SYNTHESIS, *IN VIVO* ANTI-DEPRESSANT EVALUATION AND COMPUTATIONAL STUDIES OF NOVEL BENZIMIDAZOLE-PYRIMIDINONE DERIVATIVES

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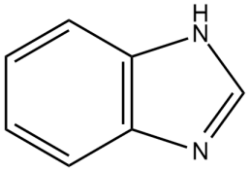
Abstract - In the present work, an attempt has been made to synthesize some novel benzimidazole-pyrimidinone derivatives with significant antidepressant, anti-inflammatory, antioxidant, and antimicrobial activity along with their *in-silico* studies.

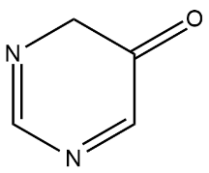
To acquire the desired molecules, the synthetic route commenced with condensation of o-phenylenediamine with 4-methoxybenzoic acid to form a benzimidazole ring. Subsequently, the resulting compound underwent a reaction with acetyl chloride to form an ethanone which served as the key intermediate compound. Later the intermediates were substituted with different substituted benzaldehydes to get the chalcones (MBC 1-10) by claisen-schmidt condensation reaction. Finally, cyclo-condensation reaction between chalcone derivative and urea to form novel benzimidazole-pyrimidinone hybrids (ANRP 1-10).

The formation of the final product was characterized by UV, IR, LCMS, 'H NMR, ^{13}C NMR and Elemental analysis. *In-silico* studies demonstrated high binding affinities of ANRP-9, ANRP-3, ANRP-8, and ANRP-10 towards 1Q3D, 1HD2, 1M17, 1KZN correlating with significant anti-depressant, anti-oxidant, anti-inflammatory and anti-microbial activities.

The synthesized compounds were screened for *in-vivo* antidepressant activity and compound ANRP-6 exhibited excellent activity in the Forced swim test. The synthesized compounds had also exhibited good *in-vitro* anti- inflammatory, antioxidant and antimicrobial activity. Overall, the synthesized pyrimidinone derivatives demo demonstrated significant therapeutic potential.

Keywords: In-silico studies, anti-oxidant activity, anti-inflammatory activity, anti-microbial activity,





1. INTRODUCTION

Pyrimidinone, i.e., pyrimidin-2(1H)-one and pyrimidin-4(3H)- one, the carbonyl derivative of diazine heterocycle pyrimidine, occupies a vantage position among nitrogen containing heterocyclic compounds. The privileged scaffold has displayed critical value as a precursor in many synthetic and drug design protocols[1].

These compounds displayed therapeutic applications, as anticancer[2,3], antihypertensive[4], hypoglycemic[5], antiviral[6], anticonvulsive[7], anti-inflammatory and analgesic drugs[8].

Pyrimidine nucleus is the main reason of many therapeutic applications. Substitution in pyrimidine moiety offers an opportunity for development of newer drugs with better efficacy[9].

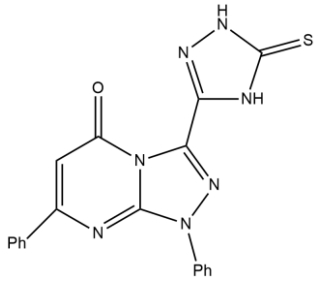
Benzimidazole, also known as 1*H*-benzimidazole, benzoglyoxaline, or 1,3-benzothiazole, is a bicyclic compound bearing a benzene ring fused to a five-membered imidazole that contains two nitrogen atoms[10].

2. OBJECTIVES

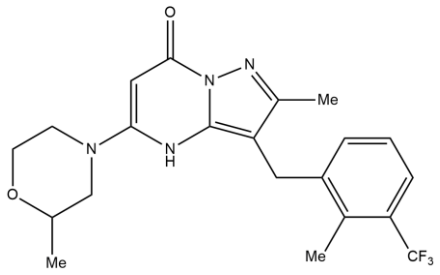
[1] Synthesis of some novel pyrimidinone derivatives.
[2] Characterization of newly synthesized derivatives using spectral studies.
[3] Drug likeliness property of the derivatives by using Swiss ADME and Molinspiration web servers.
[4] Online toxicity prediction.
[5] Molecular Docking studies by using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

3. LITERATURE REVIEW

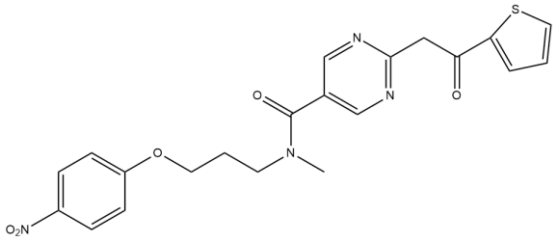
[1] Elkanzi N A and co-workers reported the efficient procedures for the synthesis of pyrimidinedione and pyrimidinethione derivatives. The structure of these compounds was established based on spectral and elemental analysis like IR, ^{1}H NMR, ^{13}CNMR. The experimental results shows that the prepared product displayed outstanding pharmacological activities when screened *in-vitro* against gram positive and gram-negative strains and could be further exploited in medicinal chemistry[11].



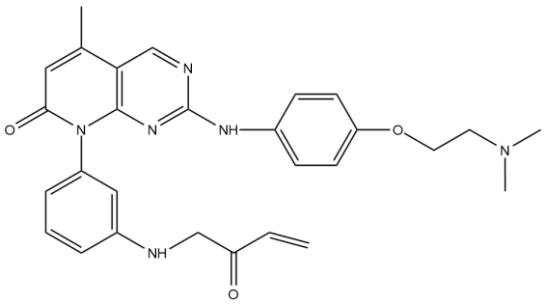
[2] Kelada M and co-workers had developed a new facile method for the one-pot microwave-assisted synthesis of substituted pyrazolo pyrimidinones. The structures of the pyrazolo pyrimidinones were characterized using ^{1}H and ^{13}C NMR spectral data, HRMS and IR spectroscopy[12].



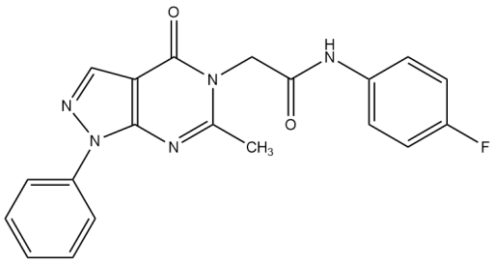
[3] Han C and co-workers, designed, synthesized and evaluated a novel series of pyrimidinone derivatives as acetylcholinesterase inhibitors (AChEIs) for the treatment of Alzheimer's disease (AD). The target compounds were characterized by ^{1}H NMR, ^{13}C NMR, HRMS, and IR spectra. Biological activity results demonstrated that compound showed the best inhibitory activity against AChE, which was better than that of Huperzine-A[13].



[4] Alasfoury R and co-workers, synthesized a new series of 6,8- diaryl pyrido thiazolo pyrimidinones. Anticancer evaluation was performed through screening for these compounds against MCF-7, PC-3, HCT-116 and A-549 cancerous cell lines at a dose of 100 in comparison with erlotinib[14].



[5] Zaki W and co-workers, designed and synthesized a series of new pyrazolo[3,4-d] pyrimidine analogs and evaluated as anticancer agents against the CRC and HCC cells, HCT116, and HepG2. *In silico* molecular docking provided insights into the molecular interactions with important amino acids within the ATP-binding site of CDK2[15].



4. METHODOLOGY

SYNTHETIC SCHEME:

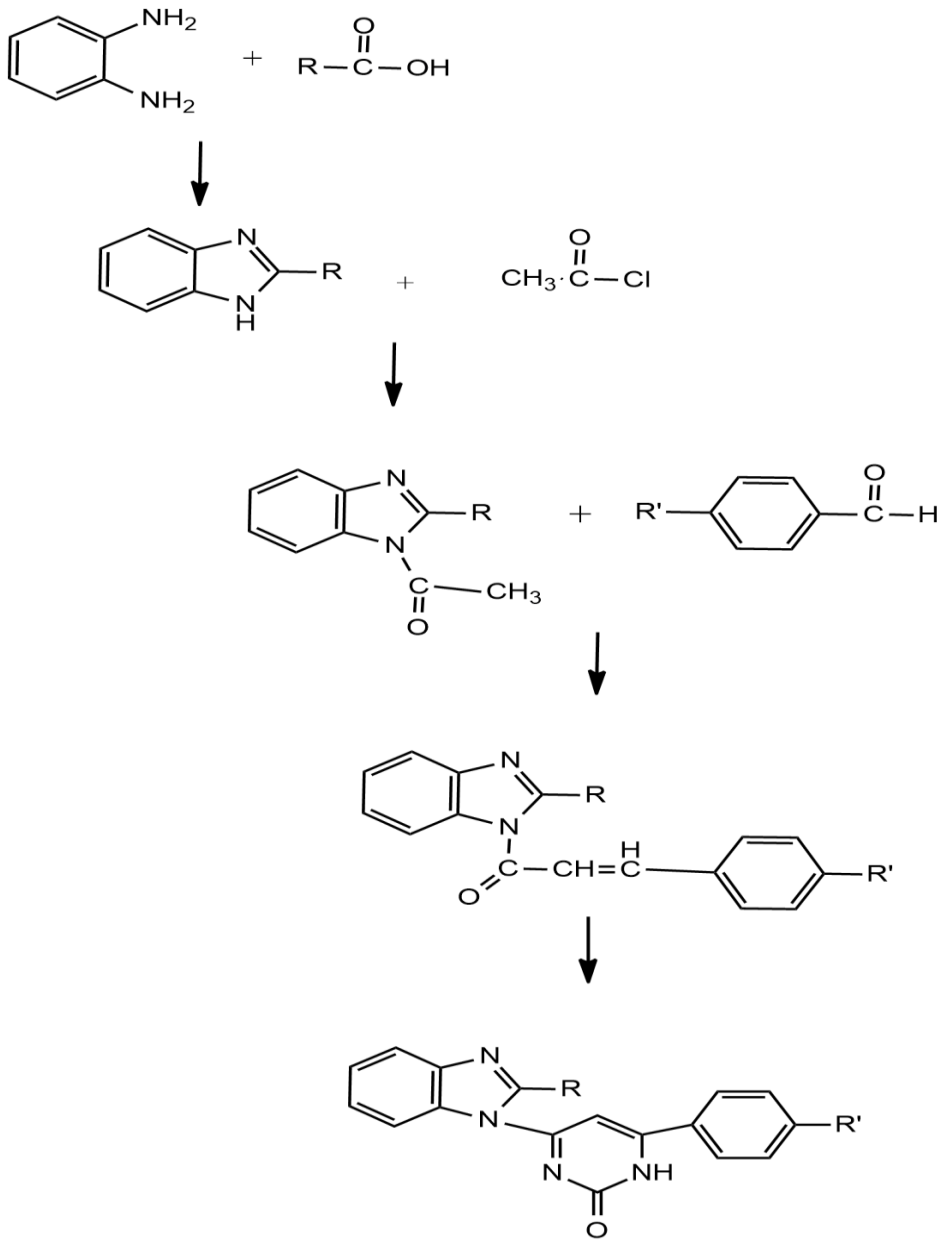
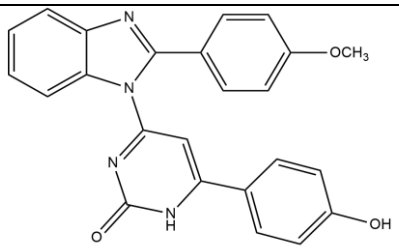
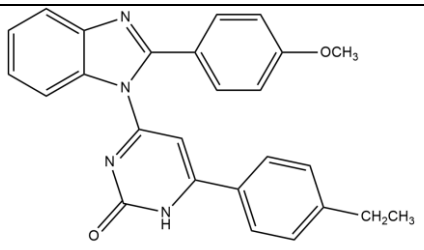
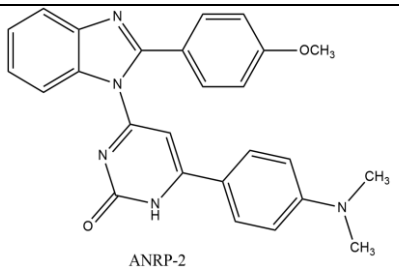
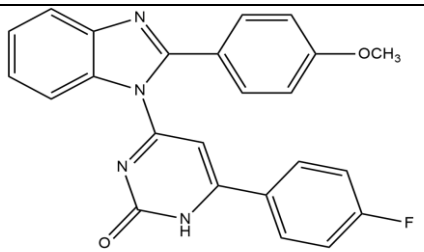
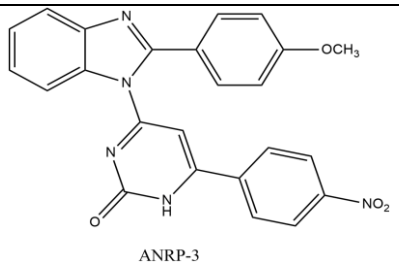
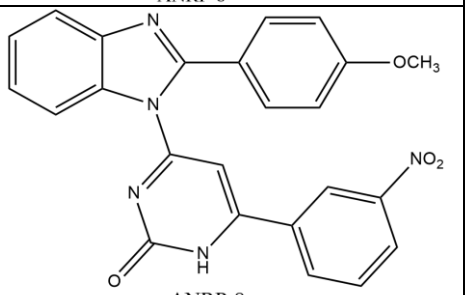
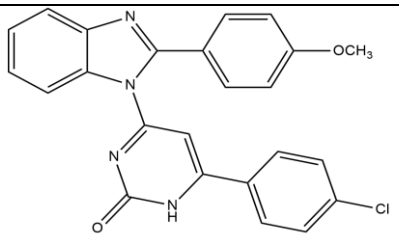
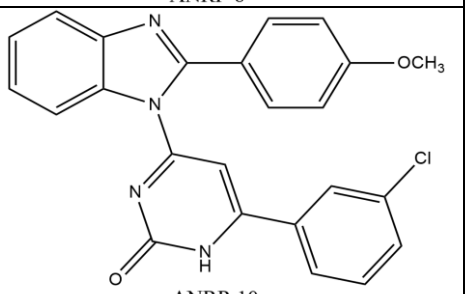
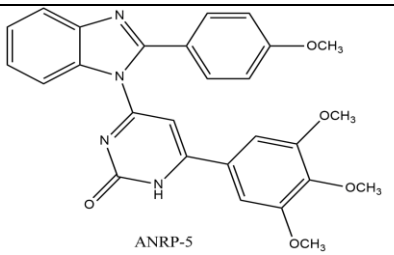
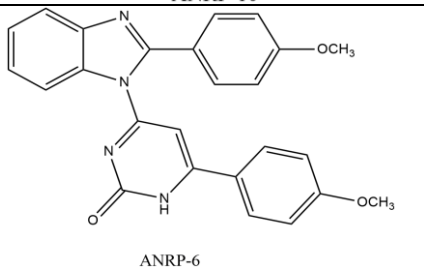


Table 1: List of the Novel Pyrimidinone derivatives (ANRP 1-10)

CODE	STRUCTURE	CODE	STRUCTURE
ANRP-1	 ANRP 1	ANRP-7	 ANRP-7
ANRP-2	 ANRP-2	ANRP-8	 ANRP-8
ANRP-3	 ANRP-3	ANRP-9	 ANRP-8
ANRP-4	 ANRP-4	ANRP-10	 ANRP-10
ANRP-5	 ANRP-5	ANRP-6	 ANRP-6

Procedure for the synthesis of novel Benzimidazole-Pyrimidinones:

STEP-1: *o*-Phenylene diamine (15 mmol) and substituted benzoic acid (30 mmol) were stirred in 4N HCl (40ml) under reflux for 5 hours. After cooling at room temperature, the pH was djusted to 7.0 with NaOH (Solid). The resulting solid was filtered, washed with cold water, dried in vacuum pump and recrystallized from the alcohol[16].

STEP-2: 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[17].

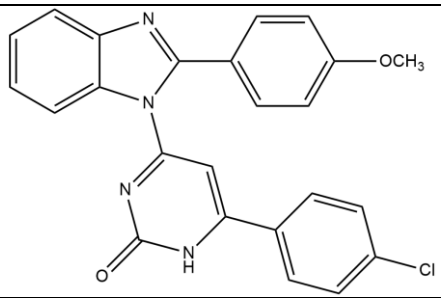
STEP-3: N-Acetyl- benzimidazole derivative (0.01 mole, 1.73g) was dissolved in ethanol and sodium hydroxide solution (30 ml, 40%) was added and the mixture was cooled. To this was added (0.01 mole, 1.51g) nitro-benzaldehyde dissolved in a minimum quantity of ethanol and then the reaction mixture was stirred for a period of 4-5 hours and was left overnight. Conc.HCl was added drop by drop till the solution was slightly acidic. The solid separated was filtered, washed with water and dried. The crude product was crystallized from aqueous ethanol (50%). Purity was confirmed by thin layer chromatography using the solvent system, hexane: chloroform: methanol (60: 20: 20).

STEP-4: Chalcone (0.01mole), thiourea (0.02 mole; 1.52 g) and KOH (0.02 mole; 1.12 g) were taken in a 100 ml round bottom flask. To the above reaction mixture ethanol (30ml) was added then the reaction mixture was refluxed for 5 hrs. It was then cooled and poured into cold water. The product which appeared after acidification with dill HCl, was filtered, washed with water and dried, then was recrystalized from ethanol[18].

5. RESULTS

I. Physical properties and spectral data's of synthesized pyrimidinone derivatives

Table 2: Physical properties of 6-(4-chlorophenyl)-4-(2-(4-methoxyphenyl)-1H-benzo[d]6midazole-1-yl)pyrimidin-2(1H)-one (ANRP-4

Compound code	ANRP-4
Structure	
IUPAC name	6-(4-chlorophenyl)-4-(2-(4-methoxyphenyl)-1H-benzo[d]6midazole-1-yl)pyrimidin-2(1H)-one
Molecular formula	$C_{24}H_{17}ClN_4O_2$
Molecular weight	428.87

Colour	Off-white
M.P. range	166-169^{0}C
Rf value	0.80
Solubility	DMSO, Methanol, Ethanol
% yield	66.3%

Spectra data of 6-(4-chlorophenyl)-4-(2-(4-methoxyphenyl)-1H-benzo[d]7midazole-1-yl)pyrimidin-2(1H)-one (ANRP-4)

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

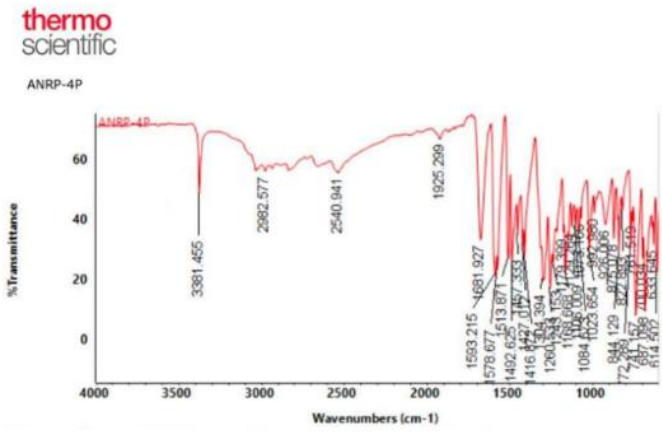


Fig 1: IR spectra of ANRP-4

3381 (N–H str), 2982 (C–H str), 1681 (C=O str), 1593 (C=C str), 1304 (C–N str), 1260 (C–O–C str), 822 (C–H oop bend).

2. **MS m/z**

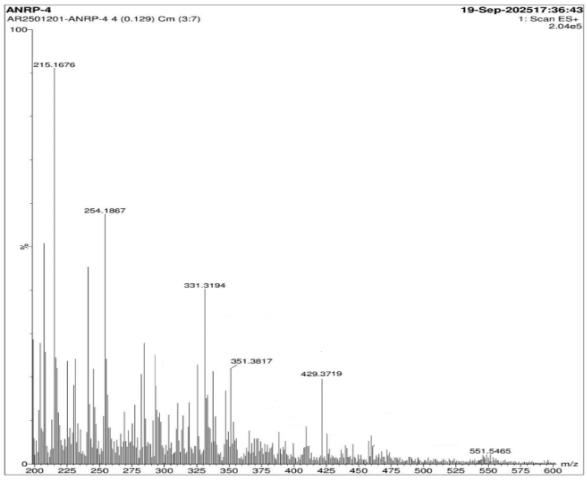


Fig 2: MS spectrum of ANRP-4

M^{+1} = 429.37

3. ^{1}H NMR

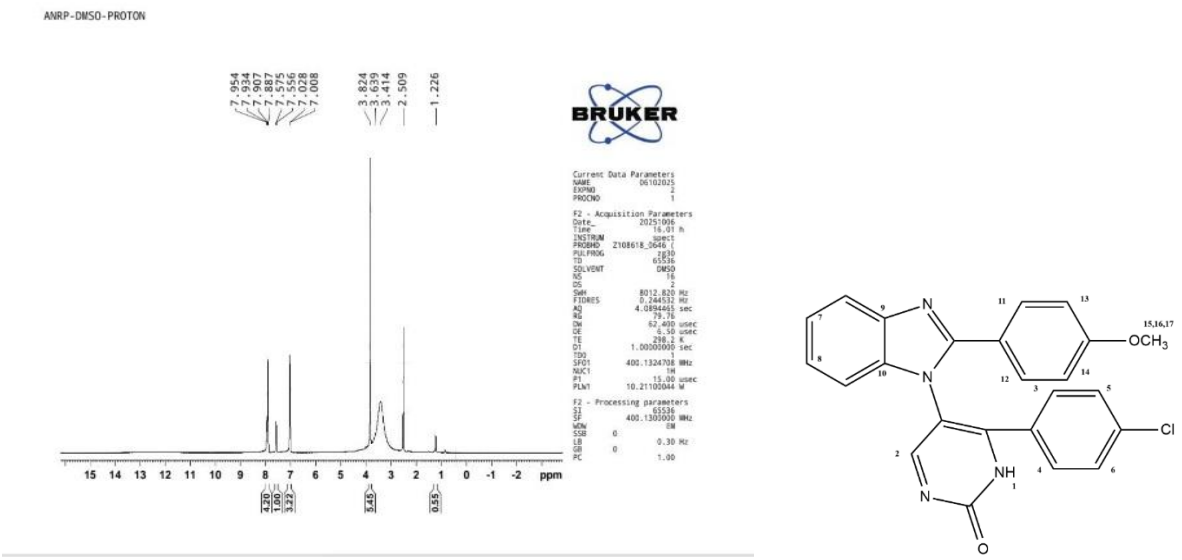


Fig 3: ^{1}H NMR spectrum of ANRP-4

δ:7.954(1H,H_1),7.887(1H,H_2),3.639(3H,H_{15},H_{16},H_{17})

4. ^{13}C NMR

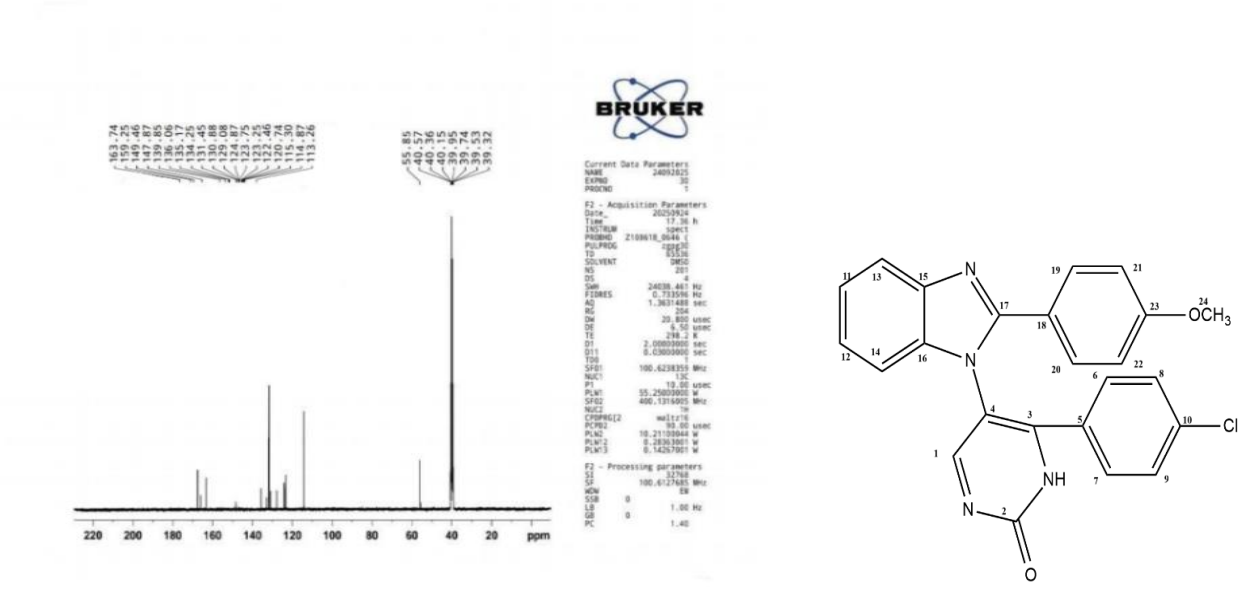
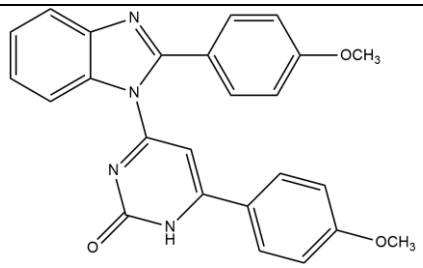


Fig 4: ^{13}C NMR spectrum of ANRP-4

δ: 163.74(C-1), 149.46(C-2), 113.26(C-4), 133.5(C-10), 159.25(C-23), 55.85(C-24).

Table 3: Physical properties of 6-(4-methoxyphenyl)-4-(2-(4-methoxyphenyl)-1H-benzo[d]imidazole-1-yl)pyrimidin-2(1H)-one (ANRP-6)

Compound code	ANRP-6
Structure	
IUPAC name	6-(4-methoxyphenyl)-4-(2-(4-methoxyphenyl)-1H-benzo[d]9midazole-1-yl)pyrimidin-2(1H)-one
Molecular formula	$C_{25}H_{20}N_4O_3$
Molecular weight	424.15
Colour	Dark yellow
M.P. range	177-180^{0}C
Rf value	0.82
Solubility	DMSO, Methanol, Ethanol
% yield	74.8%

Spectra data of 6-(4-methoxyphenyl)-4-(2-(4-methoxyphenyl)-1H-benzo[d]9midazole-1-yl)pyrimidin-2(1H)-one

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

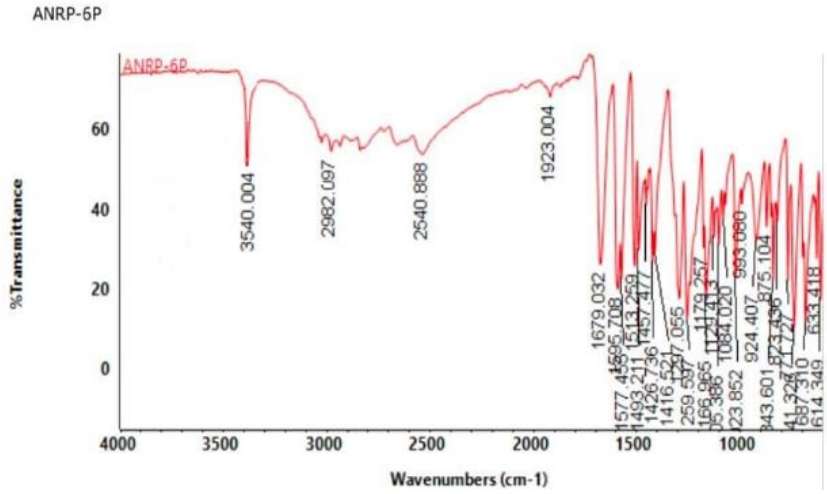


Fig 5: IR spectra of ANRP-6

3540 (N–H str), 2982 (C–H str), 1679 (C=O str), 1595 (C=C str), 1259 (C–O–C str), 823 (C–H oop bend).

2. MS m/z

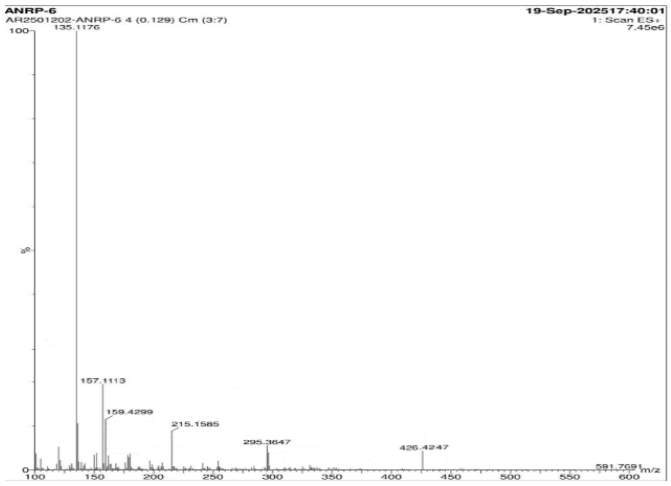


Fig 6: MS spectrum of ANRP-6

$M^{+1} = 426.42$

3. ^{1}H NMR

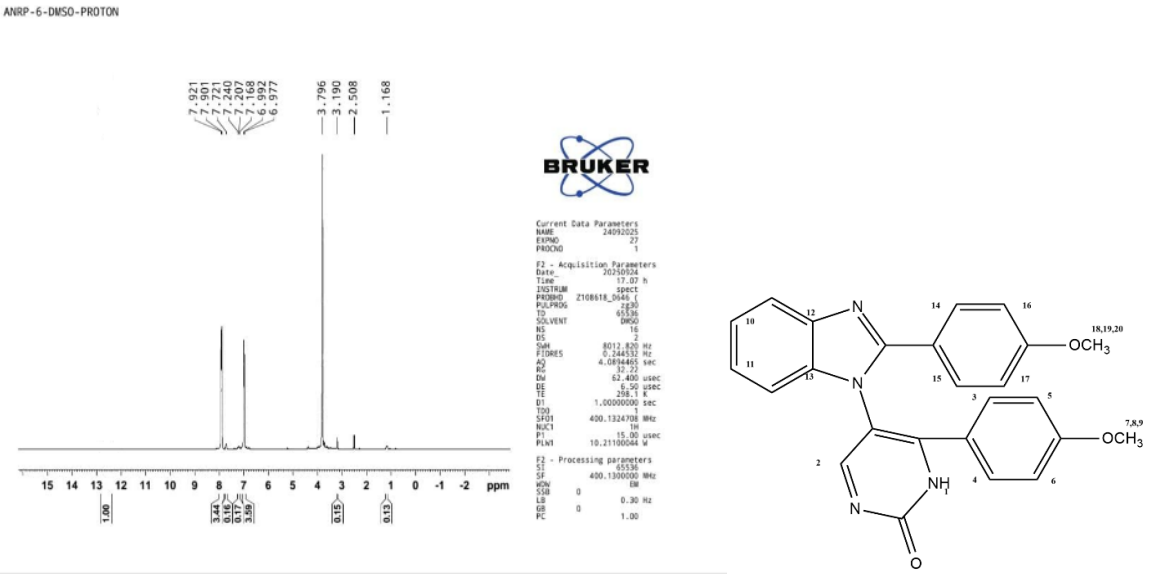


Fig 7: ^{1}H NMR spectrum of ANRP-6

δ: 7.921(1H,H_1), 7.207(1H,H_2), 7.240-6.977, 3.796(6H,$H_{7,8,9}$,$H_{18,19,20}$)

4. **^{13}C NMR**

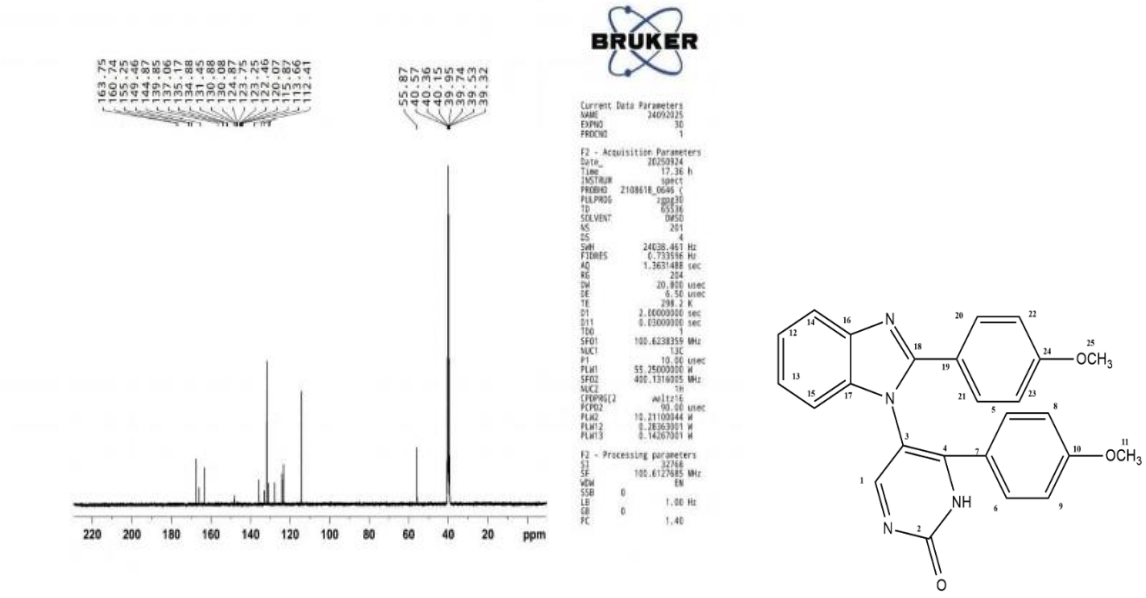
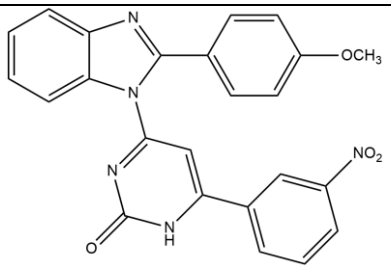


Fig 8: ^{13}C NMR spectrum of ANRP-6

δ: 163.75(C-1), 149.46(C-2), 112.41(C-3), 155.25(C-10), 55.87(C-11,25), 160.74(C-24).

Table 4: Physical properties of 4-(2-(4-methoxyphenyl)-1H-benzo[d]11midazole-1-yl)-6-(3-nitrophenyl)pyrimidin-2(1H)-one

Compound code	**ANRP-9**
Structure	
IUPAC name	4-(2-(4-methoxyphenyl)-1H-benzo[d]11midazole-1-yl)-6-(3-nitrophenyl)pyrimidin-2(1H)-one
Molecular formula	$C_{24}H_{17}N_5O_4$
Molecular weight	439.42
Colour	Cream
M.P. range	167-170^{0}C
Rf value	0.74
Solubility	DMSO, Methanol, Ethanol
% yield	60.9%

Spectra data of 4-(2-(4-methoxyphenyl)-1H-benzo[d]12midazole-1-yl)-6-(3-nitrophenyl)pyrimidin-2(1H)-one

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

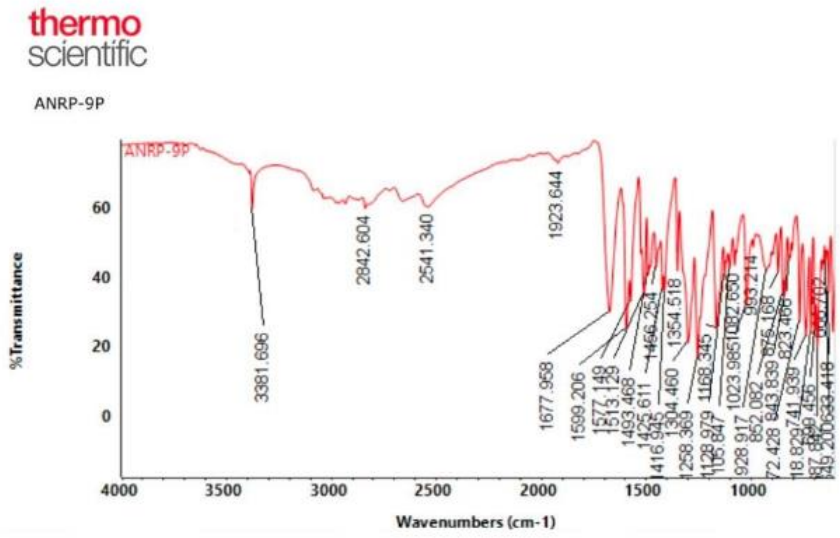


Fig 9: IR spectra of ANRP-9

3381 (N–H str), 2842 (C–H str), 1677 (C=O str), 1599 (C=C str), 1354 (C–N str), 1258 (C–O–C str), 823 (C–H oop bend).

2. **MS m/z**

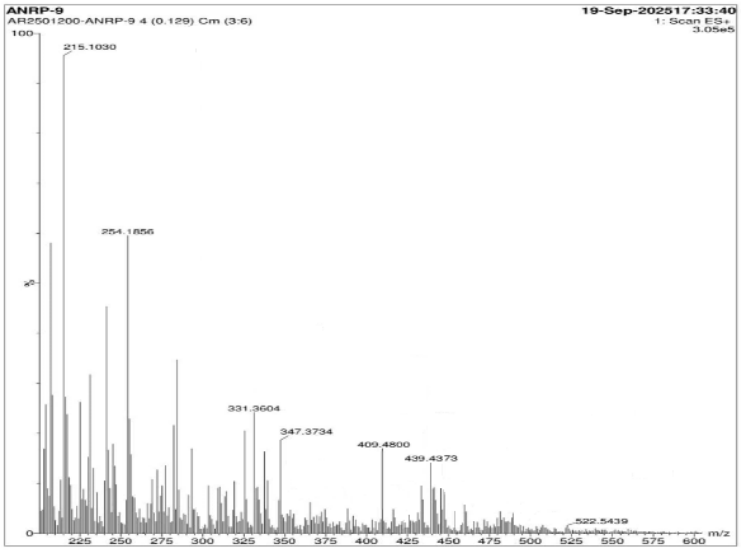


Fig 10: MS spectrum of ANRP-9

$M^{+1} = 439.43$

3. ^{1}H NMR

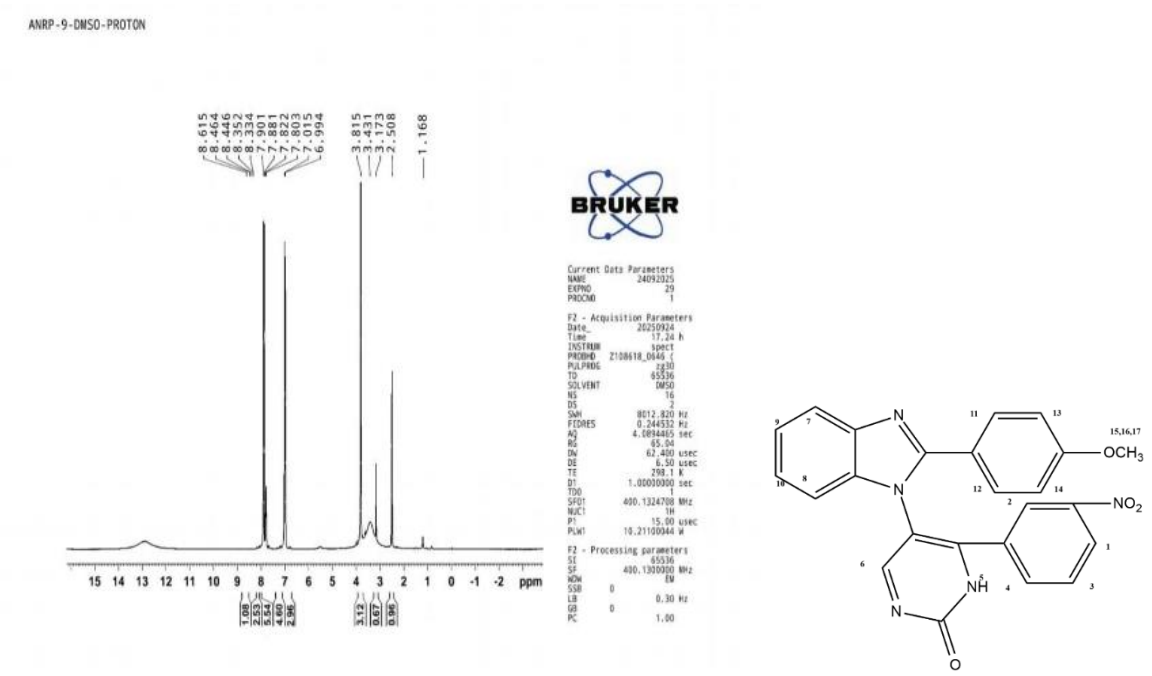


Fig 11: ^{1}H NMR spectrum of ANRP-9

δ: 8.615(1H,H_1), 8.446(1H,H_4), 8.352(1H,H_5), 7.803(1H,H_6), 3.815(3H,H_{15}-H_{17})

4. ^{13}C NMR

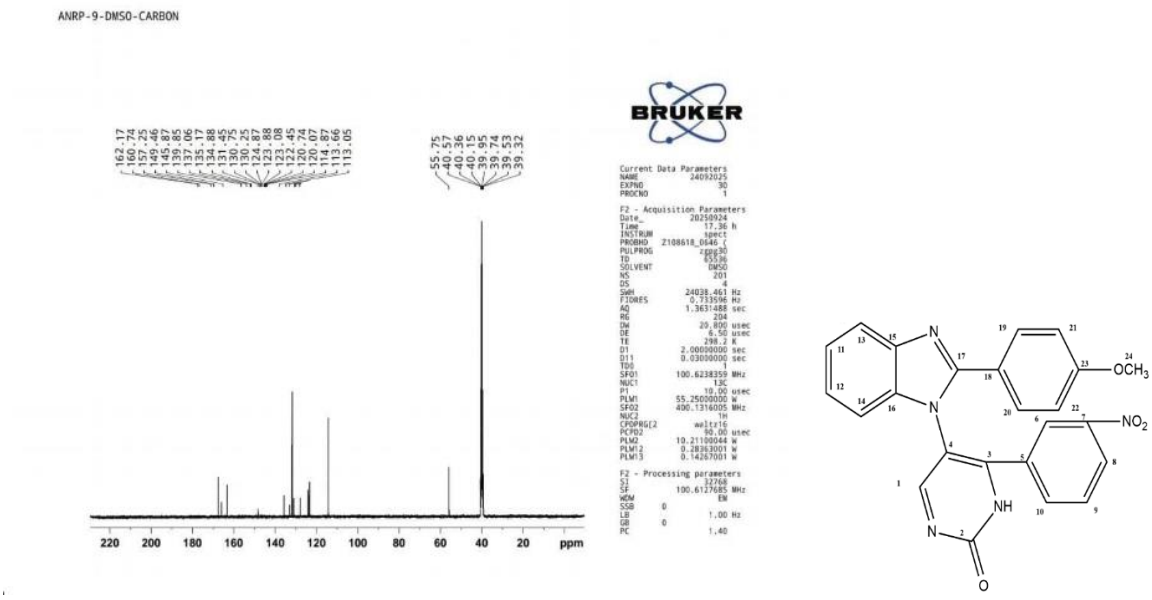


Fig 12: ^{13}C NMR spectrum of ANRP-9

δ: 162.17(C-1), 157.25(C-2), 113.05(C-4), 149.46(C-7), 160.74(C-23), 55.75(C-24).

II. DOCKING STUDIES

The molecular interaction of the synthesized compounds was studied in complementary for each in-vivo, *in-vitro* and *in-silico* biological activity with respective governing the mechanism of action.

***In-Silico* Screening for Antidepressant activity:**

Table 5: Binding energy of synthesized compounds with 1Q3D (Antidepressant activity):

LIGAND	BINDING AFFINITY (kcal/mol)
ANRP-1	-8.2
ANRP-2	-8.3
ANRP-3	-8.6
ANRP-4	-9
ANRP-5	-8.7
ANRP-6	-8.9
ANRP-7	-7.7
ANRP-8	-7.8
ANRP-9	**-9.5**
ANRP-10	-7.7
IMIPRAMINE	-7

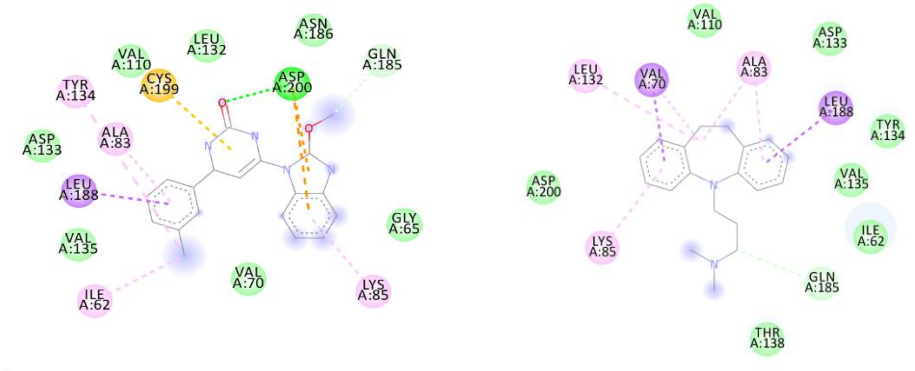


Fig 13: 2D interaction of ANRP-9 and Imipramine with 1Q3D having binding affinity of -9.5, -7 kcal/mol respectively with 1Q3D receptor.

***In-Silico* Screening for Antioxidant activity:**

Table 6: Binding energy of synthesized compounds with 1HD2 (Antioxidant activity):

LIGAND	BINDING AFFINITY (kcal/mol)
ANRP-1	-6.9
ANRP-2	-7.4
ANRP-3	-7.7
ANRP-4	-7.4
ANRP-5	-6.8
ANRP-6	-7.2
ANRP-7	-7.5
ANRP-8	-7.3
ANRP-9	-7.7
ANRP-10	-7.2
ASCORBIC ACID	-5.5

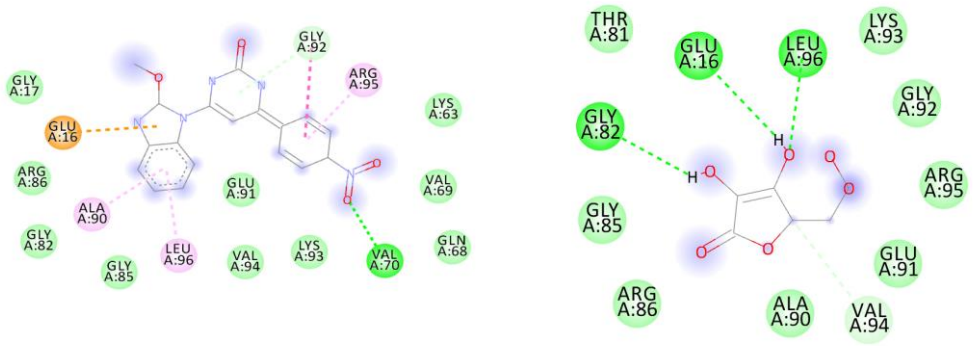


Fig 14: 2D interaction of ANRP-9 and Ascorbic acid with 1HD2 having binding affinity of -7.7, -5.5 kcal/mol respectively with 1HD2 receptor.

***In-Silico* Screening for Anti-inflammatory activity:**

Table 7: Binding energy of synthesized compounds with 1M17 (Anti-inflammatory activity):

LIGAND	BINDING AFFINITY (kcal/mol)
ANRP-1	-8.6
ANRP-2	-8.5
ANRP-3	-9
ANRP-4	-8.6
ANRP-5	-8
ANRP-6	-8.3
ANRP-7	-8.2
ANRP-8	-8.7
ANRP-9	-8.6
ANRP-10	-8.7
INDOMETHACIN	-8

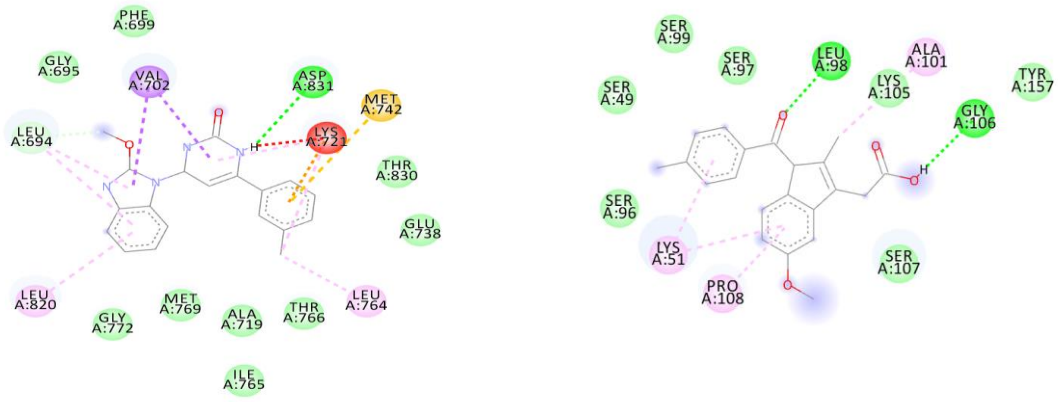


Fig 15: 2D interaction of ANRP-8 and Indomethacin with 1M17 having binding affinity of -8.7, -8 kcal/mol respectively with 1M17 receptor.

III. *In-silico* Toxicity Studies

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

COMPOUND CODE	*Predicted LD50 (mg/kg*	*Predicted Toxicity Class*	*Hepa totoxicity*	*Carcino genicity*	*Immuno toxicity*	*Cytotoxicity*
ANRP 1	1800	4	+	-	-	-
ANRP 2	1800	4	-	-	+	+
ANRP 3	1800	4	+	+	+	-
ANRP 4	1000	4	+	-	-	-
ANRP 5	1000	4	-	-	+	-
ANRP 6	1800	4	+	-	-	-
ANRP 7	759	4	-	-	-	-
ANRP 8	759	4	+	-	+	-
ANRP 9	759	4	+	+	+	-
ANRP 10	1000	4	+	-	+	-

IV. *In-silico* Physicochemical Studies

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

Table 35: *In-Silico* Physicochemical studies obtained from Molinspiration

Compound Code	*miLogP*	*TPSA*	*natoms*	*MW (g/mol)*	*nON*	*nOHNH*	*n violation*	*n rotb*	*Volume*
ANRP-1	2.83	93.04	25	334.33	7	2	0	3	285.44
ANRP-2	3.41	76.05	27	361.40	7	1	0	4	323.33
ANRP-3	3.26	118.64	27	363.33	9	1	0	4	300.76
ANRP-4	3.98	72.81	25	352.78	6	1	0	3	290.26
ANRP-5	2.94	100.52	30	408.41	9	1	0	6	354.06
ANRP-6	3.36	82.05	26	348.36	7	1	0	4	302.97
ANRP-7	4.22	72.81	26	346.39	6	1	0	4	310.78
ANRP-8	3.47	72.81	25	336.33	6	1	0	3	282.35
ANRP-9	3.24	118.64	27	363.33	9	1	0	4	300.76
ANRP-10	3.96	72.81	25	352.78	6	1	0	3	290.96

V. *In-silico* ADME properties

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

Compound Code	ANRP -1	ANRP -2	ANRP -3	ANRP -4	ANRP -5	ANRP -6	ANRP -7	ANRP -8	ANRP-9	ANRP-10
Num. heavy atoms	25	27	27	25	30	26	26	25	27	25
Num. Arom. Heavy atoms	21	21	21	21	21	21	21	21	21	21
Num. Rotatable bonds	3	4	4	3	6	4	4	3	4	3
Num. H-bond acceptors	5	4	6	4	7	5	4	5	6oo	4
Num. of H-bond donors	2	1	1	1	1	1	1	1	1	1
Molar refractivity	93.44	105.62	100.24	96.42	110.89	97.91	101.19	91.37	101.24	96.42
Total Polar Surface Area(Å)	93.03	76.04	118.62	72.80	100.49	82.03	72.80	72.80	118.62	72.80
Log Po/w (ilogp)	2.36	3.03	2.03	2.83	3.29	2.99	3.18	2.73	2.28	2.66
Water solubility	Moderately soluble	Poorly soluble	Moderately soluble	Poorly soluble	Poorly soluble	Poorly soluble	Poorly soluble	Poorly soluble	Moderately soluble	Poorly soluble
GI absorption	High	High	high	High	High	High	High	High	High	High
BBB permeant	No	Yes	No	Yes	No	No	Yes	Yes	No	Yes
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)	Yes	No; (1), MW> 350	No; (1), MW> 350	No; (1), MW> 350	No; (1), MW> 350	Yes	No; (1) MW> 350	Yes	No; (3), MW> 350	No; (1) MW> 350

VI. BIOLOGICAL ACTIVITY

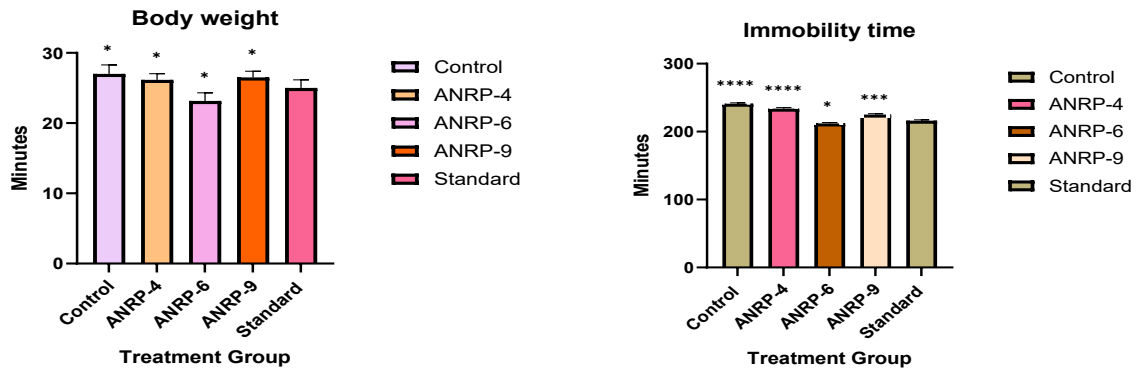
In-vivo antidepressant activity:

Forced swim test – The synthesized novel pyrimidinone derivatives (ANRP-4,6,9) which showed good binding affinity as compared to the standard Imipramine in molecular docking were tested for their anti-depressant activity using Forced swim test.

Table 11: *In-vivo* Antidepressant activity of synthesized pyrimidinone derivatives

SL. NO.	GROUPS	BODY WEIGHT (g)	IMMOBILITY TIME (s)	CHANGE FROM CONTROL (%)
1	Control	17.333±1.29	241±1.366	0
2	Standard	19.333±1.15	216±1.366	10.351±0.60
3	ANRP-4	26.166±0.87	233.83±1.424	24.333±1.03
4	ANRP-6	18.333±1.13	212±1.414	12.018±0.65
5	ANRP-9	26.5±0.88	226.66±1.460	25.333±0.46

Fig 16: Anti-depressant experimentation by Forced swim test



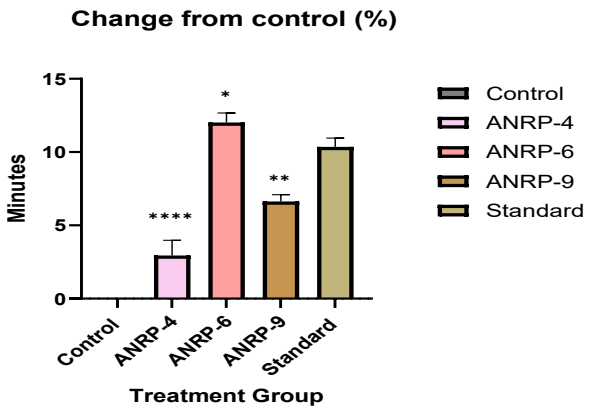


Fig 17: Graphical representation of *In-vivo* anti-depressant activity

***In-vitro* antioxidant activity**

2,2-diphenyl-1-picrylhydrazyl (DPPH Free Radical Scavenging Assay) Assay- All the synthesized compounds were tested for antioxidant activity by DPPH free radical reduction method at concentrations of 50µg, 100 µg, 200 µg, 400 µg and 600 µg. Ascorbic acid was used as a positive control.

Table 12: *In-vitro* antioxidant activity of synthesized Pyrimidinone substituted compounds.

SI.	***Compound***	***% inhibition***					
No	***Code***	**50µg**	**100 µg**	**200 µg**	**400 µg**	**600 µg**	**IC_{50}**
1	***ANRP-1***	20.65	32.75	45.84	50.98	68.03	390.7
2	***ANRP-2***	18.40	22.13	35.57	42.43	65.71	417.36
3	***ANRP-3***	18.47	22.40	37.14	48.50	60.49	426.69
4	***ANRP-4***	16.20	18.89	32.51	39.74	50.36	594.2
5	***ANRP-5***	20.92	28.89	38.01	45.54	55.35	514.53
6	***ANRP-6***	18.89	21.62	35.10	30.76	66.52	409.23
7	***ANRP-7***	22.86	35.06	40.55	55.96	62.06	430.79
8	***ANRP-8***	25.41	35.72	47.69	54.98	72.90	334.78
9	***ANRP-9***	29.64	36.22	74.64	81.62	88.13	245.23
10	***ANRP-10***	16.94	39.06	40.55	61.45	72	380
11	**Ascorbic acid**	20.89	37.89	62.78	75.97	96.81	260.88

Fig 18: Antioxidant evaluation by DPPH assay

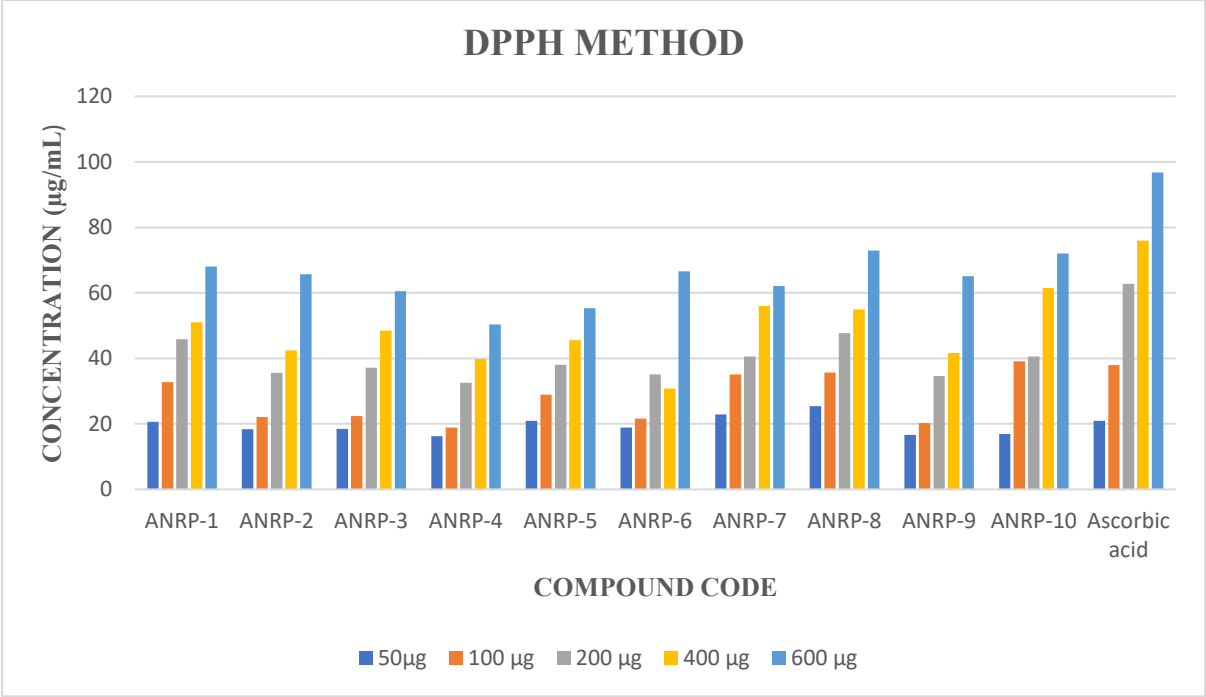


Fig 19: Graphical representation of Anti-oxidant activity

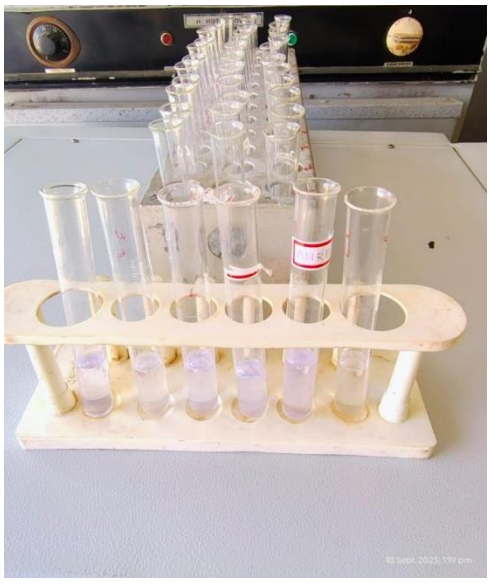
***In-vitro* antiinflammatory activity**

Bovine Serum Albumin (BSA) Denaturation Method: -

All the synthesized compounds were analyzed for their anti-inflammatory activity by the ability to protect Bovine Serum Albumin protein against heat induced denaturation method at a different concentration 100µg, 200µg, 400µg, 600µg, 800µg and 1000µg. Diclofenac sodium was used as a positive contro.

Table 13: *In-vitro* anti-inflammatory activity of synthesized pyrimidinone derivatives

SI. No	*Compound code*	*% inhibition*				
		50µg	*100µg*	*200µg*	*400µg*	*IC_{50}*
1	***ANRP-1***	8.41	27.52	57.26	78.25	258.44
2	***ANRP-2***	8.64	51.19	84.34	78.20	258.10
3	***ANRP-3***	6.70.	10.24	20.21	48.62	411.52
4	***ANRP-4***	16.09	48.85	87.91	99.19	197.88
5	***ANRP-5***	5.19	24.29	42.49	89.19	236.70
6	***ANRP-6***	15.41	68.14	69.29	57.39	338.38
7	***ANRP-7***	9.13	64.21	84.62	91.18	224.33
8	***ANRP-8***	6.37	6.29	9.39	57.19	350.48
9	***ANRP-9***	14.58	41.27	62.20	69.62	275.23
10	***ANRP-10***	4.47	11.59	21.15	25.40	811.37
11	**Indomethacin**	14.11	44.80	77.70	96.69	202.26



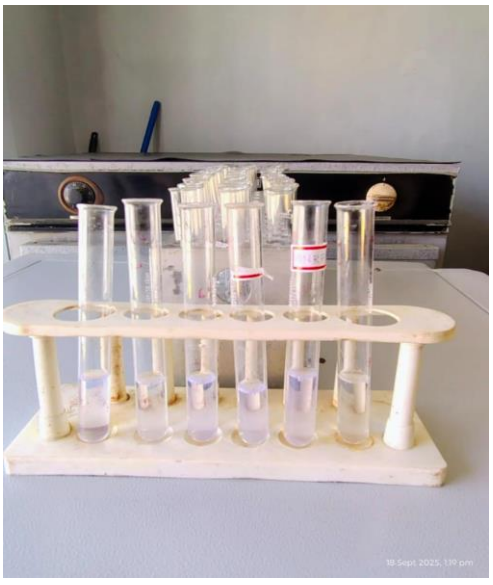


Fig 20: Antiinflammatory activity by BSA method

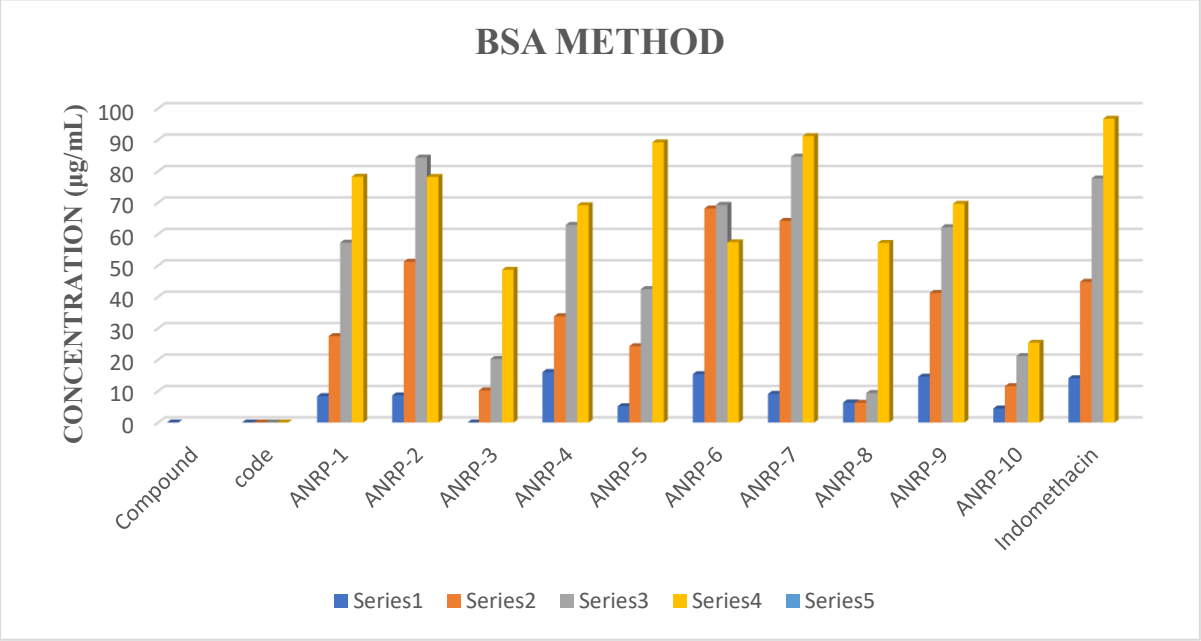


Fig 21: Graphical representation of Anti-inflammatory activity

6.CONCLUSION

[1] The main objective of the present dissertation was to synthesize, characterise, to evaluate in-silico studies and biological activity of novel Pyrimidinone derivatives.

Step-1: Condensation of o-phenylendiamine with 4-methoxybenzoic acid to form a benzimidazole ring.
Step-2: N-acetylation of the methoxy phenyl benzimidazole using acetyl chloride to form an ethanone which is a N-acetylated product.
Step-3: Claisen-Schmidt condensation reaction between methoxy phenyl benzimidazole ethanone and an aromatic aldehyde to form chalcone derivative.
Step-4: Cyclocondensation reaction between chalcone derivative and urea to form novel benzimidazole-pyrimidinone hybrids.

[2] The structure of all synthesized pyrimidinone derivatives was established by IR, ^{1}HNMR, ^{13}CNMR and LCMS.

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

Anti-depressant activity:

Compound ANRP-4(4-Cl), ANRP-6(4-OCH3), ANRP-9(3-NO2) showed binding affinity of -9 kcal/mol, -8.9 kcal/mol and -9.5 kcal/mol respectively with protein 1Q3D. The immobility

time of the synthesized compound ANRP-6 was found to be 212±1.414 seconds which is less than that of standard Imipramine having immobility time of 216±1.366 seconds.

Anti-oxidant activity:

Compounds ANRP-3 (4-NO2), ANRP-7 (4-CH2CH3) and ANRP-9 (3-NO2) showed binding affinity of -7.7kcal/mol, -7.5kcal/mol and -7.7 kcal/mol with protein 1HD2 and exhibited higher binding affinity than ascorbic acid (-5.5 kcal/mol). All the synthesized derivatives exhibited moderate inhibition of the free radicals. Compound ANRP-9 showed excellent anti-oxidant activity with IC_{50} of 245.23µg/mL.

Anti-inflammatory activity:

In-silico activity of the compounds was studied with the protein 1M17. CompoundANRP-8 (4-F), ANRP-10 (3-Cl) and ANRP-9 (3-NO2) showed binding affinity of -8.7 kcal/mol and -8.6 kcal/mol greater than binding affinity of indomethacin (-8 kcal/mol). Compound ANRP-4 showed excellent anti-inflammatory activity with IC_{50} of 197.88µg/mL.

Anti-microbial activity:

Compounds ANRP-8 (4-F), ANRP-10 (3-Cl) and ANRP-3 (4-NO2) showed binding affinity of -9.2, -9.5 and -8.6 kcal/mol respectively. Compound ANRP-2 (N(CH3)2), ANRP-5 ((OCH3)3) produced good zone of inhibition as compared to standard streptomycin against gram-positive bacteria. Compound ANRP-7 (CH2CH3) produced good zone of inhibition as compared to standard streptomycin against gram-negative bacteria.

[4] The *in-silico* studies of the synthesized pyrimidinone derivatives (ANRP-1-10) indicate promising potential for therapeutic use, highlighted by their favourable bioactivity and safety profiles. Toxicity predictions using ProTOX-II demonstrate that most compounds fall into Class IV, suggesting a high level of safety with no significant immune toxic, cytotoxic, carcinogenic properties

[5] 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 pyrimidinone derivatives, paving the way for further experimental validation and development into effective drug candidates. The lipophilic profiles, adherence to Lipinski's rule of five further support their drug-like properties.

Thus, the objective of the present work has been met with.

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