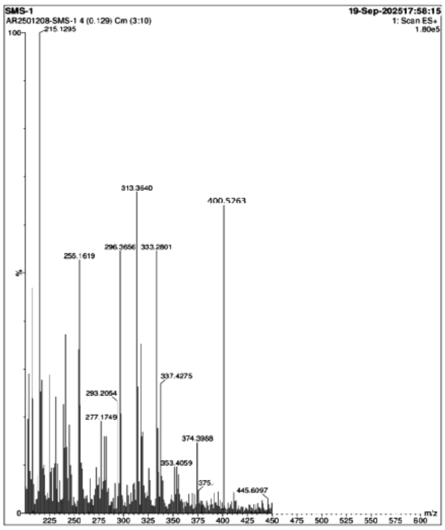


Fig-3: ^{13}C-NMR spectra of SMS-1

δ: 8.85 (2H, H_{10} H_{11}), 7.315 (2H, H_5 H_{16}), 7.148 (2H, H_{12} H_{13}), 7.931-7.430(12H, Ar-CH).

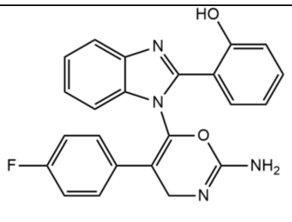
d) Mass spectra



(m/z): 400.5236 M^{2+}

Fig-4: Mass spectra of SMS-1

Table-3: Physical properties and Spectral data of compound SMS-3

Compound code	**SMS-3**					
Structure	 SMS-3					
IUPAC Name	2-(1-(2-amino-5-(4-fluorophenyl)-4H-1,3-oxazin-6-yl)-1Hbenzo[d]imidazol-2-yl)phenol					
Physical Properties	Mol formula & weight	Colour	M.P range	Rf Value	Solubility	%Yield
	$C_{23}H_{17}FN_4O_2$ 400.41g/mol	Pale green	160-163^{0}C	0.83	Methanol, DMSO	71.22%

a) IR (KBr) cm^{-1}

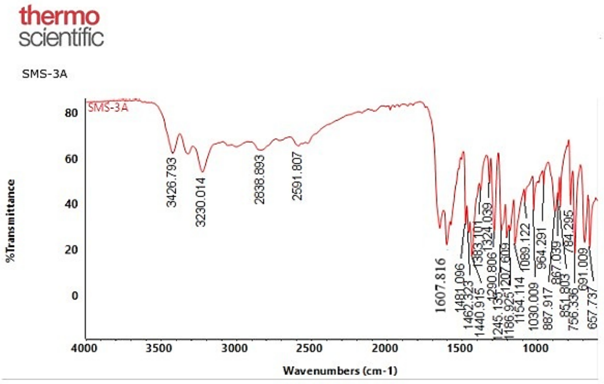
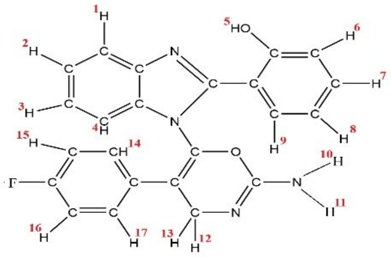


Fig-5: IR spectra of SMS-3

3426.793(NH str), 3230 (OH Str), 2838 (Ar C-H Str), 1607 (C=C Str), 1481 (C=N str), 1290 (C-F str), 1245 (C-O-C str).

b) ^{1}H-NMR



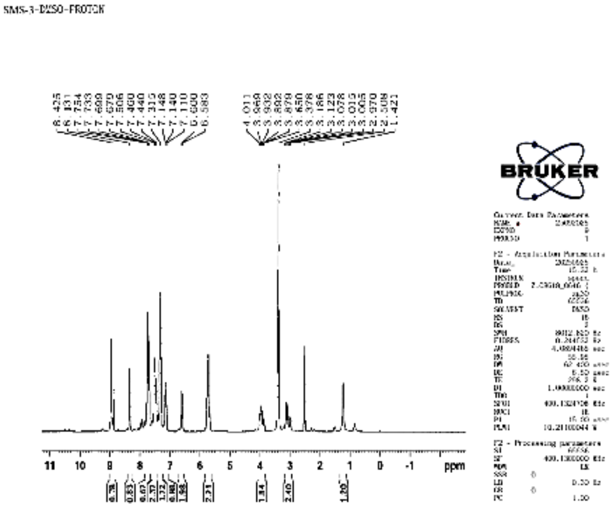


Fig-6: ^{1}H-NMR spectra of SMS-3

δ:8.426 (1H,H_4), 8.131-7.440 (11H, Ar-H), 7.315 (2H, H_{10} H_{11}), 7.148 (1H, H_5), 7.140(2H, H_{12} H_{13}).

c) ^{13}C-NMR

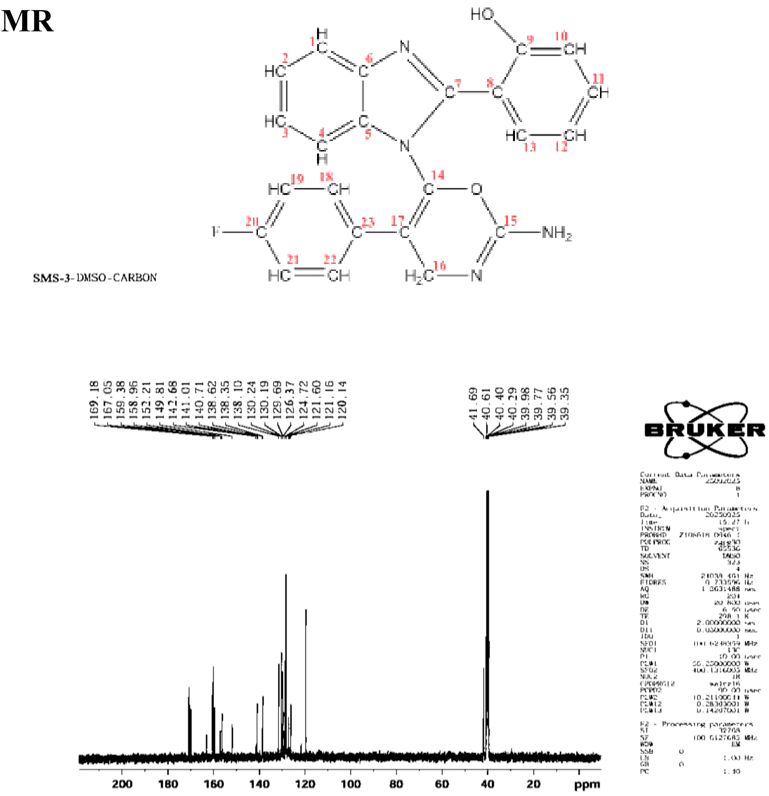
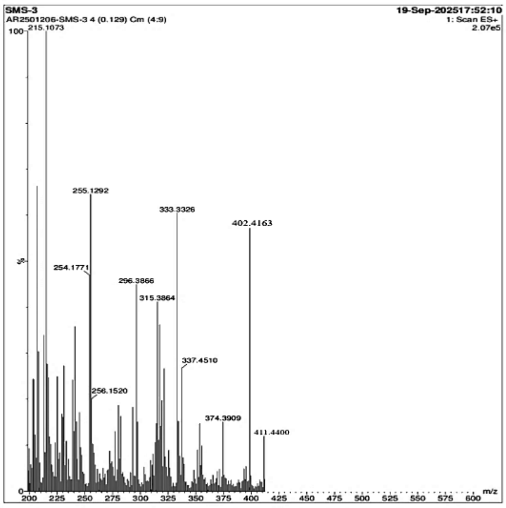


Fig-7: ^{13}C-NMR spectra of SMS-3

δ: 169.18 (C$_{14}$), 167.05 (C$_{20}$), 159.38 (C$_9$), 158.96 (C$_{15}$), 152.21-121.60 (17 Ar-C), 121.16 (C$_{17}$), 120.14 (C$_{16}$).

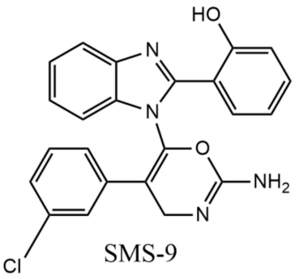
d) Mass spectra



(m/z): 402.4163 M^{2+}

Fig-8: Mass spectra of SMS-3

Table-4: Physical properties and Spectral data of compound SMS-9

Compound code	**SMS-9**					
Structure	 SMS-9					
IUPAC Name	2-(1-(2-amino-5-(3-chlorophenyl)-4H-1,3-oxazin-6-yl)-1Hbenzo[d]imidazol-2-yl)phenol					
Physical Properties	Mol formula & weight	Colour	M.P range	Rf Value	Solubility	%Yield
	$C_{23}H_{17}ClN_4O_2$ 416.87g/mol	Off-brown	136-140^{0}C	0.88	Methanol, DMSO	70.14%

a) **IR (KBr cm-^{1})**

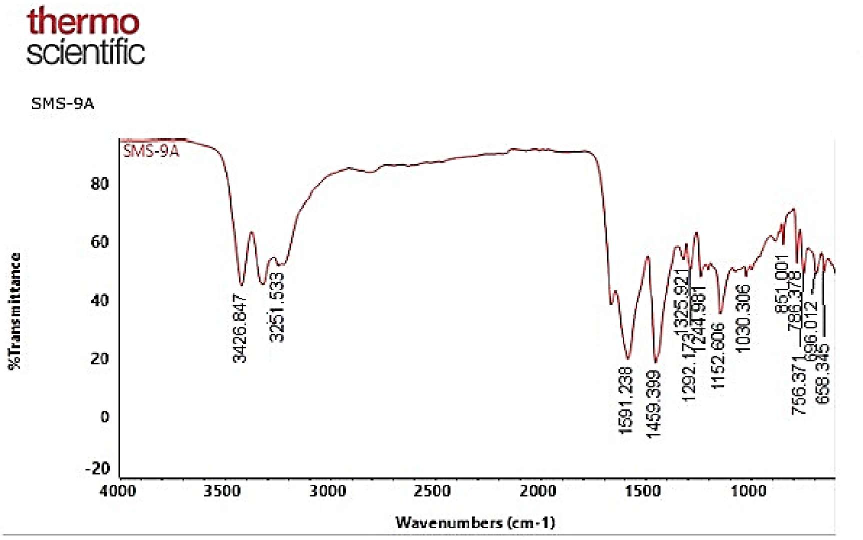
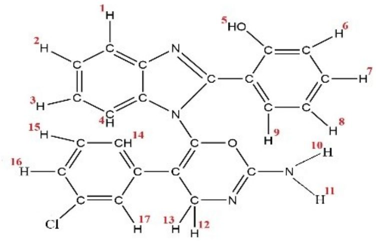


Fig-9: IR spectra of SMS-9

3426.84(NH str), 3251.53 (OH Str), 1591 (Ar C=C str), 1292 (C-O-C str),756.37 (C-Cl str).

b) ^{1}H-NMR



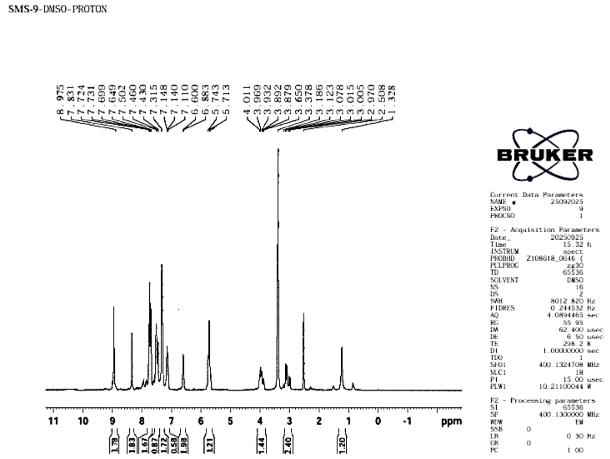
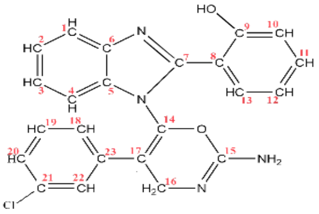


Fig-10: ^{1}H-NMR spectra of SMS-9

δ: 8.975 (2H, H_{10} H_{11}), 7.831(1H, H_9), 7.724-7.315 (10 Ar-H), 7.148 (1H, H_{14}), 7.140 (1H, H_5), 7.110 (2H, H_{12} H_{13}).

c) ^{13}C-NMR



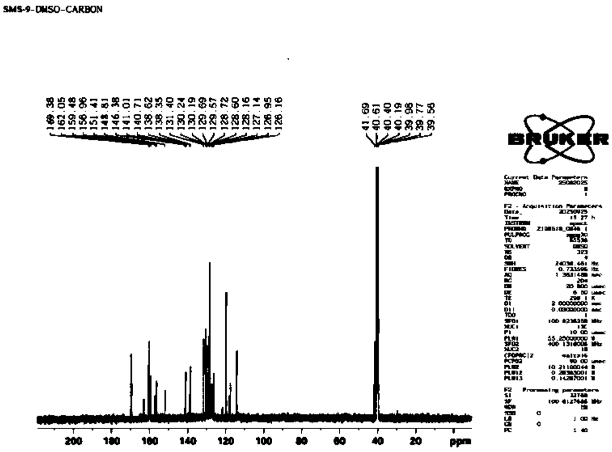
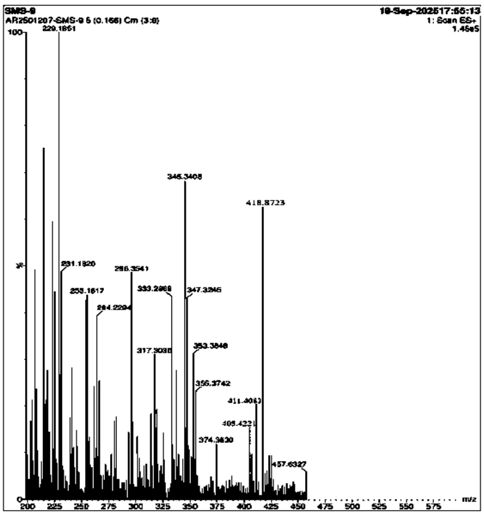


Fig-11: ^{13}C-NMR spectra of SMS-9

δ: 169.38 (C_{14}), 162.05 (C_9), 159.48 (C_{15}), 156.96-127.14(18Ar-C),126.95(C_{17}), 126.16 (C_{16}).

d) Mass spectra



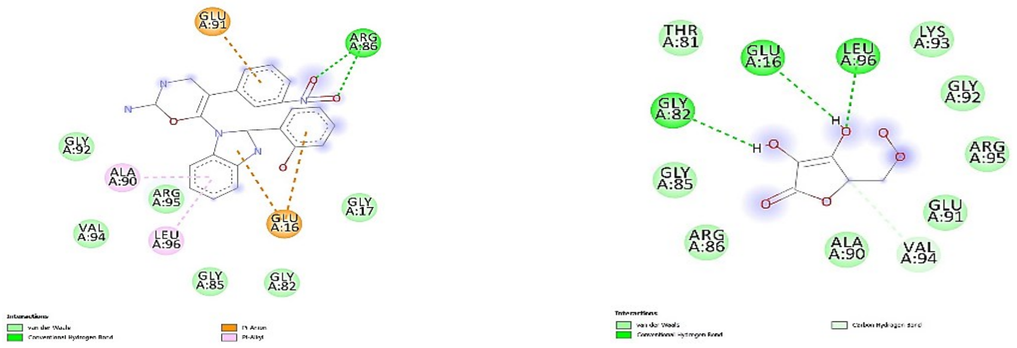
(m/z): 418.8723 M^{2+}

Fig-12: Mass spectra of SMS-9

DOCKING STUDIES

Table-5: *In-Silico* Screening for Antioxidant activity with PDB ID: 1HD2

Ligand	Binding Affinity (Kcal/mol)
SMS-1	-9.9
SMS-2	-10.7
SMS-3	-11.2
SMS-4	-10.9
SMS-5	-9.3
SMS-6	-9.7
SMS-7	-9.1
SMS-8	-9.4
SMS-9	-11.1
SMS-10	-11.6
Ascorbic acid	-8.3

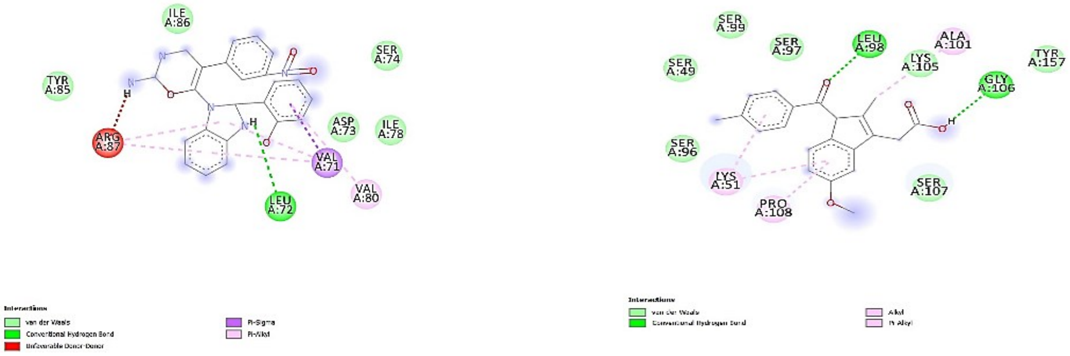


SMS-10 **STD**

Fig-13: Docking interactions for anti-oxidant activity with PDB ID:1HD2

Table-6: *In-Silico* Screening for Anti-inflammatory activity with PDB ID: 5HN1

Ligand	Binding affinity (Kcal/mol)
SMS-1	-9.6
SMS-2	-8.7
SMS-3	-9.9
SMS-4	-10.5
SMS-5	-9.8
SMS-6	-8.6
SMS-7	-9.8
SMS-8	-9.4
SMS-9	-11.5
SMS-10	-11.8
Indomethacin	-9.4



SMS-10 **STD**

Fig-14: Docking interactions for anti-oxidant activity with PDB ID: 5HN1

Table-7: *In-Silico* Screening for Analgesic activity with PDB ID: 1ZXM

Ligand	Binding affinity (Kcal/mol)
SMS-1	-9
SMS-2	-9.3
SMS-3	-9.6
SMS-4	-8.9
SMS-5	-7.3
SMS-6	-7.7
SMS-7	-8.4
SMS-8	-7.2
SMS-9	-9.4
SMS-10	-8.6
Diclofenac	-7.3

SMS-3 **STD**

Fig-15: Docking interactions for analgesic activity with PDB ID: 1ZXM

Table-8: *In-silico* Toxicity studies of synthesized compounds

Compound code	*Predicted LD_{50} (mg/kg)*	*Predicted Toxicity Class*	*Hepatotoxicity*	*Carcinogenicity*	*Immunotoxicity*	*Mutagenicity*	*Cytotoxicity*
SMS-1	800	4	+	-	+	-	-
SMS-2	595	4	+	+	+	+	-
SMS-3	595	4	+	-	+	-	-
SMS-4	1000	4	+	-	+	-	-
SMS-5	1190	4	-	-	+	-	-
SMS-6	1190	4	+	-	+	-	-
SMS-7	1190	4	+	-	+	-	-
SMS-8	1190	4	+	-	+	-	-
SMS-9	1000	4	+	-	+	-	-
SMS-10	1000	4	+	+	+	+	-

Active = ‘+’ and Inactive = ‘–’

Table-9: *In-Silico* Physicochemical studies obtained from Molinspiration

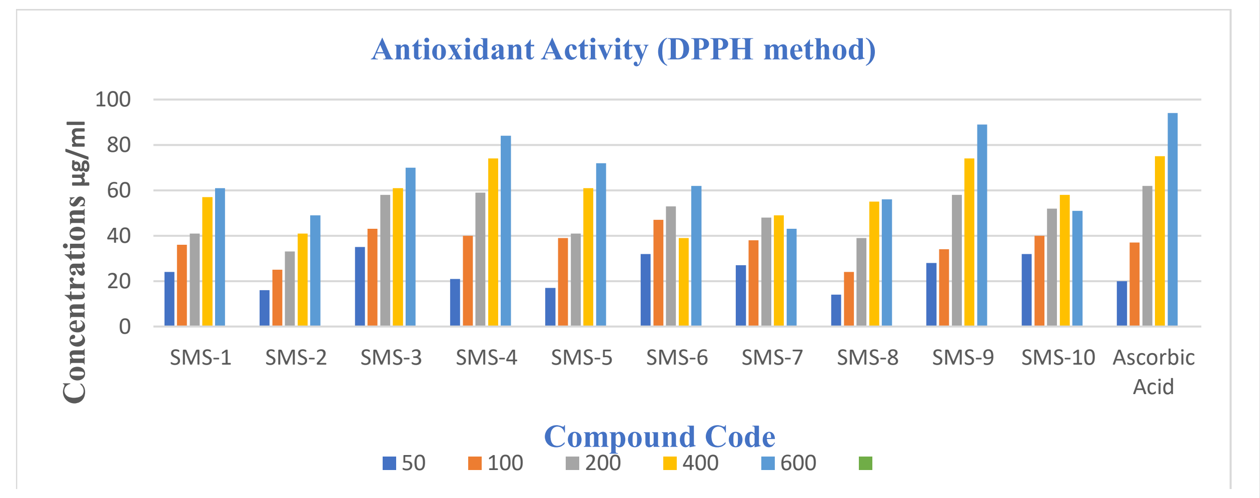
Compound Code	*miLogP*	*TPSA*	*natoms*	*MW (g/mol)*	*nON*	*nOHNH*	*n violation*	*n rotb*	*Volume*
SMS-1	3.60	105.90	30	398.42	7	4	0	3	345.01
SMS-2	4.04	131.50	32	427.42	9	3	0	4	360.33
SMS-3	4.25	85.67	30	400.41	6	3	0	3	341.92
SMS-4	4.76	85.67	30	416.87	6	3	0	3	350.53
SMS-5	4.14	94.91	31	412.45	7	3	0	4	362.54
SMS-6	5.00	85.67	31	410.48	6	3	0	4	370.35
SMS-7	4.19	88.91	32	425.49	7	3	0	4	382.90
SMS-8	3.72	113.38	35	472.50	9	3	0	6	413.63
SMS-9	4.74	85.67	30	416.87	6	3	0	3	350.53
SMS-10	4.02	131.50	32	427.42	9	3	0	4	360.33

Table-10: *In-silico* ADME properties obtained from SwissADME

Compound code	**SMS-1**	**SMS-2**	**SMS-3**	**SMS-4**	**SMS-5**	**SMS-6**	**SMS-7**	**SMS-8**	**SMS-9**	**SMS-10**
No. heavy atoms	32	34	30	30	31	31	32	35	30	32
No. Ar. Heavy atoms	21	21	21	21	21	21	21	21	21	21
No. Rotatable bonds	3	4	3	3	4	4	4	6	3	4
No. H-bond acceptors	5	6	5	4	5	4	4	7	4	6
No. of H-bond donors	3	2	2	2	2	2	2	2	2	2
Molar refractivity	130.44	137.24	117.12	122.17	123.65	126.93	131.37	136.64	122.17	125.98
Total Polar Surface Area(Å)	105.89	131.48	85.66	85.66	94.89	85.66	88.90	113.35	85.66	131.48
Log Po/w (ilogp)	3.99	3.60	2.84	2.75	3.13	3.30	2.95	3.25	2.99	2.20
Water solubility	Soluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble
GI absorption	High	Low	high	High	High	High	High	High	High	Low
BBB permeant	No	No	No	No	No	No	No	No	No	No
Drug likeness (violation)	Yes; (0)	Yes; (0)	Yes; (0)	Yes; (0)	Yes; (0)	Yes; (0)	Yes; (0)	Yes; (0)	Yes; (0)	Yes; (0)
Lead likeness (violation)	No; (2), MW>350	No; (2), MW>350	No; (2), MW>350	No; (2), MW>350	No; (1), MW>350	No; (2) MW>350	No; (2) MW>350	No; (1), MW>350	No; (), MW>350	No; (1) MW>350

Table-11: *In-vitro* antioxidant activity by DPPH method

SI. No	*Compound Code*	*% Inhibition*					*IC_{50} µg/ml*
		50 µg/ml	**100 µg/ml**	**200 µg/ml**	**400 µg/ml**	**600 µg/ml**	
1	***SMS-1***	24	36	41	57	61	436.48
2	***SMS-2***	16	25	33	41	49	616
3	***SMS-3***	35	43	58	61	70	285.71
4	***SMS-4***	21	40	59	74	84	303.174
5	***SMS-5***	17	39	41	61	72	380
6	***SMS-6***	32	47	53	39	62	380
7	***SMS-7***	27	38	48	49	43	840.624
8	***SMS-8***	14	24	39	55	56	521.42
9	***SMS-9***	28	34	58	74	89	248.36
10	***SMS-10***	32	40	52	58	51	600
11	**Ascorbic acid**	20	37	62	75	94	272.94



. **Fig-16:** Graphical representation of *in-vitro* antioxidant activity by DDPH method

Fig-17: DPPH Experimentation

Table-12: *In-vitro* anti-inflammatory activity by BSA denaturation method

SI. No	***Compound code***	***% Inhibition***				***IC_{50} μg/ml***
		50 μg/ml	***100 μg/ml***	***200 μg/ml***	***400 μg/ml***	
1	***SMS-1***	7	12	26	34	607.4
2	***SMS-2***	5	10	19	31	655.769
3	***SMS-3***	8	15	38	52	384.09
4	***SMS-4***	6	12	31	41	490
5	***SMS-5***	3	12	33	51	392.7
6	***SMS-6***	9	23	35	48	419.9
7	***SMS-7***	8	11	29	39	524.19
8	***SMS-8***	6	24	38	52	384.78
9	***SMS-9***	6	10	22	38	531.25
10	***SMS-10***	9	21	30	35	601.9
11	***Indomethacin***	10	14	32	51	391.4

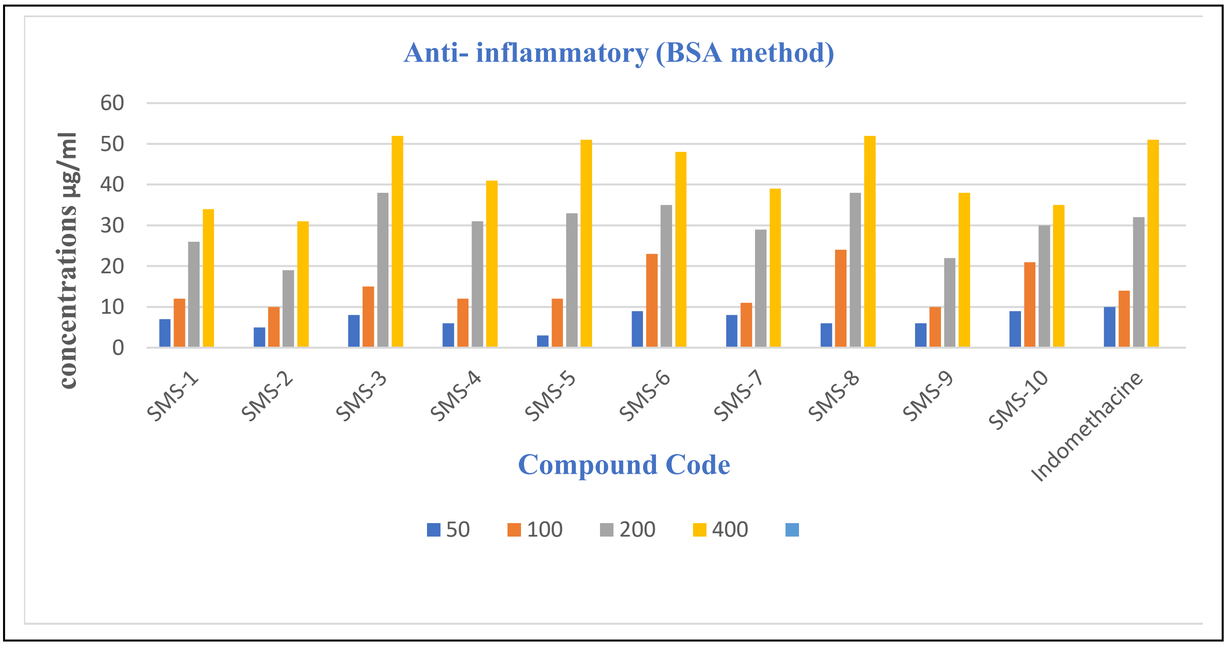


Fig-18: Graphical representation of *in-vitro* anti-inflammatory activity by BSA denaturation method

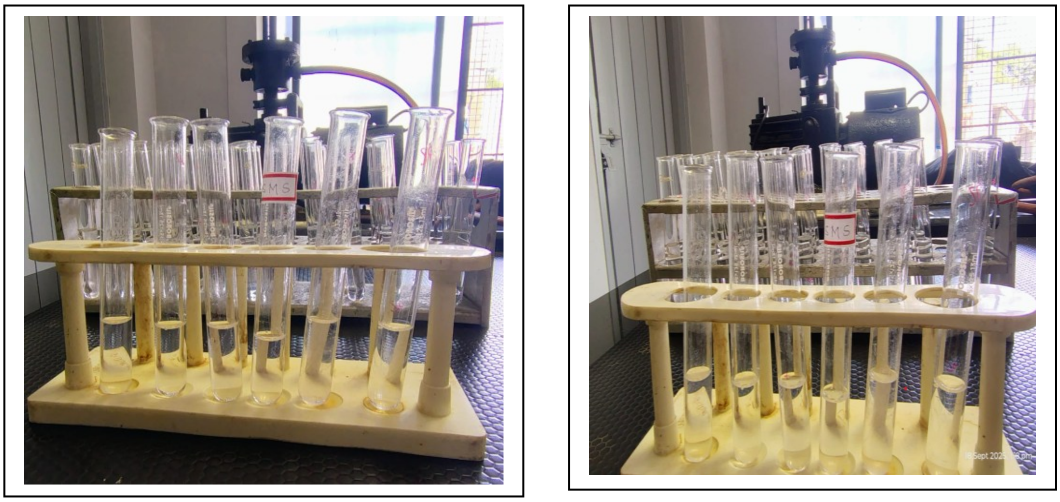


Fig-19: BSA Experimentation

In-vivo analgesic activity by acetic acid induced writhing method:

Table-13: Effect of analgesic activity of synthesized oxazine compound against acetic acid induced writhing method.

SI. No	Groups	Body weight (grams)	Pre-treatment time	Dose and route	Total Writhing Events	% Reversal
01	**Acetic acid**	28.5 ± 0.428	60-min	1ml/100gm (IP)	23.833±0.792	0
02	**Standard**	28.333±0.843	60-min	20mg/kg (IP)	13.5±0.48	59.16 ± 3.187
03	**SMS-1**	28.833±0.654	60-min	20mg/kg (IP)	10.333±0.428	43.58 ± 2.640
04	**SMS-3**	29 ± 0.632	60-min	20mg/kg (IP)	16.833±0.703	71.29 ± 4.793
05	**SMS-9**	29.333±0.714	60-min	20mg/kg (IP)	12 ± 0.856	58.26 ± 4.666

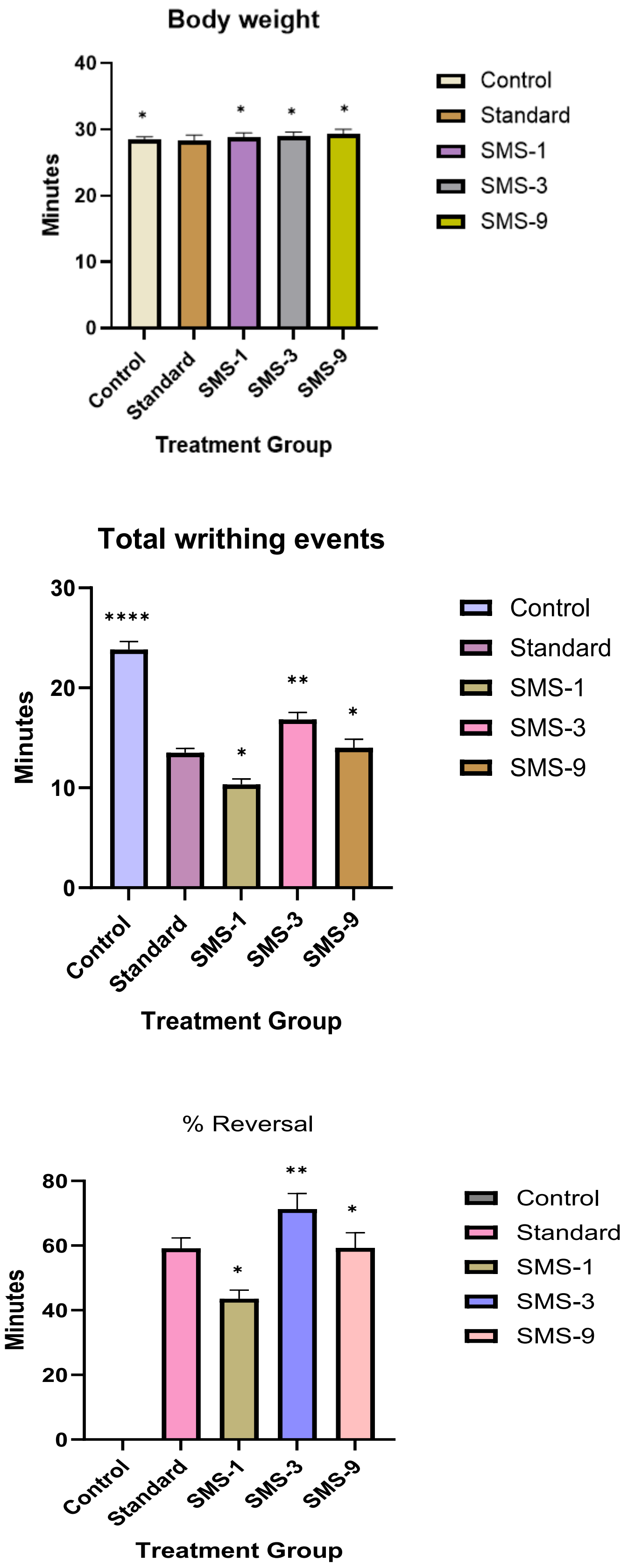


Fig-20: Graphical representation of *in-vivo* analgesic activity by acetic acid induced writhing method

6. CONCLUSION:

The main objective of the present dissertation was to synthesize, characterise, to evaluate *in-silico* studies and biological activity of novel oxazine derivatives.

STEP-1: The reaction was carried out between *ortho*-Phenylenediamine and Salicylic acid to obtain of 2-(1H-Benzimidazole-2-yl) phenol. It is a type of condensation reaction, where the two amine groups (*o*-phenylenediamine) react with the carboxylic acid (Salicylic acid) to form an Benzimidazole derivative.

STEP-2: Acetylation reaction with substituted Benzimidazole is known as Schotten-Baumann reaction. This reaction typically forms an α, β-unsaturated carbonyl compound.

STEP-3: Claisen-Schmidt reaction (aldol-condensation) reaction with substituted Benzaldehydes, this reaction involves the synthesis of Chalcone intermediate.

STEP-4: The reaction between chalcones and Urea to form oxazine moieties is known as the Cyclization reaction. Here which then undergoes intramolecular cyclization to form Oxazine ring.

The structure of all synthesized oxazine derivatives was established by IR, ^{1}H-NMR, ^{13}C-NMR and MS.

The synthesized compounds were screened for *in-silico, in-vitro* and *in-vivo* studies on different biological activities as following;

Antioxidant activity:

- ***In-silico*: PDB ID-1HD2**

  Compounds SMS-10(M-NO_2), SMS-3(P-F) and SMS-9(M-Cl) showed binding affinity of –-11.6 kcal/mol, -11.2 kcal/mol and -11.1 kcal/mol with **1HD2** and exhibited higher binding affinity than ascorbic acid (-8.3 kcal/mol).
- **In *in-vitro* antioxidant activity**

  compound (SMS-9) displayed an excellent activity among the synthesized compounds by IC_{50} = 248.36µg/ml in DPPH assay.

Anti-inflammatory activity:

- ***In-silico*: PDB ID-5HN1**

  Compound SMS-10(M-NO_2) and SMS-9(M-Cl) showed binding affinity of -11.5 kcal/mol greater than binding affinity of indomethacin (-9.4 kcal/mol).

- ***In-vitro* anti-inflammatory activity**

  Among all the synthesized compounds screened for *in-vitro* anti-inflammatory activity by BSA method, compounds SMS-3(F) and SMS-8(3,4,5-OCH_3) exhibited excellent activity by IC_{50} = 384.09µg/ml and 384.78 µg/ml in BSA assay while standard (Indomethacin) exhibited IC_{50} = 391.4µg/ml preventing denaturation of BSA protein respectively.

Analgesic activity:

- ***In-silico*: PDB ID-1ZXM**

  Compound SMS-3(P-F), SMS-9(M-Cl), SMS-2(P-NO_2), SMS-1(P-OH) showed binding affinity of -9.6 kcal/mol, -9.4 kcal/mol, -9.3 kcal/mol and -9.0 kcal/mol respectively with protein **1ZXM** (Crystal Structure of ATPase domain Human DNA Topoisomerase-II) and exhibited higher binding affinity than Diclofenac (-7.3 kcal/mol).

- ***In-vivo* studies: Acetic acid induced writhing method**

  SMS compounds and Diclofenac were effective in managing the pain induced by Acetic acid in mice. SMS-1 and SMS-9 treatment showed comparable efficacy to Diclofenac. SMS-1 and SMS-9 successfully reducing the number of writhing’s in mice to 10.333±0.5577 and 12±0.856 when compared to standard Diclofenac 13.5±0.4281 recorded for 15-mins of time interval.

The *in-silico* studies of the synthesized oxazine derivatives (SMS 1-10) indicate promising potential for therapeutic use, highlighted by their favourable bioactive safety profiles. Toxicity predictions using ProTOX-II demonstrate that most compounds fall into Class IV, suggesting a high level of safety with no significant hepatotoxic, cytotoxic, mutagenic, or carcinogenic properties. The lipophilic profiles, adherence to Lipinski's rule of five, and moderate solubility in water further support their drug-like properties.

Specifically, compounds SMS-10, SMS-9 and SMS-3 show promising scores correlating with their *in-vitro* and *in-vivo* efficacy in antioxidant and anti-inflammatory applications.

The SwissADME analysis confirms that these novel compounds possess acceptable pharmacokinetic profiles, with moderate solubility and favourable absorption characteristics. Overall, these findings underscore the therapeutic potential of the synthesized oxazine derivatives, paving the way for further experimental validation and development into effective drug candidates.

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