3.5.1 *Number of Collaborative activities for research, Faculty exchange, Student exchange/ internship*

Vishnu Institute of Pharmaceutical Education and Research
Narsapur, Medak District – 502 313, TS

LIST OF COLLABORATIVE ACTIVITIES

S.No.	Title of the collaborative activity	Name of the collaborating agency with contact details	Name of the participant	Year of collaboration	Duration in days
1	Determination of anti-bacterial and anti-fungal activity for various synthetic molecules	Mathrusri Engineering College, Saifabad, Hyderabad.	Mrs. B. Lakshmi Satya	2022-2023	45
2	RP-HPLC method development and validation for the simultaneous estimation of azelnidipine and telmisartan in bulk and pharmaceutical dosage form	Spectrum Pharma Research Solutions, Addagutta, Kukatpally, Hyderabad.	K. Swetha	2022-2023	180
3	Development of new RP-HPLC for the estimation of lamivudine and abacavir in tablet dosage form	Clintech solutions, Chaintayapuri, Dilsukhnagar, Hyderabad.	D. Sahithi	2022-2023	210
4	Method development and validation for the determination of fexofenadine and montelukast in active pharmaceutical ingredient and combined tablet dosage form by RP-HPLC	Sura labs, SS Towers, Dilsukhnagar, Hyderabad.	N. Ashwini	2022-2023	225
5	Analytical method development and validation for simultaneous estimation of metformin and linagliptinin bulk and pharmaceutical formulation using RP-HPLC	Pharma Life Research Lab, Rajalakshmi Lakshmi Colony, Hyderabad-500068, Telangana.	K. Mounika	2022-2023	160
6	Development and validation	Spectrum Pharma Research	B. Shireesha	2022-2023	180

	of new analytical method for the simultaneous estimation of remofgliflozin and teneligliptin in bulk and pharmaceutical dosage form	Solutions, Addagutta, Kukatpally, Hyderabad.			
7	Development and validation of a RP-HPLC method for simultaneous determination of remogliflozinetabonate and vildagliptin in pure form and its pharmaceutical dosage form	Sura labs, SS Towers, Dilsukhnagar, Hyderabad.	K. Hiranmai	2022-2023	180
8	Development of Nitric oxide releasing Quinoline derivatives as inhibitors of doxorubicin resistance in cancer cells	BogaR Laboratories, Peddapuram - Rayabhupalapatnam Rd., Andhra Pradesh.	Dr. VVS Rajendra Prasad	2022-2023	30
9	Development of Antiinflammatory Agents from Natural Products	Chemiloids Life Sciences, Auto nagar, 7th line, Vijayawada, Andhra Pradesh	Dr. VVS Rajendra Prasad	2022-2023	30
10	A validate stability indicating RP-HPLC method development and validation for simultaneous estimation of cabotegravir and rilpivirine in pharmaceutical dosage form	Spectrum Pharma Research Solutions, Addagutta, Kukatpally, Hyderabad.	I. Srilatha	2022-2023	150
11	Newer RP-HPLC method development and validation for the simultaneous estimation of lafutidine and rabeprazole in dosage form	Sura labs, SS Towers, Dilsukhnagar, Hyderabad.	K. Ankitha	2022-2023	180
12	Validated RP-HPLC method for the simultaneous determination of montelukast and bilastine in bulk and pharmaceutical formulations	Spectrum Pharma Research Solutions, Addagutta, Kukatpally, Hyderabad.	D. Ravalika	2022-2023	150

13	Skin sensitivity test	Sanmed Healthcare Pvt. Ltd., Begumpet, Hyderabad – 500081	Mr. P. Rajashekar	2022-2023	365
14	Formulation & evaluation of telmisartan& amlodipine bilayered tablets by HPLC	DelExcel Pharma Pvt. Ltd., Kucharam Village, Manoharabad.	V. Nikitha	2022-2023	180
15	Method development and validation for quantitative estimation of capecitabine tablets dosage form by using HPLC method	DelExcel Pharma Pvt. Ltd., Kucharam Village, Manoharabad.	Sk. Neha Afrin	2022-2023	180
16	Cytotoxic activity for organic molecules	CMCR, VIPER, Narsapur	Dr. VVS Rajendra Prasad	2022-2023	45
17	Manufacturing assistance	Granules India Ltd., Jinnaram Rd, Bonthapalle, Telangana 500043	Dr. A. Ramesh	2022-2023	60
18	SMT Training	DrReddys Labs Miyapur - Janapriya West City Rd, Jaya Prakash Narayan Nagar, Miyapur, Telangana 500049	Dr. A. Ramesh	2022-2023	45
19	Identification of pharmacophore of quinazolines to target lung cancer	Incozen Therapeutics Pvt Limited13th Floor, Manjeera Corporate, Trinity, K P H B Phase 3, Kukatpally, Hyderabad, Telangana 500072	Dr. VVS Rajendra Prasad	2022-2023	365

Vishnu Institute of Pharmaceutical Education & Research ★ Narsapur Medak Dist. 502313

PRINCIPAL

Principal
Vishnu Institute of Pharmaceutical Education & Research
Narsapur, Medak dist -502313

Matrusri Engineering College

(Sponsored by: MATRUSRI ERDUCATION SOCIETY, Estd: 1980)
(Approved by AICTE, Affiliated to Osmania University)
16-1-486, Saidabad, Hyderabad-500059. Ph: 040-24072764

NATIONAL BOARD OF ACCREDITATION

Email: thumma.vishnu@matrusri.edu.in

Website: www.matrusri.edu.in

Dt. 17.10.2022

To

The Principal,
Vishnu Institute of Pharmaceutical Education and Research,
Narsapur, Medak -502313

Dear Sir/Madam,

Subject: Project proposal titled "*Determination of anti-bacterial and anti-fungal activity for various synthetic molecules*" reg.

Ref: Following up on our earlier discussion and proposed protocol for the study of 500 samples.

With respect to the above-stated subject. In accordance with our discussion via email and telephone regarding timelines and price quotes for consultancy work related to antimicrobial activity. Now we are sending 100 mg of each sample for the determination of activity. We expect you to strictly follow the timeline for the completion of the study and to keep the results of the project confidential. In order to proceed with the proposed study as soon as possible, we request that you initiate it at VIPER as soon as possible.

We look forward to continuing to work with you on a collaborative basis.

Thanking You,

With regards,

Vishnu Thumma
Assistant Professor,
Department of Sciences and Humanities,
Matrusri Engineering College,
Hyderabad, Telangana – 500059

Email: thumma.vishnu@matrusri.edu.in
Mobile: 9676172776

SPECTRUM
PHARMA RESEARCH SOLUTIONS

CERTIFICATE

This is to certify that this dissertation entitled **"RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF AZELNIDIPINE AND TELMISARTAN IN BULK AND PHARMACEUTICAL DOSAGE FORM "** Submitted by **KAPPALA SWETHA**; Roll no: **(20DH1S1204)** towards partial fulfilment for the requirement of **Master of Pharmacy** for the period of 08th May 2022 to 08th October 2022, this work carried out in **Pharmaceutical Analysis Department of Spectrum Pharma Research Solutions, Hyderabad.**

With Best Regards.

HR-Manager

Spectrum Pharma Research Solutions Hyderabad

Vishnu Institute of Pharmaceutical Education & Research Narsapur Medak Dist 502 313. T.S.

Praveen D

Research Scholar,
Department of Chemistry
Mahatma Gandhi University
(www.mguniversity.ac.in)
Yellareddy gudem,
Nalgonda – 508 254

UNIVERSITY COLLEGE OF SCIENCE & INFORMATICS
MAHATMA GANDHI UNIVERSITY
(www.mguniversity.ac.in)
Yellareddy gudem, Nalgonda – 508 254
Phone: 08682 – 221914

19-12-22

To

The Principal,
Vishnu Institute of Pharmaceutical Education and Research,
Narsapur, Medak-502313.

Dear Sir/Madam

Sub: Requesting Letter for Antimicrobial studies at Dept. of Pharmaceutical Analysis reg…

With respect to above stated subject. In accordance with our discussion via telephone regarding timelines and price quotes for consultancy work related to antimicrobial activity. Now we are sending about 21 samples with qty. 10 mg of each for the determination of activity. We expect you to strictly follow the timeline for the completion of the study and to keep the results of the project confidential. In order to proceed with the proposed study we request you to initiate it at VIPER as soon as possible.

We look forward to continuing to work with you on collaborative basis.

Thanking you,

D.P Praveen

Praveen D
Research Scholar,
Department of Chemistry
Mahatma Gandhi University
(www.mguniversity.ac.in)
Yellareddy gudem, Nalgonda – 508 254.

Vishnu Institute of Pharmaceutical Education & Research
Narsapur, Medak Dist. 502 313, T.S.

Praveen D

Research Scholar,
Department of Chemistry
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UNIVERSITY COLLEGE OF SCIENCE & INFORMATICS
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(www.mguniversity.ac.in)
Yellareddy gudem, Nalgonda – 508 254
Phone: 08682 – 221914

09-11-22

To

The Principal,
Vishnu Institute of Pharmaceutical Education and Research,
Narsapur, Medak-502313.

Dear Sir/Madam

Sub: Requesting Letter for Antimicrobial studies at Dept. of Pharmaceutical Analysis reg…

With respect to above stated subject. In accordance with our discussion via telephone regarding timelines and price quotes for consultancy work related to antimicrobial activity. Now we are sending about 20 samples with qty. 10 mg of each for the determination of activity. We expect you to strictly follow the timeline for the completion of the study and to keep the results of the project confidential. In order to proceed with the proposed study we request you to initiate it at VIPER as soon as possible.

We look forward to continuing to work with you on collaborative basis.

Thanking you,

D. P. Praveen

Praveen D
Research Scholar,
Department of Chemistry
Mahatma Gandhi University
(www.mguniversity.ac.in)
Yellareddy gudem, Nalgonda – 508 254.

V

To Vishnu institute of pharmaceutical ...

₹5,500

microbial activity

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State Bank of India
XXXXXX8763

UPI transaction ID
230676758016

To
•••• 1865

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(State Bank of India)
veerankichaitanya@oksbi

Google transaction ID
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Mode	IMPS
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Amount	₹ 5500
From Account	XX-XX-XX-XX-XX-70
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Remarks	Biology

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Amount	₹ 5500
From Account	XX-XX-XX-XX-XX-70
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Remarks	Biology

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Date: 30/10/2022

TO WHOMSOEVER IT MAY CONCERN

This is to certify that **DAROJU SAHITHI** H. No **20DH1R1213** is a bonafied student of Vishnu Pharmaceutical Education& Research Sangareddy-Narsapur Rd, Narsapur, Telangana has undergone project work in our organization from 20-03-2020 to 26-10-2022 as a part of partial fulfillment of her M. Pharmacy course.

The title of the project is **DEVELOPMENT OF NEW RP-HPLC FOR THE ESTIMATION OF LAMIVUDINE AND ABACAVIR IN TABLET DOSAIGE FORM AND VALIDATION OF THE METHOD AND VALIDATION AND CHLORTHALIDONE IN PHARMACEUTICAL FORMULATION&USING RP-HPLC**

During the aforesaid period, we found her hard working, sincere and learning attitude

CLINTECH SOLUTION Hyderabad

Vishnu Institute of Pharmaceutical Education & Research Narsapur, Medak Dist. 502 313, T.S.

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Ref.Number:SPL/MPCL/81135

Date: 15-10-2022.

CERTIFICATE

This is to certify that Mr/Miss. NEERUDI ASHWINI (HT.NO: 20DH1S1209) pursuing his/her M. Pharmacy in VISHNU INSTITUTION OF PHARMACEUTICAL EDUCATION AND RESEARCH he/she carried out his/her project work in our Organization entitled "METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF FEXOFENADINE AND MONTELUKAST IN ACTIVE PHARMACEUTICAL INGREDIENT AND COMBINED TABLET DOSAGE FROM BY RP-HPLC" in the department of Pharmaceutical Analysis from 1ST APRIL 2022 TO 15TH OCTOBER 2022.

During his/her tenure he/she was sincere, hardworking and Punctual in his/her Project work.

We wish him/her to success in his/her future career.

Authorized Signature

Rajini Sura
Managing Director

Narsapur
Medak Dist.
502 313, TS.

Pharma Lite Research Lab
[illegible] Colony,
[illegible] Telangana State
[illegible]
[illegible]

PHARMA

Date: 27/09/2022

TO WHOMSOEVER IT MAY CONCERN.

This is to certify that **Konda Mounika** H. No **20DH1S1205** is a bonafied student of Vishnu Institute of Pharmaceutical Education & Research **(VIPER)** Sangareddy-Narsapur Rd, Narsapur, Telangana has undergone from project work in our organization from 20-03-2020 to 26-08-2022 asa part of partial fulfillment of her M. Pharmacy course.

The title of the project is **ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND LINAGLIPTIN IN BULK AND PHARMACEUTICAL FORMULATION USING RP-HPLC**

During the aforesaid period, we found her hard working, sincere and learning attitude .

Narsapur
Medak Dist
502 313, TS.

Pharma Life Research Lab
7569073455
Hyderabad

CERTIFICATE

This is to certify that this dissertation entitled " **DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR THE SIMULTANEOUS ESTIMATION OF REMOGLIFLOZIN AND TENELIGLIPTIN IN BULK AND PHARMACEUTICAL DOSAGE FORM** " Submitted by **BOYINI SHIREESHA** ; Roll no: **(20DH1S1202)** towards partial fulfilment for the requirement of **Master of Pharmacy** for the period of 04th May 2022 to 04th October 2022, this work carried out in **Pharmaceutical Analysis Department of Spectrum Pharma Research Solutions, Hyderabad.**

With Best Regards,

HR-Manager

Spectrum Pharma Research Solutions Hyderabad

Narsapur, Medak Dist. 502 313, T.S.

Ref. No- SPL|MPCL|8301

Date: 01-02-2023

CERTIFICATE

This is to certify that Mr/Miss. **K.HIRAN MAI (HT.NO:20DH1S1212)** pursuing his/her **M. Pharmacy** in **VISHNU INSTITUTE OF PHARMACEUTICAL EDUCATIONAL AND RESEARCH** he/she carried out his/her project work in our Organization entitled **"DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF REMOGLIFLOZIN ETABONATE AND VILDAGLIPTIN IN PURE FORM AND ITS PHARMACEUTICAL DOSAGE FORM"** in the department of Pharmaceutical Analysis from **01-09-2022** to **01-02-2023.**

During his/her tenure he/she was sincere, hardworking and Punctual in his/her Project work.

We wish him/her to success in his/her future career.

FROM SURA PHARMA LABS

Managing Director

Rajini Sura

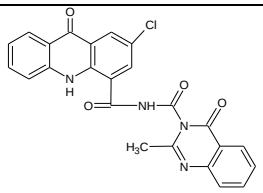
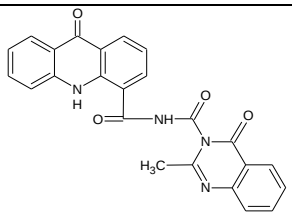
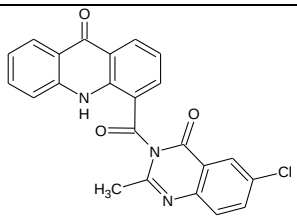
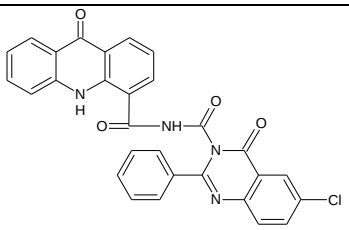
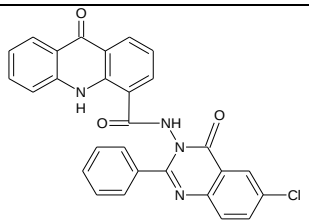
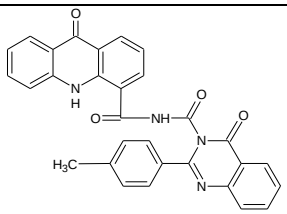
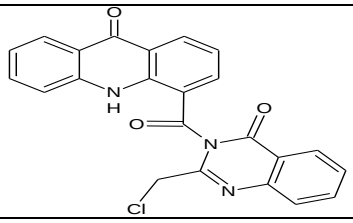
Vishnu Institute of Pharmaceutical ... Narsapur, Medak Dist 502 313, TS.

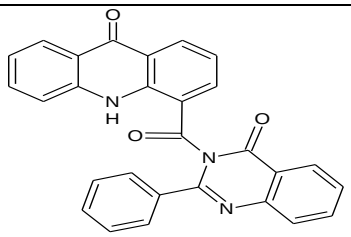
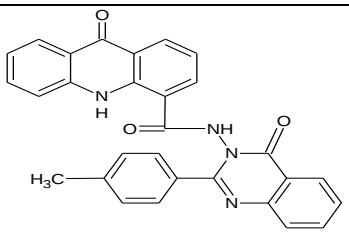
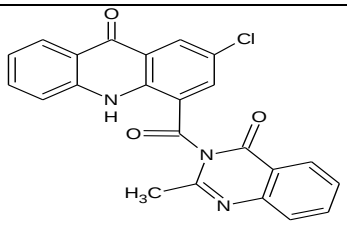
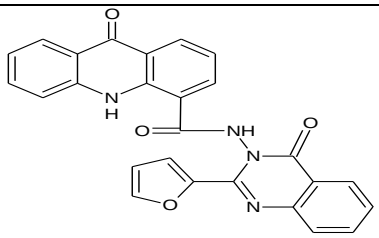
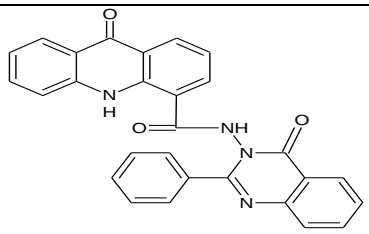
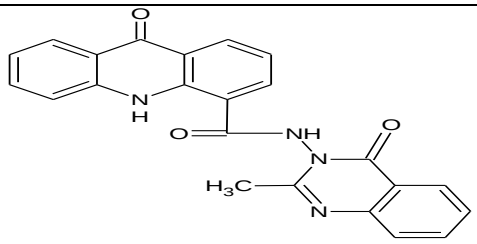
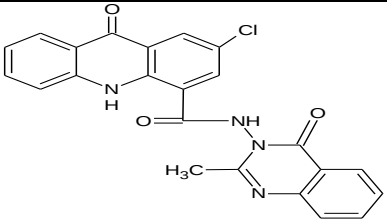
Development of nitric oxide releasing quinoline derivatives as inhibitors of doxorubicin resistance in cancer cells

Protein ligand interactions of the chlorine containing acridones with calmodulin dependent *c*AMP – Phosphodiesterase (PDE1c) enzyme were studied by employing an efficient docking protocol, GLIDE XP. Initially, a theoretically built digitalized structure of the protein PDE1C was retrieved from the protein databank with PDB ID: 1LXS. Structure of the protein was corrected by adding hydrogens to satisfy the valence and optimized by using OPLS-2005 force field (optimized potentials for liquid simulations). Binding pockets were identified by using the SITEMAP tool.Receptor grid generation was accomplished using Glide docking protocol and ligands were docked by employing XP mode of Glide. Best pose of each ligand was ranked according to the E-model energy.The docking score from Glide (Glide Score) is entirely based on Chem Score. It also includes a steric-clash term, adds polar terms featured by Schrodinger to correct electrostatic mismatches.

GScore = 0.065 x Van der Waals energy + 0.130 x Coulomb energy + Lipophilic term (Hydrophobic interactions) + H bonding + Metal binding + BuryP (Penalty for buried polar groups) + RotB (Penalty for freezing rotatable bonds) + Site (Polar interactions in the active site)

Table 1: Structures and Docking result

Compound	Structure	Dock Score
31		-6.95975
27		-6.50991
22		-5.43411
15		-5.26858
13		-5.1381
7		-5.12026
34		-5.1168

2		-5.07641
5		-4.94562
30		-4.85447
17		-4.8065
1		-4.59469
25		-4.50395
29		-4.39855

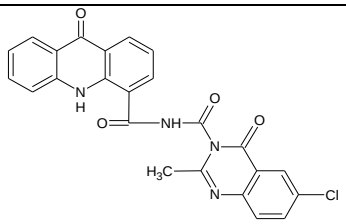
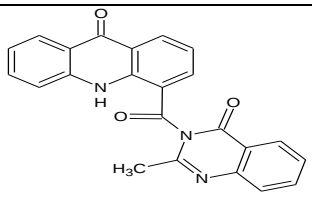
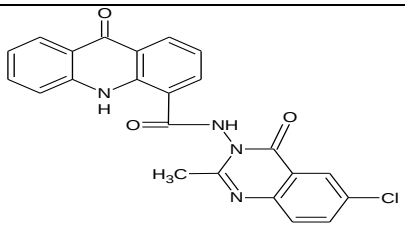
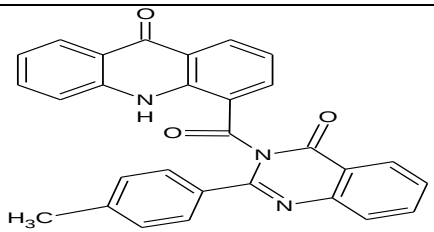
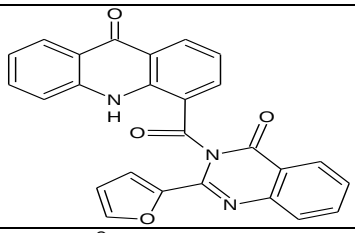
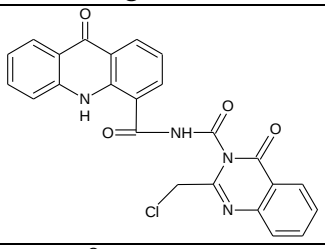
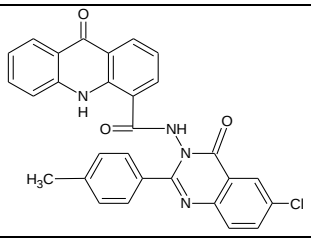
23		-4.35577
26		-3.88481
21		-3.80914
6		-3.78187
18		-3.63449
35		-3.56668
9		-1.06874

Table 2: Molecular Properties

Molecule	MW	Dipole	SASA	FOSA	FISA	PISA	WPSA	Volume
31	458.86	11.039	658.349	70.563	157.026	359.325	71.436	1230.942
27	424.415	8.66	629.351	77.161	151.305	400.884	0	1183.541
22	415.835	6.198	656.94	79.116	122.556	383.686	71.582	1176.015
15	520.931	6.794	747.129	0	152.374	536.597	58.158	1423.571
13	492.92	9.201	707.719	0	123.415	512.658	71.646	1337.1
7	500.512	7.376	767.625	88.161	150.923	528.54	0	1450.461
34	415.835	6.487	650.65	47.848	111.776	430.403	60.623	1168.089
2	443.461	7.605	728.319	0	97.518	630.801	0	1310.486
5	472.502	7.522	732.85	82.362	123.984	526.504	0	1361.525
30	415.835	6.966	663.243	76.295	110.553	407.046	69.348	1180.372
17	448.437	7.167	721.472	0	129.216	592.256	0	1295.234
1	458.475	6.013	723.914	0	108.808	615.106	0	1335.377
25	396.404	6.763	671.211	73.812	153.163	444.236	0	1214.115
29	430.849	6.856	675.721	73.187	155.319	408.472	38.741	1227.895
23	458.86	7.6	651.175	77.096	164.548	350.14	59.391	1222.537
26	381.39	6.457	644.47	76.561	109.386	458.523	0	1139.713
21	430.849	4.657	669.92	57.369	131.282	409.689	71.58	1207.827
6	457.487	6.821	732.655	88.049	121.623	522.983	0	1349.379
18	433.422	5.903	681.657	0	113.21	568.447	0	1246.227
35	458.86	8.331	680.817	45.996	161.111	411.045	62.665	1230.239
9	506.947	9.143	689.689	56.737	124.562	436.846	71.543	1338.67

Recommended range: MW – molecular weight (130-725), dipole (1-12.5), SASA- solvent accessible surface area (300-1000), FOSA – hydrophobic component of SASA (0-750), FISA – hydrophilic component of SASA (7-330), PISA -π (carbon and attached hydrogen) component of the SASA (0.0 – 450.0), WPSA - Weakly polar component of the SASA (halogens, P, and S) (0.0 – 175.0),volume (500-2000).

Table 3:Predicted Pharmacokinetic (ADME) profiles of compounds

Molecule	CNS	QPlog Po/w	QPlogS	QPlog HERG	QPP Caco	QPlog BB	QPP MDCK	QPlog Kp	QPlog Khsa	% Human Oral Absorption
31	-1	3.628	-5.438	-4.4	240.341	-0.792	356.954	-3.052	0.356	90.802
27	-1	3.135	-4.549	-4.338	268.094	-0.861	165.928	-2.8	0.218	88.761
22	0	4.163	-5.936	-6.271	681.864	-0.536	806.72	-2.331	0.518	100
15	-1	5.074	-6.918	-5.643	268.846	-0.928	336.946	-2.245	0.873	74.222
13	0	4.887	-6.294	-6.738	669.197	-0.638	791.177	-1.7	0.727	100
7	-2	5.028	-7.071	-5.797	280.164	-1.101	167.433	-2.247	0.951	74.274
34	0	4.199	-5.769	-6.426	862.827	-0.445	906.119	-1.967	0.495	100
2	0	5.23	-6.813	-7.854	1177.977	-0.585	590.528	-0.903	0.866	100
5	-1	4.778	-6.442	-7.107	660.931	-0.875	316.199	-1.662	0.791	100
30	0	4.293	-6.045	-6.476	886.179	-0.438	1041.151	-2.027	0.531	100
17	-1	4.162	-6.169	-7.65	589.582	-0.915	279.47	-1.623	0.526	100
1	-1	4.819	-6.278	-7.538	920.605	-0.723	452.395	-1.07	0.718	100
25	-2	3.537	-5.479	-6.563	349.506	-1.076	158.81	-2.585	0.424	93.178
29	-1	3.716	-5.73	-6.382	333.431	-0.999	246.037	-2.751	0.463	93.863
23	-1	3.465	-5.251	-4.269	204.091	-0.873	256.757	-3.223	0.332	88.573
26	0	3.832	-5.387	-6.651	909.058	-0.585	446.264	-1.824	0.413	100
21	0	3.88	-5.767	-6.419	563.58	-0.687	656.569	-2.304	0.406	100
6	-1	5.191	-6.896	-7.165	695.904	-0.805	334.322	-1.727	0.979	95.256
18	0	4.386	-5.83	-7.13	836.237	-	407.753	-	0.588	100

						0.615		1.507		
35	-1	3.622	-5.96	-5.102	236.863	-0.958	290.196	-2.945	0.354	90.655
9	0	4.806	-5.965	-6.015	652.642	-0.587	769.041	-1.988	0.737	92.504

Recommended range: CNS Predicted central nervous system activity on a –2 (inactive) to +2 (active) scale; QPlogPo/w: Predicted octanol/water partition coefficient (–2.0 - 6.5); QPlogS: Predicted aqueous solubility (-6.5 – 0.5); QPlogHERG: Predicted IC50 value for blockage of HERG K+ channels (below -5); QPPCaco: Predicted apparent Caco-2 cell permeability in nm/sec. Caco- 2 cells are a model for the gut-blood barrier (<25: poor, >500: great); QPlogBB: Predicted brain/blood partition coefficient (-3 – 1.2); QPPMDCK: Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the blood-brain barrier (<25: poor, >500: great); QPlogKp: Predicted skin permeability, log Kp (–8.0 – –1.0); QPlogKhsa: Prediction of binding to human serum albumin (–1.5 – 1.5); %Human- Oral Absorption (>80% is high, <25% is poor).

Table 4: Binding energy predictions using MMGBSA calculations

Compound	Prime MMGBSA Ligand Energy	Prime MMGBSA Receptor Energy	Prime MMGBSA Complex Energy	Prime MMGBSA DG bind
31	10.614942	-12400.48218	-12461.73689	-71.869657
27	31.020423	-12400.48218	-12436.37635	-66.914599
22	26.875696	-12400.48218	-12417.01052	-43.404036
15	24.361114	-12400.48218	-12438.3363	-62.215234
13	12.897055	-12400.48218	-12457.31489	-69.729772
7	35.067764	-12400.48218	-12418.86285	-53.44844
34	29.497186	-12400.48218	-12429.6953	-58.710309
2	44.695166	-12400.48218	-12422.17456	-66.387554
5	19.412305	-12400.48218	-12437.80588	-56.736014
30	17.752203	-12400.48218	-12450.17266	-67.442686
17	21.947572	-12400.48218	-12431.30236	-52.767752
1	22.618603	-12400.48218	-12441.6908	-63.827227
25	12.295664	-12400.48218	-12436.40754	-48.221029
29	-7.729278	-12400.48218	-12461.50121	-53.289759
23	18.010575	-12400.48218	-12445.86514	-63.39354
26	38.163555	-12400.48218	-12417.89096	-55.572336
21	-4.800136	-12400.48218	-12470.05472	-64.772412
6	36.72764	-12400.48218	-12389.21948	-25.464948
18	40.025004	-12400.48218	-12398.78225	-38.325076
35	25.292569	-12400.48218	-12403.00388	-27.814275
9	10.790788	-12400.48218	-12437.26277	-47.57138

The Prime MM-GBSA approach is used to predict the free energy of binding for a receptor anda set of ligands. MM-GBSA is an acronym for a method that combines OPLS molecularmechanics energies (EMM), an SGB solvation model for polar solvation (GSGB), and a nonpolarsolvation term (GNP) composed of the nonpolar solvent accessible surface area and van derWaals interactions. The total free energy of binding is then expressed as:

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand})$$

where

$$G = E_{MM} + G_{SGB} + G_{NP}$$

The ligand in the unbound state is minimized in SGB solvent but is not otherwise sampled. Inthe calculation of the complex, the ligand is minimized in the context of the receptor. Theprotein is currently held fixed in all calculations. The following descriptors generated by thePrime MM-GBSA approach:

MM-GBSA_DG_bind: Ligand binding energy, ΔG_{bind}

MM-GBSA_E_complex: Energy of the complex, $G_{complex}$

MM-GBSA_E_protein: Energy of the receptor without the ligand, $G_{protein}$

MM-GBSA_E_ligand: Energy of the unbound ligand, G_{ligand}

Table 5: **Embrase calculations for ligand binding energy**

Compound	MBAE Complex Total Energy-OPLS-2005	MBAE Rec Total Energy-OPLS-2005	MBAE Lig Total Energy-OPLS-2005	MBAE Del Total Energy-OPLS-2005
31	-4464.179668	-4365.702312	93.740479	-192.217834
27	-4363.533169	-4365.702312	176.034424	-173.86528
22	-4453.182247	-4365.702312	149.049454	-236.529388
15	-4465.980438	-4365.702312	155.900314	-256.17844
13	-4486.879223	-4365.702312	70.90551	-192.08242
7	-4442.784595	-4365.702312	193.26413	-270.346413
34	-4412.546429	-4365.702312	166.637756	-213.481873
2	-4328.60968	-4365.702312	218.52951	-181.436878
5	-4477.749912	-4365.702312	97.529282	-209.576881
30	-4387.141613	-4365.702312	109.180939	-130.620239
17	-4420.547894	-4365.702312	110.716797	-165.562378
1	-4470.624641	-4365.702312	98.782898	-203.705227
25	-4580.207062	-4365.702312	50.148129	-264.652878
29	-4675.097286	-4365.702312	-26.948503	-282.44647
23	-4449.573925	-4365.702312	130.186203	-214.057816
26	-4316.43198	-4365.702312	193.465561	-144.195229
21	-4694.094612	-4365.702312	-12.645243	-315.747057
6	-4308.456825	-4365.702312	209.598465	-152.352978
18	-4363.897919	-4365.702312	178.150131	-176.345737
35	-4335.764233	-4365.702312	162.9133	-132.97522
9	-4540.806538	-4365.702312	58.089577	-233.193802

Embrace calculates ligand-receptor binding energies by molecular mechanics energy minimization of the complex and the separated receptor and ligand, with or without continuum solvation. The Embrace calculation is run in energy difference mode. The following descriptors aregenerated from the calculation:

Embrace_Total_Energy_without_constraints: Ligand binding energy

Embrace_Valence_Energy: Valence energy difference

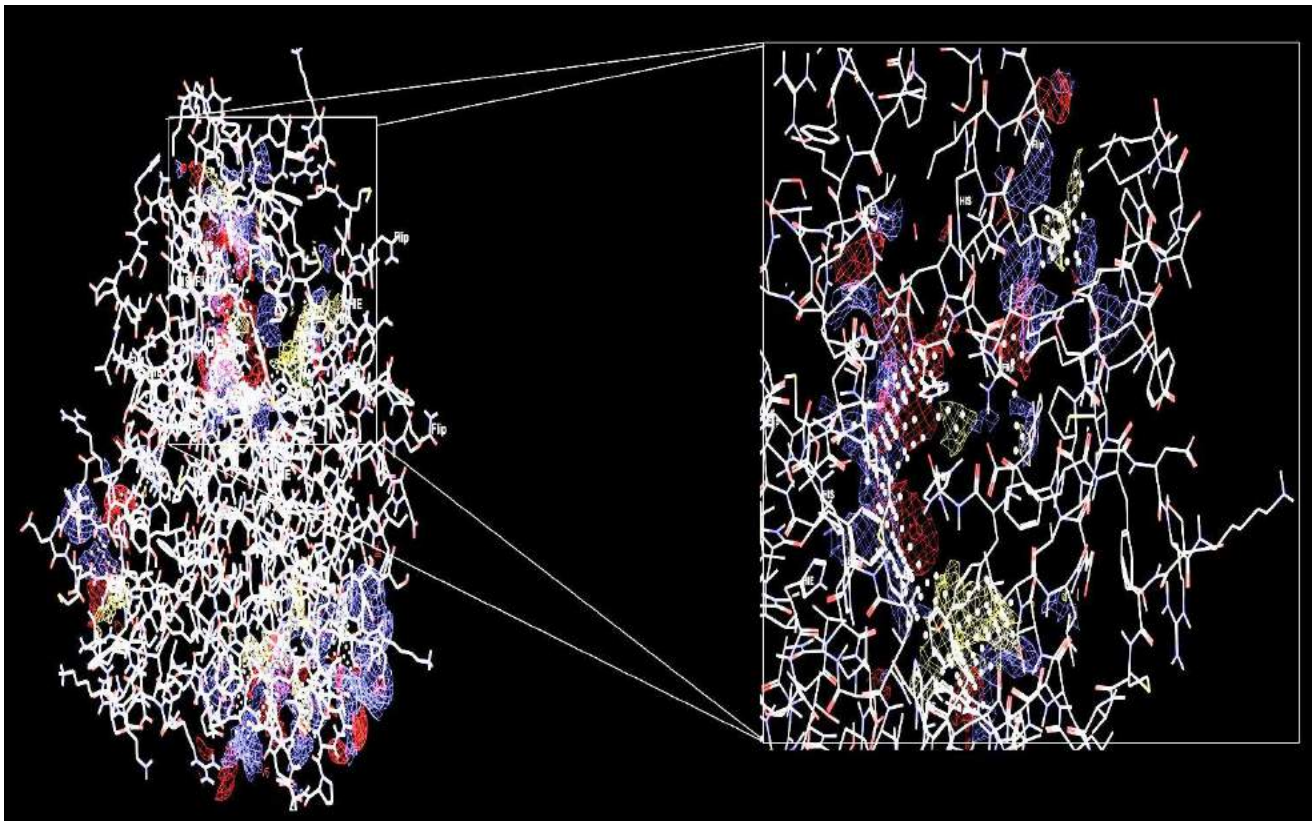
Embrace_vdW_Energy: van der Waals energy difference

Embrace_Electrostatic_Energy: Coulomb energy difference

Embrace_Solvation_Energy: Solvation energy difference

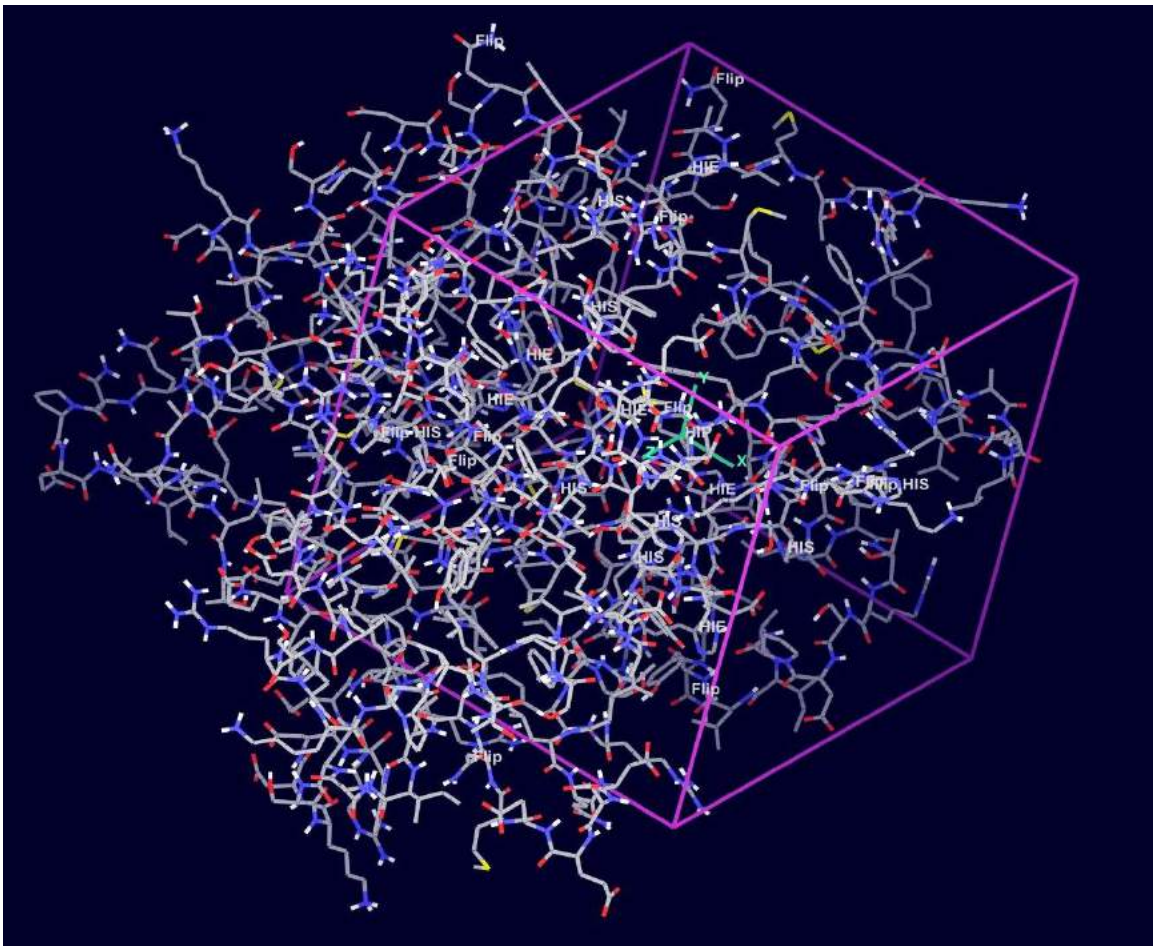
Embrace_Constraint_Energy: Constraint energy difference

Figure 1: site map calculations for the protein 1LXS.



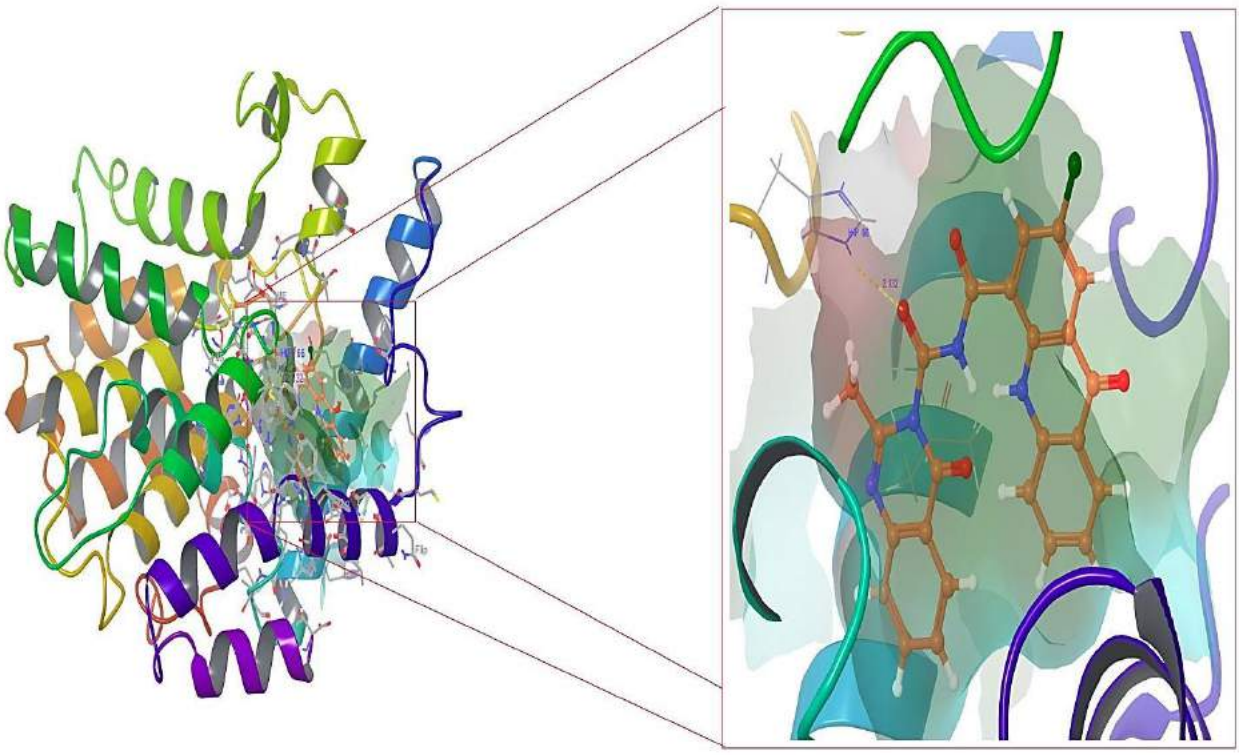
- Site map calculation identifies the possible receptor pockets along with their area and volumes.
- Site map is useful for binding site characterization and also in SBDD.
- Red colour: hydrogen bond acceptor region (high conc of oxygen)
- Blue color: hydrogen bond donor region (high conc of nitrogen)
- Yellow colour: hydrophobic region.

Figure 2: 1LXS Grid position for docking



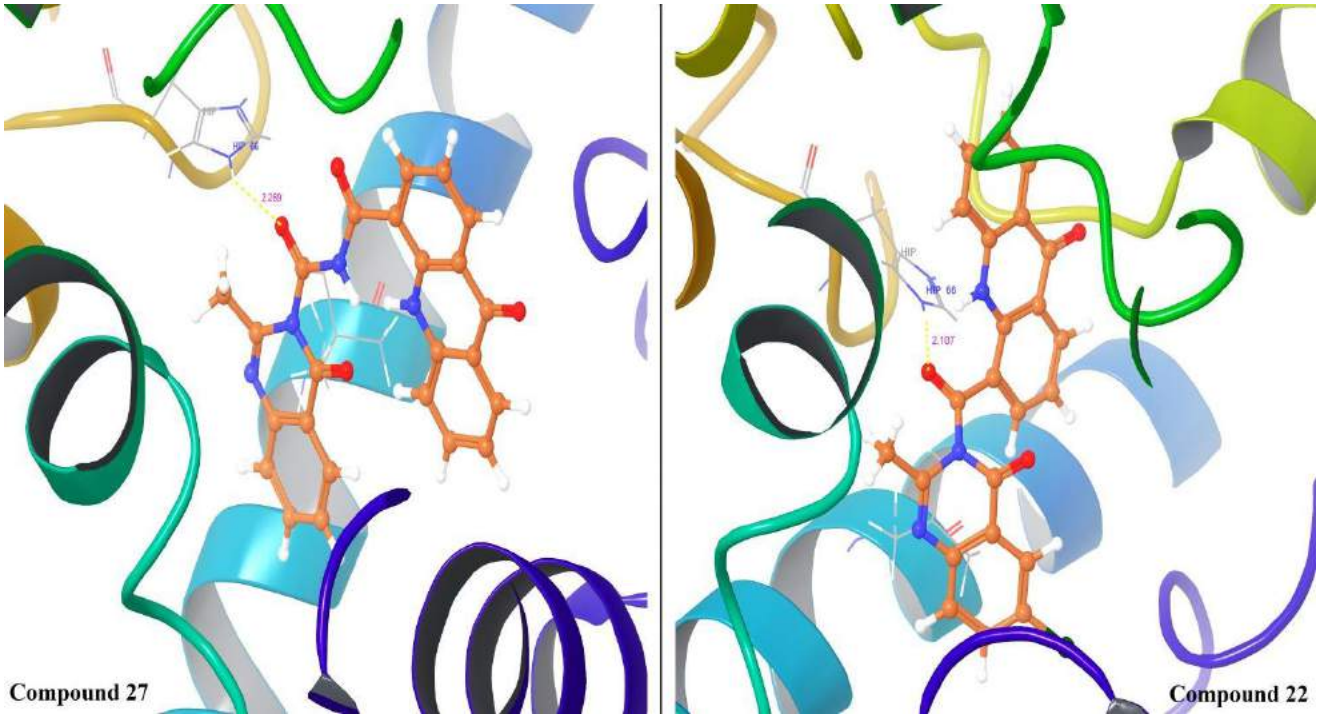
- Magenta colour cube represents the grid established for docking.
- Ligands with maximum of 20Å can fit into the grid.

Figure 3: protein ligand interactions of the docked PDE1c enzyme and ligand 31 complex.



- 1 hydrogen bond with Histidine 66 with a bond length of 2.132 Å
- Hydrogen bond is represented in yellow dotted lines.
- Magnified image shows the ligand conformational fitting into the receptor pocket.

Figure 4: Docking conformations of compound 27 and 22.



- Compound 27, and 22 interact with Histidine 66 residue of protein at hydrogen bond distance of 2.269Å and 2.107 Å respectively.

CERTIFICATE

This is to certify that this dissertation entitled " **A VALIDATE STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CABOTEGRAVIR AND RILPIVIRINE IN PHARMACEUTICAL DOSAGE FORM** " Submitted by **ILITEM SRILATHA** ; Roll no: **(20DH1S1203)** towards partial fulfilment for the requirement of **Master of Pharmacy** for the period of 04th May 2022 to 04th October 2022, this work carried out in **Pharmaceutical Analysis Department of Spectrum Pharma Research Solutions, Hyderabad.**

With Best Regards,

HR-Manager

Spectrum Pharma Research Solutions Hyderabad

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During his/her tenure he/she was sincere, hardworking and Punctual in his/her Project work.

We wish him/her to success in his/her future career.

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Managing Director

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Spectrum Pharma Research Solutions Hyderabad

HR-Manager

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She has successfully completed her project in 6 months stipulated period from 02nd November 2021 to 02nd April 2022.

We found her sincere and hardworking during the project period.

We wish her all the best in future career.

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India-502313.
cmcr@viper.ac.in
www.viper.ac.in

Study No: GNC2018-002
Sample Type (Matrix): Serum
Assay Kit: 25(OH) Vitamin D Kit: Krishgen Biosystems Cat# KBH501

Animal No.	Sample ID	25(OH) Vit-D Concn. (ng/ml) in Serum [Final Day (Day 84)]
1	S1	31.72
2	S2	46.23
3	S3	23.27
4	S4	24.38
5	S5	26.42
6	S6	21.49
7	S7	24.50
8	S8	25.95
9	S9	24.44
10	S10	23.10
11	S11	20.79
12	S12	24.69
14	S13	20.26
16	S14	26.42
17	S15	24.26
18	S16	23.91
19	S17	23.85
20	S18	21.75
21	S19	16.43

Prepared by:

(Mr.Y. Vishwanadham)

Authorized by:

(Dr. V.V.S Rajendra Prasad)
Principal Investigator,
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(CMCR)

Dr. V.V.S. Rajendra Prasad, Ph.D
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Sub: Request for payment of Charges for Research work done at our Laboratory - Reg.

***

We are happy to support Elisa testing for biological samples at our Research Laboratory i.e. Centre for Molecular Cancer Research (CMCR). The testing charges for the same is amounting Rs.50,000/- We request for the payment of **Rs. 50,000/-(Fifty thousand Rupees only).**

With regards,

PRINCIPAL

Encl: Invoice

VISHNU INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH					
VISHNUPUR, NARSAPUR, MEDAK Dt-502313					
Sl.No	Batch No	Amount Received	Start date	End Date	No.of Employees
1	SBT-1	250000	25-May-22	18-Jul-22	29
2	SBT-2	250000	23-Aug-22	02-Nov-22	32
3	SBT-3	250000	24-Nov-22	18-Feb-23	25
4	SBT-4	250000	25-Apr-23	30-Jun-23	28
TOTAL		1000000			114

Vishnu Institute of Pharmaceutical Education & Research
Narsapur
Medak Dist.
502313

Principal
Vishnu Institute of Pharmaceutical
Education & Research
Narsapur, Medak dist -502313


Pharma
Pathashala


Pharma
Pathashala

VISHNU INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH					
VISHNUPUR, NARSAPUR, MEDAK Dt-502313					
Sl.No	Batch No	Amount Received	Start date	End Date	No.of Employees
1	DRL-1	568750	25-Mar-22	19-May-22	42
TOTAL		568750			42

Principal
Vishnu Institute of Pharmaceutical
Education & Research
Narsapur, Medak dist -502313


VALEDICTORY FUNCTION
Dr.Reddy's
Of
VISHNU
Technical Training Program


GPS Map Camera
Tuljaraopet, Telangana, India
Vishnu Institute of Pharmaceutical Education and Research, P7H4+J6X, SH 17,
Tuljaraopet, Telangana 502313, India
Lat 17.729174°
Long 78.255583°
27/09/23 11:46 AM GMT +05:30
Google

INCOZEN THERAPEUTICS PRIVATE LIMITED
&
Vishnu Institute of Pharmaceutical Education & Research

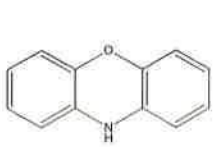
6. Methodology:

i. *In-Silico* drug design:

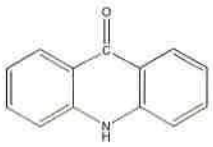
Initially a data set ligand library consisting acridone/phenoxazine as major scaffold was designed and prepared for all the computational studies. These data set ligands also include few ligands that were already synthesized in our lab and screened for MDR reversal activity. These ligands were used for the identification of possible potential pharmacophore responsible for MDR reversal activity using PHASE module of Schrodinger Suite 2012. Thus obtained model was validated using 3D-QSAR studies. All the ligands were evaluated for inhibition of Calmodulin dependent cAMP phosphodiesterase (PDE1c).

Digital protein structure of PDE1c was retrieved from protein data bank (http://www.rcsb.org/pdb/home) with a PDB ID 1LXS. All the ligands were geometrically optimized by minimizing the energy using LIGPREP module of Schrodinger Suite 2012. Metal binding and ionization states were generated where ever necessary. In the same way retrieved protein was also geometrically optimized by satisfying valencies and filling the missing amino acid groups and a grid was generated for docking studies. All the ligands were docked into the developed grid using GLIDE module of Schrodinger Suite 2012. Compounds with better fitness were synthesized and characterized. Synthetic scheme was shown in **Annexure: I**.

ii. Synthesis of designed molecules:



Phenoxazine



Acridone

A series of phenoxazines were synthesized by tagging quinazolinones **(Scheme I & II)** using standard synthetic procedure with optimized techniques. Quinazolinone derivatives were synthesized by following previously described method shown in **Scheme I**. Briefly 2-substituted-1,2-dihydro-4*H*-3,1-benzoxazin-4-one was prepared by the condensation of anthranilic acid with different acid chlorides followed by cyclization in the presence of acetic anhydride gave compounds (III a-c). Quinazoline derivatives (IV a-l) were synthesized by the treatment of compounds (III a-c) with hydrazine at high temperature. Total of nine Phenoxazine derivatives were synthesized by reacting p-amino phenol and diiodobenzinitrle followed by

N-alkylation. NO donating group was fused as N-alkyl substitution. Cyano group attached to phenyl ring is oxidized to an acid group for which previously listed qunizaolinones were tagged (**Scheme II**).

All the acridones were synthesized (**Scheme III**) by Ullman condensation reaction of animation of aryl halides (by using 2-chloro benzoic acid and anthranilic acid) in the presence of Cu as catalyst. Followed by the cyclization of intermediate in the presence of freshly prepared poly phosphoric acid at room temperature for three hours to get corresponding acridone carboxylic acid derivatives. Condensation of acridone carboxylic acid with different secondary amines was done in the presence of thionyl chloride in dry toluene and pyridine. The reaction mixture was stirred at room temperature for 3 hrs and then combined with excess of p-toluidine and triethylamine and stirred for another 3 hrs. The reaction was monitored by TLC. Then solvent was removed under vacuum and water was added to the solid residue. The precipitate was filtered, washed with water and dried.

Further *N*-alkylation of acridone carboxylic amide derivatives with alkyl halides (1-chloro 3-bromo propane & 1-chloro 4-bromo butane) were done by using phase transfer catalyst (PTC) tetra butyl ammonium bromide in the presence of KOH in tetra hydro furan for 24 hours. Completion the reaction is evidence by TLC the product was recovered and extracted with chloroform and purified by using column chromatography. In addition introduction of nitric oxide donating group into *N*-alkylated acridone was done by using silver nitrate in acetonitrile to get target lead molecules. Physical and chemical characterization of the molecules were done and shown in the **Table 1**.

iii. *In-vitro* cytotoxic activity evaluations by SRB assay:

Cytotoxic activity of the selected sixteen acridones was done by using following methodology and results were displayed in **Table 2**. The acridone derivatives were evaluated for cytotoxicity against cancer cell lines by using the sulforhodamine B (SRB) assay. The cells were cultured in RPMI 1640 (Gibco) supplemented with 10% fetal calf serum (Gibco), and cultures were passed once or twice a week using trypsin–EDTA to detach the cells from their culture flasks. The rapidly growing cells were harvested, counted, and plated at appropriate concentrations in 96-well microplates. After incubation for 24 h, the compounds, dissolved in the culture medium, were added to the culture wells in triplicate and incubated for 72 h at 37° C under a 5% CO_2 atmosphere. The cultures were fixed with cold TCA and stained with 0.4% SRB dissolved in 1% acetic acid. After dissolving the bound stain with 150 ll of 10 mM unbuffered Tris base (Tris(hydroxymethyl) aminomethane) solution using gyratory shaker, absorbance at 540 nm was measured using a microplate reader. The cytotoxic activity cytotoxicity was evaluated by measuring the concentration needed to inhibit protein synthesis by 50% (i.e., IC_{50}) as comparison. Each value represents the mean of triplicate experiments.

iv. Determination of rate nitric oxide release (Detection of nitrite):

The levels of nitrate/nitrite formed from individual compounds in the cells were determined by the colorimetric assay using the nitrate/nitrite colorimetric assay kit. Then nitrite production was measured by mixing 100 µl of cell lysates with 100 µl of Griess reagent in a 96-well plate, and after a (30-300) minute incubation at 37^0 C in the dark, absorbance was measured at 540 nm with a Tecan microplate reader, then 50 µl of cell suspension used for measurement of cellular proteins. A blank was prepared for each experimental condition in the absence of cells, and its absorbance was subtracted from that obtained in the presence of cells. Nitrite concentration was expressed as nano moles of nitrite per 24 h/mg cellular protein. The results are displayed in the **Table 3**.

7. Salient Research Achievements:

- We have designed novel nitric oxide donating acridones & phenoxazines and performed virtual screening (*In silico*) against calmodulin dependent cAMP Phosphodiesterase (PDE1c).
- Efficient pharmacophore hypotheses have identified for the MDR reversal activity from acridones among the dataset ligands.
- We have synthesized and characterized desired molecules with good yields by using novel synthetic strategies.
- The studies to detect the percentage of nitrile indicate that these molecules have shown considerable NO releasing pattern when compared with a standard NO donating molecule.
- Molecules screened for the anti-cancer and MDR reversal studies shown significant results and proved to be efficient and further studies are to performed to correlate NO donating pattern and MDR reversal activity.

7.1 Summary of Progress:

Various computational and *In silico* studies like pharmacophore modeling, 3D QSAR and molecular docking were performed for designing and identification of molecules with potential anticancer and MDR reversal activity from which most efficient molecules from the library were synthesized by using novel synthetic strategies with good yields. Desired molecules were tested and proved for the considerable release patterns of nitric oxide. These molecules were

screened for anticancer and MDR reversal activity against various drug sensitive and resistant cell lines and found to be significant. Further studies have to performed to identify the correlation between NO release and anticancer and MDR reversal activity and also further mechanistic studies have to be performed for the above stated biological activities.

7.2 New Observations:

- Efficient pharmacophore has been identified for MDR reversal.
- Synthetic strategies have been developed like N-alkylation under phase transfer catalyst with good yields.
- Synthesized molecules have shown better release patterns of NO when compared with the standards.
- Physico chemical properties of the molecules were found to be favorable and molecules shown significant cytotoxic properties.

7.3 Innovations:

- In general, pharmacophore modeling and 3D QSAR studies will be performed with respect to ligand and its receptor but, in the present investigation same principle has be implemented for ligand and cell lines (sensitive and drug resistant).
- Optimized synthetic methodologies were developed for development of nitric oxide releasing phenoxazines and acridones.
- Synthesized molecules were shown to have good nitric oxide donating property.

7.4 Application Potential:

7.4.1 Long Term

- Since targeting MDR is an emerging strategy in cancer chemotherapy, the present investigation focused on regulation of indirect iNOS pathway by designing NO donating molecules. These molecules will be a promising hit to reverse multidrug resistance in cancer chemotherapy.

7.4.2 Immediate:

- These investigations lead to design more efficient other NO donating scaffolds.
- The results of this investigation strongly support the role of iNOS pathway and exogenous nitric oxide release in the cellular apoptosis.

7.5 Any other: None

8. Research work which remains to be done under the project (for on-going projects)

<table>
<tr><td colspan="3">

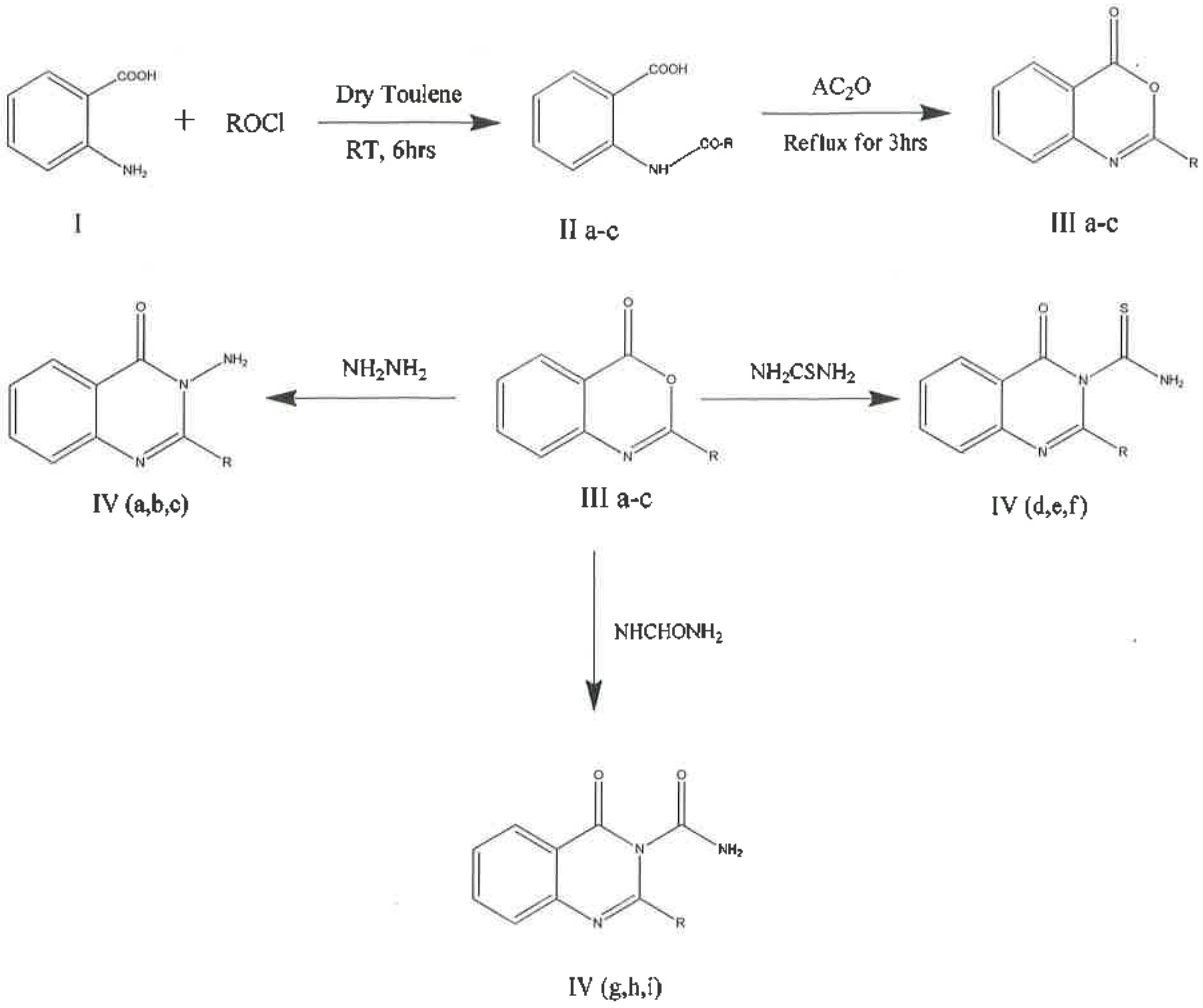
Remaining designed molecules of the library have to be synthesized by using efficient synthetic strategy.
Plan to elucidate anticancer mechanism of novel molecules by targeting other possible cancer drug targets.
Evaluation of in vitro cytotoxic activity against various sensitive and resistant cancer cell lines.
Intracellular doxorubicin accumulation studies.
Mechanistic studies and 3D QSAR studies.

</td></tr>
<tr><td>Ph.Ds Produced no:

One scholar currently working</td><td>Technical Personnel trained:

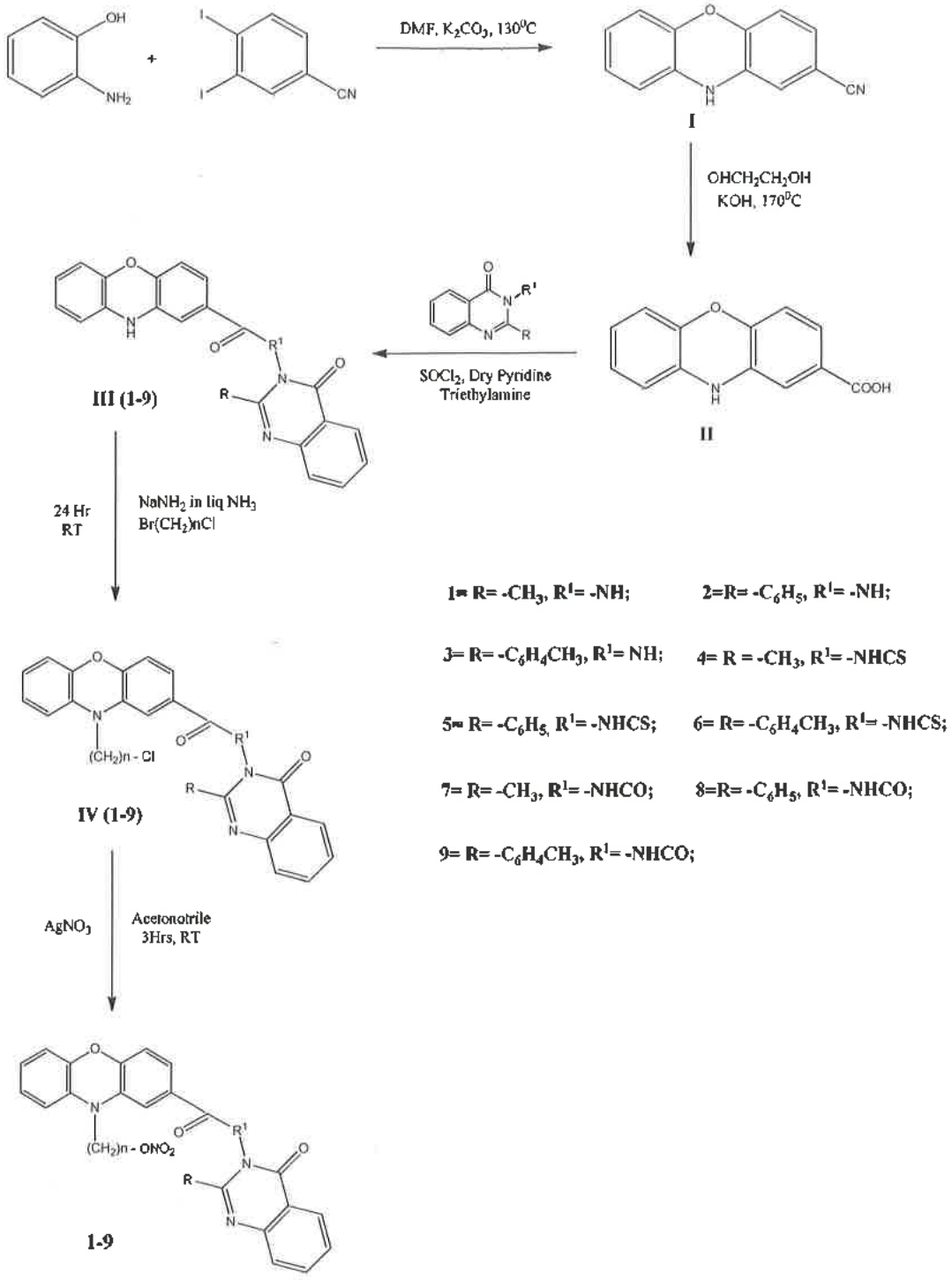
NO</td><td>Research Publications arising out of the present project:
One publication in cited journal (Elsevier publications) with impact factor 2.33
One paper accepted for publication in Journal Archive der Pharmazie with Impact factor of 1.54.
One papers are under review in reputed journals.</td></tr>
</table>

Scheme I: Synthesis of quinazoline derivatives



R: $-CH_3$, $-C_6H_5$, $-C_6H_4CH_3$

Scheme 2: NO donating Quinazolinone linked Phenoxoazines



1= R= $-CH_3$, R^1= -NH; **2=R= $-C_6H_5$, R^1= -NH;**

3= R= $-C_6H_4CH_3$, R^1= NH; **4= R = $-CH_3$, R^1= -NHCS**

5= R= $-C_6H_5$, R^1= -NHCS; **6= R= $-C_6H_4CH_3$, R^1= -NHCS;**

7= R= $-CH_3$, R^1= -NHCO; **8=R= $-C_6H_5$, R^1= -NHCO;**

9= R= $-C_6H_4CH_3$, R^1= -NHCO;

Scheme 3: Synthesis of NO donating acridone carboxamide derivatives

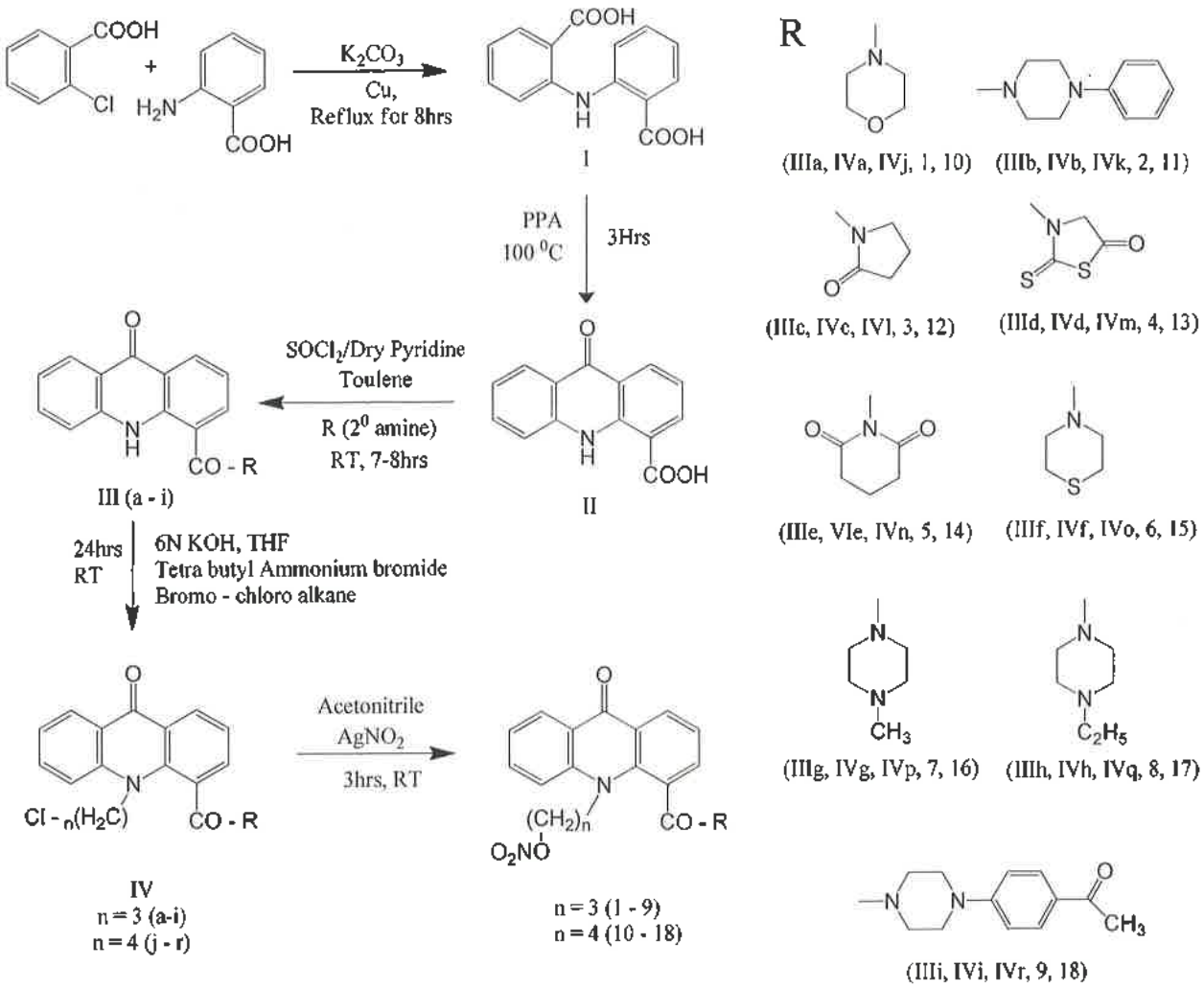
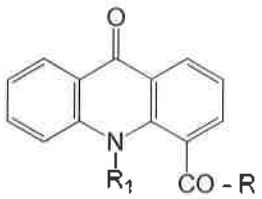
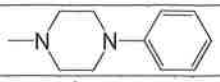
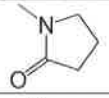
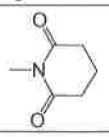
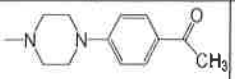
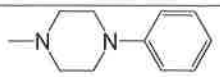
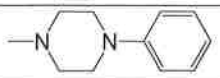
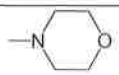
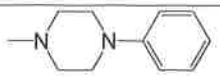
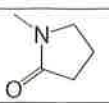
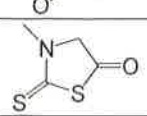
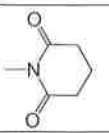


Table 1: Physical characterization data of acridone carboxamide derivatives.



Molecule	R1	R	MW	Volume	LogP
II a	$-(CH_2)_3-ONO_2$	-OH	342	1181.482	1.481
III b	H		383	1224.549	2.453
III c	H		306	1261.474	1.214
III e	H		334	1297.145	1.985
III i	H		425	1168.487	1.687
IV b	$-(CH_2)_3-Cl$		459	1304.678	1.049
IV k	$-(CH_2)_4-Cl$		473	1138.013	1. 650
1	$-(CH_2)_3-ONO_2$		411	1163.379	1.217
2	$-(CH_2)_3-ONO_2$		486	1405.695	3.463
3	$-(CH_2)_3-ONO_2$		409	1151.482	1.814
4	$-(CH_2)_3-ONO_2$		457	1244.549	1.685
5	$-(CH_2)_3-ONO_2$		437	1231.474	1.087

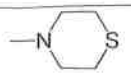
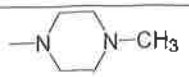
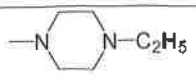
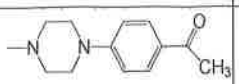
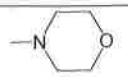
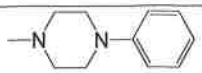
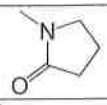
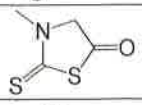
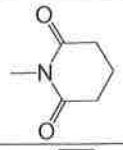
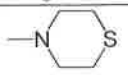
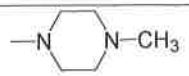
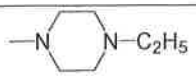
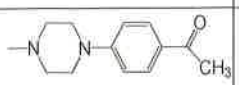
6	$-(CH_2)_3-ONO_2$		427	1233.797	2.347
7	$-(CH_2)_3-ONO_2$		424	1264.463	1.082
8	$-(CH_2)_3-ONO_2$		438	1318.14	1.465
9	$-(CH_2)_3-ONO_2$		528	1528.458	2.842
10	$-(CH_2)_4-ONO_2$		425	1247.593	1.642
11	$-(CH_2)_4-ONO_2$		500	1491.145	3.749
12	$-(CH_2)_4-ONO_2$		423	1268.487	2.304
13	$-(CH_2)_4-ONO_2$		471	1304.678	2.076
14	$-(CH_2)_4-ONO_2$		451	1313.067	1.604
15	$-(CH_2)_4-ONO_2$		441	1280.458	2.636
16	$-(CH_2)_4-ONO_2$		438	1338.013	1.530
17	$-(CH_2)_4-ONO_2$		452	1362.198	1.888
18	$-(CH_2)_4-ONO_2$		542	1611.872	3.163

Table 2: Cytotoxic activity of acridone carboxamide derivatives against various drug sensitive and resistant cancer cell lines.

Compound	Cell lines/IC_{50} (μM) ± SEM[a]					
	MCF7/Wt [1]	MCF7/Mr [2]	MCF7/Dx [3]	SW1398 [4]	WIDR [5]	LS174T [6]
II a	3.1 ± 0.7	3.4 ± 0.9	3.9 ± 1.1	7.9 ± 1.3	4.7 ± 1.0	3.75 ± 0.9
III b	4.5 ± 0.8	5.1 ± 1.1	6.5 ± 1.2	-	-	-
III c	22.0	32.0	30.	-	-	-
III e	30	55	60	-	-	-
III i	8.5 ± 1.5	10.2 ± 0.9	9.5 ± 0.5	-	-	-
IV b	10.5 ± 0.8	20.3 ± 1.9	18.5 ± 1.6	-	-	-
IV k	17.5 ± 1.6	30.1 ± 2.1	26.7 ± 2.0	-	-	-
1	1.2 ± 0.4	0.9 ± 0.1	2.9 ± 0.4	2.9 ± 0.1	4.5 ± 0.8	3.8 ± 1.0
2	2.0 ± 0.3	1.4 ± 0.2	6.2 ± 0.5	8.0 ± 0.7	13.0 ± 0.8	12 ± 2.4
3	3.1 ± 0.2	2.3 ± 0.4	7.8 ± 0.3	-	-	-
7	1.6 ± 0.2	1.1 ± 0.2	3.2 ± 0.4	-	-	-
10	0.8 ± 0.1	0.7 ± 0.1	1.9 ± 0.1	1.7 ± 0.2	2.8 ± 0.7	3.1 ± 0.3
11	0.7 ± 0.2	0.8 ± 0.1	2.0 ± 0.1	2.8 ± 0.1	4.0 ± 0.5	3.7 ± 0.4
12	2.8 ± 0.3	3.8 ± 0.6	5.8 ± 0.3	7.2 ± 0.4	14.5 ± 1.1	11.5 ± 2.7
16	1.4 ± 0.6	0.7 ± 0.1	4.2 ± 0.2	11.0 ± 0.7	19.1 ± 1.5	18.2 ± 3.1
Mitoxantrone (Mr)	0.090	3.0	-	-	-	-
Doxorubicin (Dx)	0.098	-	3.7	-	-	-

[a] SEM - standard error of the mean
[1] MCF7 – WT: Human breast cancer cell line
[2] MCF7-MR: BCRP expressed Mitoxantrone resistant breast cancer cell line
[3] MCF7-DX: P-gp expressed doxorubicin resistant breast cancer cell line
[4] SW 1398: Human colorectal cancer cell line
[5] WiDr: Colon adenocarcinoma cell line
[6] LS 174T: Human Caucasian colon adenocarcinoma cell line

Table 3: Nitrite release of NO-acriones in MCF7 cancer cell lines

Compound	Nitrite µM/mg protein	
	MCF7/Wt	MCF7/Dx
2	5.21	4.31
7	4.65	3.49
10	7.02	6.64
11	6.11	6.24
12	5.51	4.94