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DESIGN, SYNTHESIS AND STUDIES OF DELTA AND COX-2 SPECIFIC ANALGESIC ANTI-INFLAMMATORY ACTIVITY OF SOME LINEAR AND CYCLIC PEPTIDES

by

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- *Established in 1955 by visionary-industrialist Mr. B.M. Birla, it is today one of the most premier Institute in India.*
- *Main Campus Size - 780 acres, situated in a natural ,environment friendly campus.*
- *BIT, Mesra is a "Deemed University" under Sec. 3 of the U.G.C. Act 1956.*

- *BIT ,Mesra currently has many departments with other Research Centers offering various undergraduate and postgraduate courses like B.Tech, B.Pharm,B.Arch., M.Tech. M.Pharm, MBA, M.Sc and PhD in almost all disciplines.*
- *Mr. C.K Birla is the present Chairman of this premier Institute.*

PRESENTATION OVERVIEW

- **INTRODUCTION**
  - **ANALGESIC AGENTS**
  - **OPOID RECEPTOR**
  - **ANTIINFLAMMATORY AGENTS**

- **NEED FOR PRESENT INVESTIGATION**
- **OBJECTIVES**
- **EXPERIMENTAL WORK**
- **SUMMARY AND CONCLUSION**
- **FUTURE SCOPE OF THE WORK**
- **REFRENCES**

Analgesia

- **International Association for the Study of Pain(Algesia) defines pain as: "Pain is an unpleasant sensory *and* emotional experience that is associated with actual or potential tissue damage or described in such terms**."
- The term "analgesia" derived from Greek word Ava (without) and Avos (pain).
- **A drug which depresses pain without arising loss of consciousness is called an analgesic agent.[1]**
- **Major Classes of Analgesic Agents are:**
- **NSAIDs**

• **COX-2 inhibitors:**These drugs have been derived from NSAIDs. The COX- 2 inhibitors were developed to inhibit only the COX -2 enzyme

• **Flupirtine:** is a centrally acting K+ channel opener with weak NMDA antagonist properties. It is used in Europe for moderate to strong pain and migraine and its muscle-relaxant properties.

• **Opioids:** Opioids, while very effective analgesics, may have some unpleasant side-effects. opioids and similar narcotic analgesics are otherwise safe and effective, however risks such as addiction and the body's becoming used to the drug (tolerance) can occur.

• The identification of opioid peptides in mid 1970 opened up a whole new area for the development of opioid receptor.[2]

• The naturally occurring endogenous opioid peptides Enkephalin are rapidly degraded by a variety of peptidases such as aminopeptidase, peptidyl dipeptidase-A, endopeptidase-24.11. Therefore, one major goal for structural modification of these small peptides has been to increase metabolic stability.[3]

•Analgesic drugs act in various ways on the peripheral and central nervous systems.

PERIPHERAL PAIN PATHWAYS

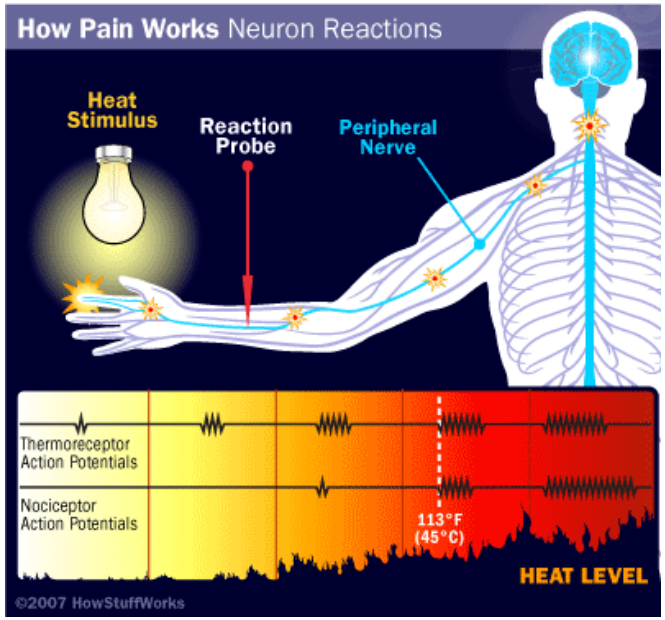


Fig.1 Pain Signal Reception
Reproduced from, www.ampainsoc.org/ce/enduring.ht [Last visited 28.11.09]

SPINAL PAIN PATHWAYS

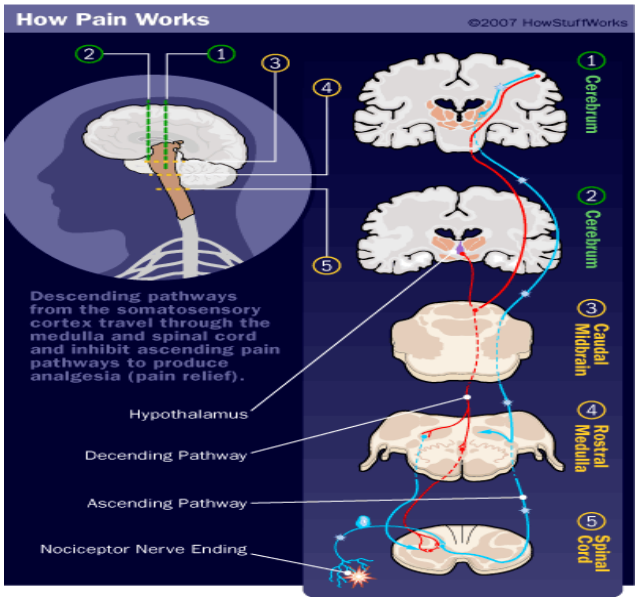


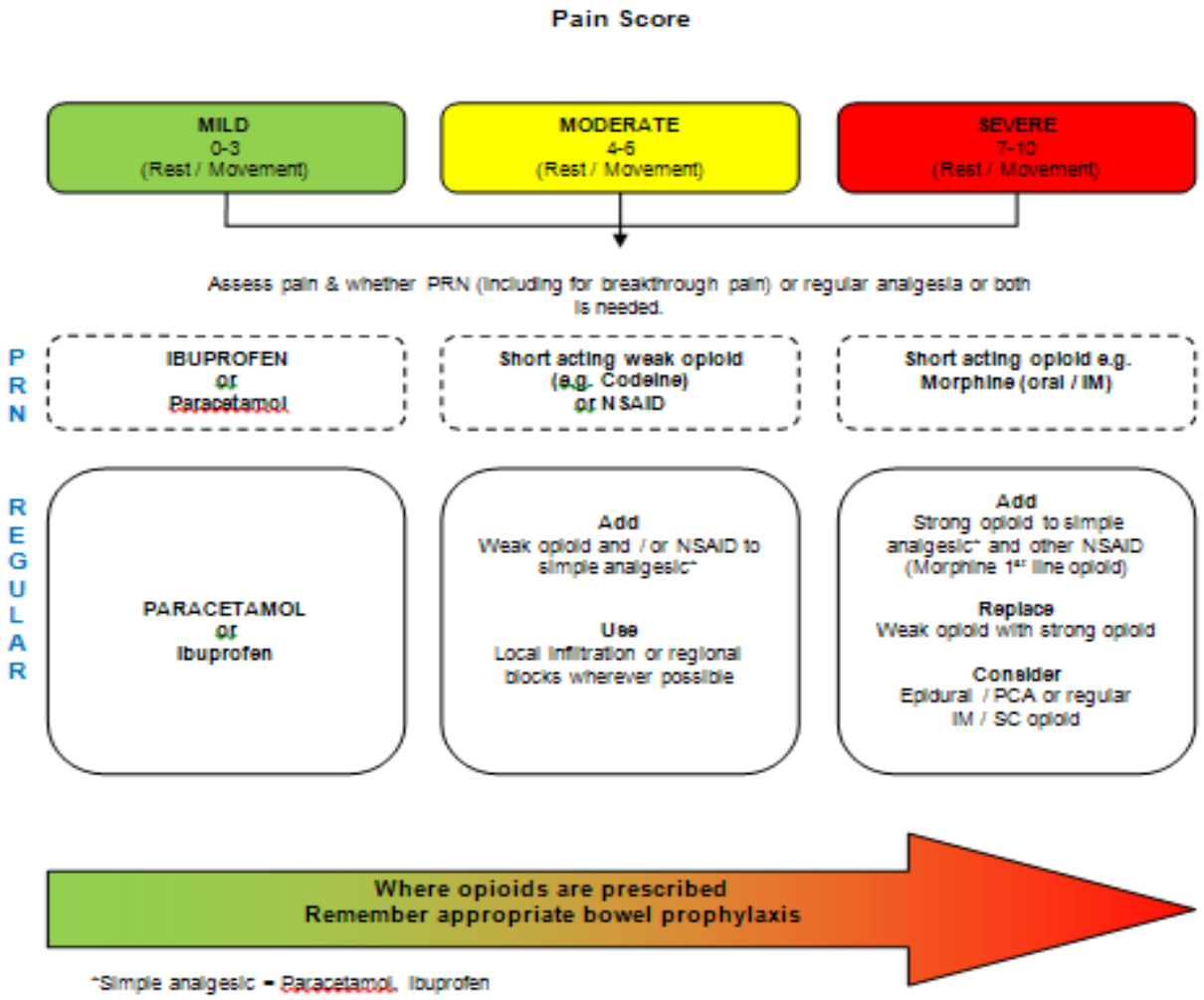
Fig.2 pain-influencing neural pathways, Reproduced from, www.ampainsoc.org/ce/enduring.htm [Last visited 28.11.09]

PERIPHERAL PAIN PATHWAYS

- Nociceptor neurons travel in peripheral sensory nerves. Their cell bodies lie in the dorsal root ganglia of peripheral nerves just inside the spine
- These neurons are lightly or non-myelinated and slower.
- Nociceptors-A δmechano-sensitive
  -Aδmechano-thermal
  -Polymodal nociceptors (C fibers)
- The pain (from Aδ fiber activation) is sharp and rapid &(from C fiber activation) is dull, burning and delayed.

SPINAL PAIN PATHWAYS

- The descending pathways originate in the **somatosensory cortex** (which relays to the thalamus) and the **hypothalamus**. Thalamic neurons descend to the midbrain.
- There, they synapse on ascending pathways in the medulla and spinal cord and inhibit ascending nerve signals. This produces pain relief (analgesia).
- Ascending pain pathway →**neuropathic pain (**damage to peripheral nerves, spinal cord or the brain itself).



Source: http://www.elmmb.nhs.uk/formularies/joint-medicines-formulary/4/4-7/(11.8.2015)

Activation of Opioid Receptor 4-5

- The μ-, δ- and κ-opioid and orphanin receptors are the rhodopsin-like G protein-coupled receptors (GPCRs) involved in pain management and regulation of mood, reward, motivation, and response to stress.

- The activation of opioid receptors enables their transient association with Gi/o proteins, which triggers signal transduction through inhibition of adenylate cyclase and regulation of ion channels and MAP kinases.

- Opioid receptors are naturally activated by endogenous opioid peptides, but also can interact with exogenously administered opiates, some of which are addictive drugs of abuse.

- The binding of opioid ligands may have different functional outcomes. Opiates can act as agonists, antagonists, partial agonists, or inverse agonists.

.

Opioid Receptor

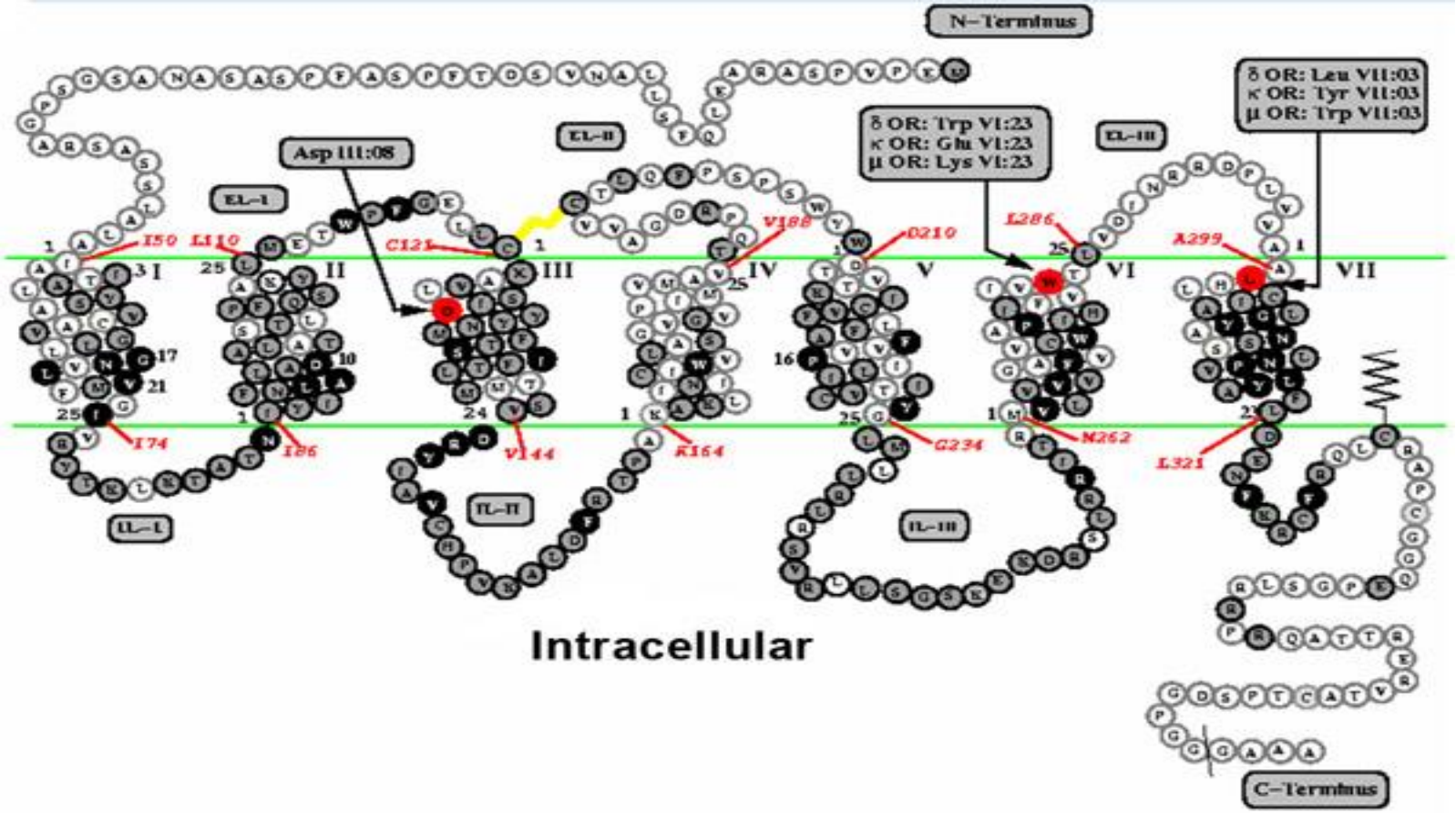


Figure 3. Serpentine model of the δ receptor. Circles contain the 1-letter code for the given amino acid. Green lines indicate the beginning and ends of the helices. The gray circles indicate the residues that are conserved among all 3 receptor types (μ, δ, and κ), while the black circles indicate the residues that are highly conserved among the rhodopsin subclass of G-protein coupled receptors. Each transmembrane (TM) region is indicated by a roman numeral.

[Reprodued from Kane, B. E.; Svensson, B.; and Ferguson, D.M. Molecular Recognition of Opioid Receptor Ligands, *The AAPS Journal*, 2006, 8(1), E128]

Opioid Ligands [6]

RECEPTOR SUBTYPE	SELECTIVE LIGANDS		NONSELECTIVE LIGANDS		PUTATIVE ENDOGENOUS LIGANDS
	Agonists	Antagonists	Agonists	Antagonists	
μ	DAMGO Morphine Methadone	CTOP	Levorphanol Etorphine	Naloxone Naltrexone	Enkephalin Endorphin
δ	DPDPE Deltorphin DSLET	Naltrindole NTB BNTX	Levorphanol Etorphine	Naloxone Naltrexone	Enkephalin
κ	Spiradoline U50,488 DynorphinA	Nor-BNI	Levorphanol Etorphine EKC	Naloxone Naltrexone	DynorphinA

Development of Delta Opioid Peptides as Nonaddicting Analgesics [7-8]

Delta (δ-) opioid peptides will be unique therapeutic agents when compared to opiates for the following reasons.

1) The peptide delta agonists offer an additional level of safety for the foetus.

- ✓ The peptide analogues of enkephalins and related peptides could serve as obstetric medications, providing analgesia for the mother without exposing the foetus due to inability to cross the placenta.
- ✓ The opioid δ-receptor can not be demonstrated in human foetal brain tissue.

2) On metabolic degradation, peptides will be hydrolyzed to their constituent amino acids, and the metabolic end products, unlike the opiates, are polar, easily eliminated from the body and unlikely to cause liver or kidney damage.

3) These are likely to have decreased dependence or abuse liability and lower reinforcing efficacy.

Cont.

4) From the drug design aspects, peptides offer special advantages [9]

- ✓ As peptides are made up of subunits, amino acid residues, virtually an unlimited number of analogues can be synthesized.

- ✓ The three dimensional architecture of the conformationally labile peptides can be altered by incorporating various structural modification (peptide bond replacement, N-methyl substituent, formation of cyclic structure, etc).

- ✓ Thus, obtain desired bioactive peptide molecules with structural rigidity.

5) Endogenous peptides serve as better models for studies on biosynthesis and conformation. As peptides are polar molecules, solution phase studies can be performed in different solvents to understand the effects of solvents on conformation.

6) Opioid δ-agonists do not demonstrate significant cross-tolerance to opiates acting at the μ-receptor such as morphine; thus they are likely to be useful pain relievers in patients undergoing prolonged therapy or high dose treatments with μ -opiates.

ANTI-INFLAMMATORY AGENT

➢Inflammation (Latin, *inflammatio*, a setting on fire) is the complex biological response of vascular tissues to harmful stimuli, such as pathogen, damaged cells, or irritants.

➢A drug which inhibits or suppresses most inflammatory response is called Anti-inflammatory Agent.

➢They include corticosteroids and NSAIDS.

TYPES OF INFLAMMATION [9,10]

Causative agent	*Acute*	*Chronic*
	Pathogens, injured tissues	Persistent acute inflammation due to non-degradable pathogens, persistent foreign bodies, or autoimmune reactions
Major cells involved	Neutrophils, mononuclear cells (monocytes, macrophages	Mononuclear cells (monocytes, macrophages, lymphocytes, plasma cells), fibroblasts
Primary mediators	Vasoactive amines, eicosanoids	IFN-γ and other cytokines, growth factors, reactive oxygen species, hydrolytic enzymes
Onset	Immediate	Delayed
Duration	Few days	Up to many months, or years
Outcomes	Resolution, abscess formation, chronic inflammation	Tissue destruction, fibrosis

NEED FOR PRESENT INVESTIGATION

- Non-μ-opioid analgesics (mainly δ-agonists) and peripherally acting opioid analgesics have a promising clinical profile but have yet to show significant clinical impact. [11]

- Non steroidal anti-inflammatory drugs (NSAIDs) inhibit brain prostaglandin synthesis, interfere with transmitters or modulators in the nociceptive system, or their effects are mediated in part by endogenous opioid peptides. [12]
- But common adverse effects of NSAIDs are gastrointestinal (GI) bleeding, ulceration and more severely, perforation.[13]
- So δ-receptor specific Enkephalin based peptide molecule acting as peripheral analgesic with novel mechanism (COX-2 receptor specific also) deserve greater attention for evaluation in chronic pain situations since they offer the potential for greater efficacy to manage intractable pain.[3]
- There is need for δ-receptor specific analgesics which would be highly potent and safe. With the investigation of small peptides both linear and cyclic ,a new potential series can be established in this area of therapy.

OBJECTIVE OF THE WORK

- The objective of this work is to design, synthesize and screen potential small peptide based compounds for their analgesic and anti-inflammatory activity.
- Design and validation of Small linear and cyclic peptides on 4-COX and the δ opoid receptor(OPRD_HUMAN_AD_JOM-13) using:
  - Molecular Modelling and Docking Studies - Glide 5.0.(Schrodinger).
  - ADME profile studied using QIKPROP 3.1(Schrodinger).
  - In Silico Toxicity studies using OSIRIS PROPERTY EXPLORER.
- To synthesize of Small linear and cyclic peptides using Liquid Phase Method and characterized by M.Pt, TLC, FT-IR, NMR and Mass spectral analysis.
- Pharmacological screening of synthesized compound for Analgesic and Anti-inflammatory activity.

PLAN OF WORK

Designing

- Designing of some Linear and Cyclic Peptides
- Homology Modeling
- Docking of modeled analogs with the receptors 4COX and OPRD_HUMAN_AD_JOM-13 by using software- Glide5.0.ADME studies using Qikprop 3.1.
- In Silico Toxicity Studies using OSIRIS Property Explorer. (http://www.organic-chemistry.org/prog/peo/tox.html)

Synthesis

- Synthesis of selected designed molecules.
- Characterization of the synthesized compounds
  i)Physicochemical characterization (M.P, R_f value
  ii)Spectral analysis (FT-IR, NMR and Mass).

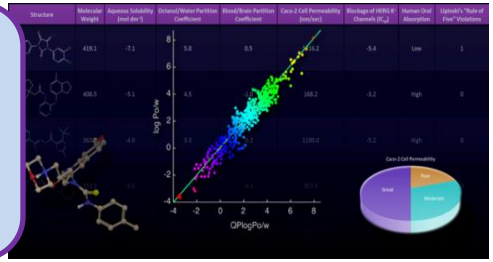
Biological activity

- Pharmacological screening:
- a)*Analgesic activity study: Acetic acid-induced writhing response in mice.*
- b)*Anti-inflammatory activity study:*
- *Carrageenan induced hind paw edema in rats*

EXPERIMENTAL WORK

Molecular modelling and Docking Studies using software Glide 5.0 and Homology modeling.

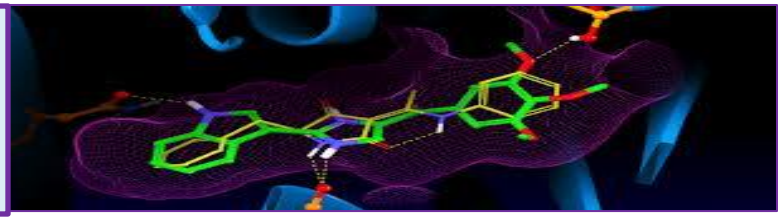
ADME prediction using software Qikprop version 3.1
In Silico Toxicity studies using OSIRIS Property Explorer.
(http://www.organicchemistry.org/prog/peo/tox.html)



Synthesis using solution phase peptide synthesis of linear and cyclic peptides.

Animal Studies:
a)Analgesic activity
b)Anti-inflammatory Activity

DOCKING STUDIES [14]



GENERAL STEPS INVOVED IN DOCKING

PROTEIN PREPARATION

The X-ray crystal structure of Cyclooxygenase-2 (Prostaglandin synthase-2) receptor 2.90A° (4cox) and Bovine Rhodopsin δ- opioid receptor 2.80 A° (1f88) were selected from Protein Data Bank (www.rcsb.org/pdb) into Maestro.

HOMOLOGY MODELLING

The Homology model for delta opioid receptor (1f88) which was used had been sent by Mosberg laboratory, University of Michigan.

LIGAND PREPARATIONS using ChemDraw Ultra 10.0 and energy minimization using OPLS 2500

RECEPTOR GRID GENERATION

-The atoms were scaled by Vander Waals radii of 1.0 Å with the partial atomic charge less than 0.25 defaults \

-active site was defined as an enclosing box at the centroid of the workspace ligand as selected in the receptor folder

LIGAND-RECEPTOR DOCKING

All the energy minimised structures were docked with the energy minimised receptors

VALIDATION OF DOCKING PROTOCOL – Every ligand was docked with both receptors (pdb: 4COX andOPRD_HUMAN_AD_ JOM-13)

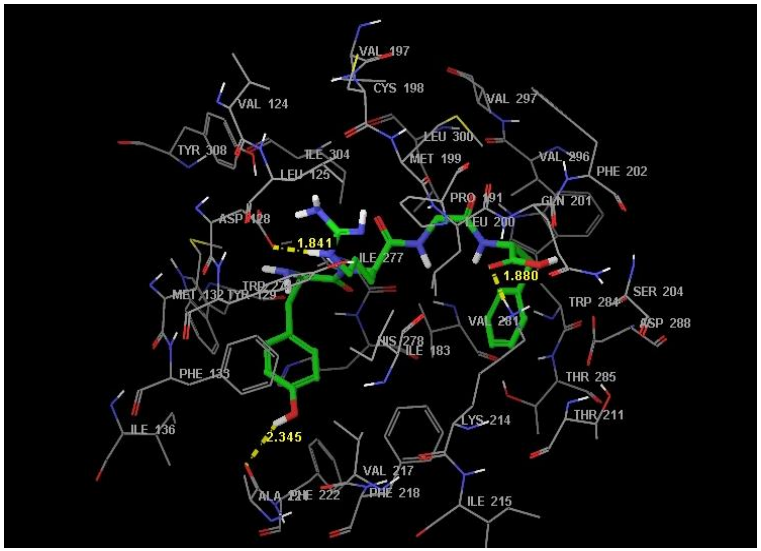
Docking Scores of Standard and designed Compounds

LIGAND CODES	Ligand Name	4COX	OPRD_HUMAN_AD_JOM-13
Standard	Indomethacin	-8.01	-7.31
Standard	Pethidine	-6.88	-4.16
Standard	DPDPE	-4.24	-10.38
Standard	JOM-13	-3.46	-10.16
SSLR-01	Tyr-Arg-Phe	-8.21	-7.19
SSLR-02	Tyr-Pro-Phe	-8.36	-8.93
SSLR-03	Tyr-Leu-Phe	-7.18	-9.43
SSLR-04	Tyr- Arg-Gly-Phe	-8.06	-8.78

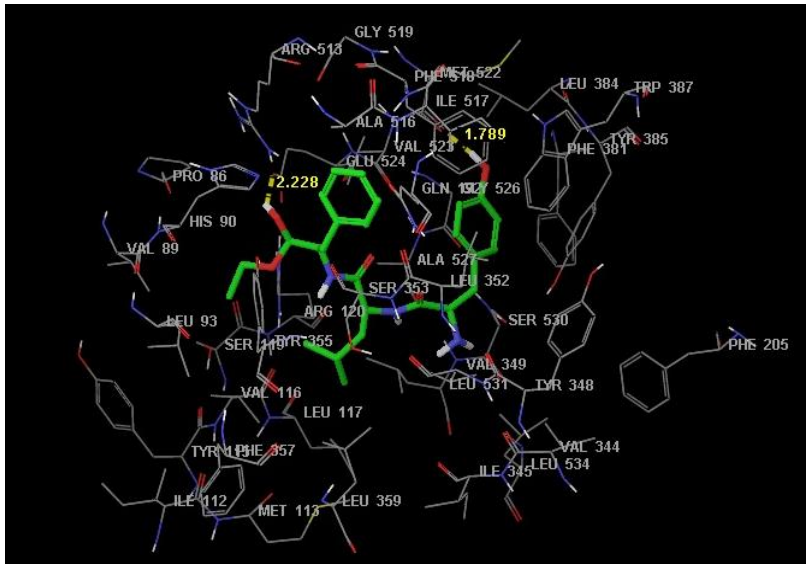
LIGAND CODES	Ligand Name	4COX	OPRD_HUMAN_AD_JOM-13
SSLR-06	Tyr-Ala-Arg-Phe	-6.99	-9.11
SSLR-07	Tyr-Ala-Phe ethyl ester	-7.18	-6.56
SSLR-08	Tyr-Pro-Phe Ethyl ester	-6.86	-6.05
SSLR-09	Tyr-Leu-Phe Ethyl ester	-6.77	-6.98
SSLR-10	C(Tyr-Gly-Phe)	-7.95	-5.30
SSLR-11	C(Tyr-Pro-Phe)	-6.82	-5.17
SSLR-12	C(Tyr-Leu-Phe)	-6.75	-9.44
SSLR-13	C(Tyr-Ala-Phe)	-4.61	-5.80

Docking poses

XP-Glide-predicted pose of molecule SSLR-4 with the receptor(OPRD_HUMAN_AD_JOM-13).



XP Glide-predicted pose of molecule SSLR-9 with the receptor (4COX).



SSLR-4 is forming three H-bonds (1.84, 1.88 & 2.35) with Asp-128, Val-281, Ala-221

SSLR-9 is forming two H-bonds (1.79 and 2.22) with Val-524, Arg-120.

Result: All the 30 designed molecules both linear and cyclic were docked using Glide5.0. SSLR-4 and SSLR-9 showed good scores of -8.06 and -6.77 in 4-COX and -8.78 and -6.98 in OPRD_HUMAN_AD_JOM-13).

ADME STUDIES using QIKPROP 3.1 [15]

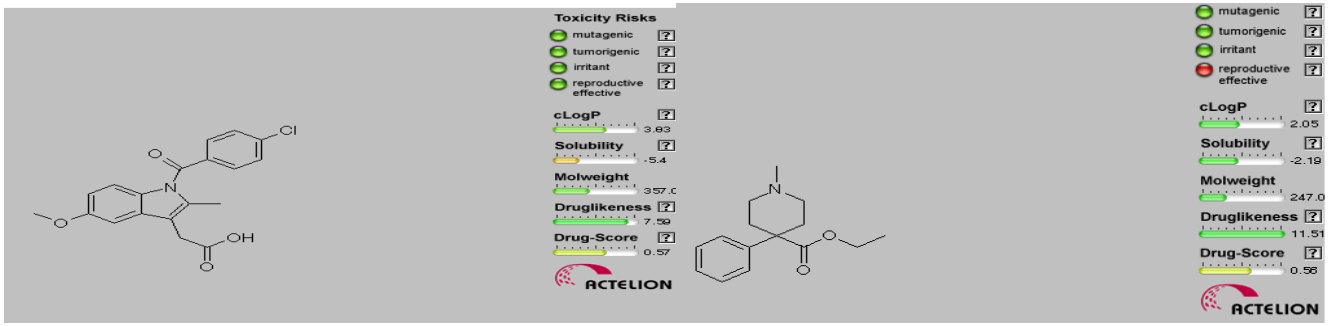
- QikProp is used as an indispensable tool for applying ADME principles in lead discovery & optimization.
- Nearly 40% of drug candidates fail in clinical trials due to poor ADME (absorption, distribution, metabolism, and excretion) properties. [27]

Standard compound	QP logP o/w	QP logS	QP logBB	CNS	MW	Human Oral absorption	Percent Human Oral absorption
Indomethacin	3.600	-4.525	-0.674	-2	357	3	90.073
Pethidine	2.662	-2.383	0.551	+2	247	3	100.000
SSLR-10	0.217	-1.293	-1.844	-2	367.404	3	49.979
SSLR-11	0.811	-2.289	-1.236	-2	407.468	3	66.402
SSLR-12	1.867	-3.274	-1.385	-2	423.511	3	73.853
SSLR-13	0.416	-2.396	-1.254	-2	381.430	3	65.743
SSLR-14	1.119	-2.888	-2.281	-2	510.589	2	28.582

QP log Po/w : Predicted octanol /water partition coefficient; Range, -2.0 to 6.5
QP logs: Predicted aqueous solubility, log S; Range, -6.5 to 0.5
QP logBB: Predicted brain/blood partition coefficient; Range, -3.0 to 1.2
CNS activity: Predicted central nervous system activity; [-2(inactive), +2 (active)]
Human Oral Absorption: Qualitative; 1→Low, 2→Medium, 3→High
Percent Human Oral absorption: 0 to 100% scale; [>80%→ High, <20%→ Poor]

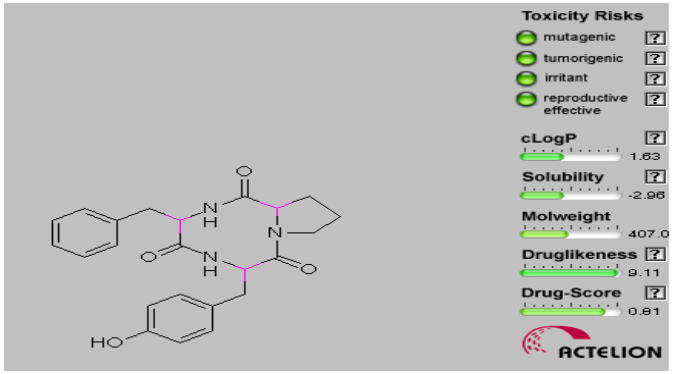
In Silico TOXICITY Studies using OSIRIS Property Explorer [16]

In Silico TOXICITY Studies using OSIRIS Software. **Toxicity related study to judge the compound's overall potential to qualify for a(Low risk ,medium risk , high risk All the designed linear and cyclic peptides selected after virtual screening, were free from toxicity using OSIRIS Property Explorer.**

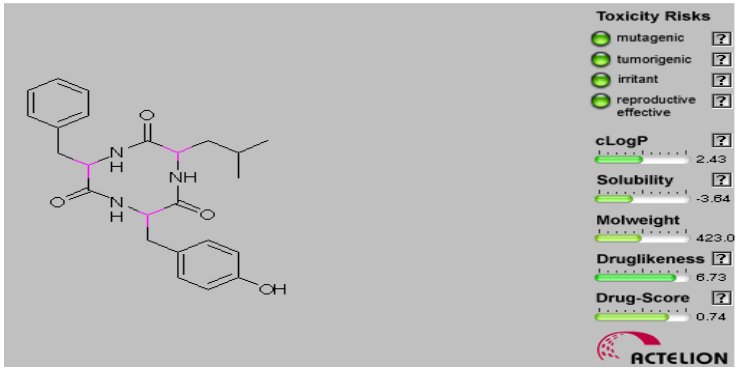


Toxicity related assessment of Indomethacin

Toxicity related assessment of Pethidine



Toxicity related assessment of SSLR-11



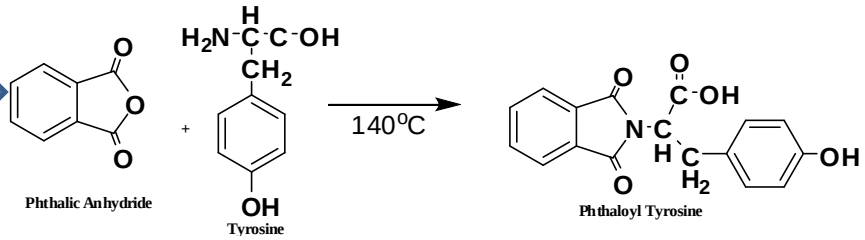
Toxicity related assessment of SSLR-12

Specific information of standard compounds (Indomethacin, Pethidine) and SSLR-11,SSLR-12 via OSIRIS Property Explorer; where green indicates safe but red unsafe. (Reproduced from http://www.organic-chemistry.org/prog/peo/ [Last visited on 26.11.09])

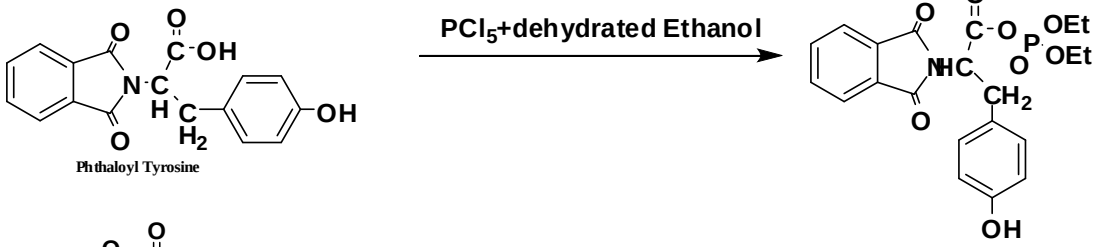
SYNTHETIC STUDIES [17,18,19]

SCHEME FOR LINEAR PEPTIDES

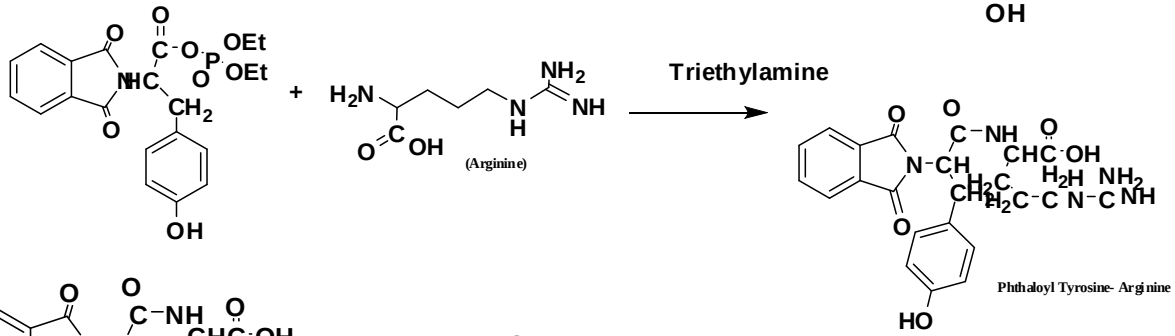
Step1 — Protection



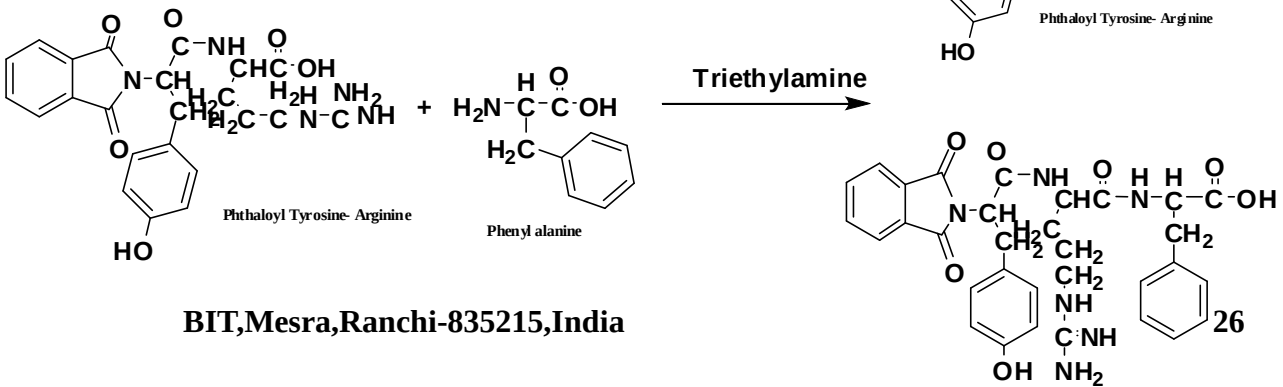
Step2 — Activation



Step 3 — Coupling

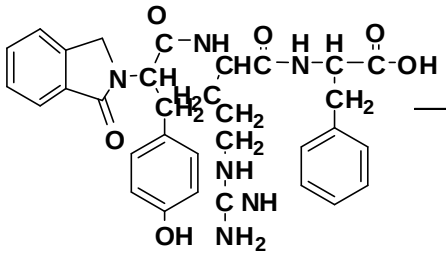


Step4 — Activation

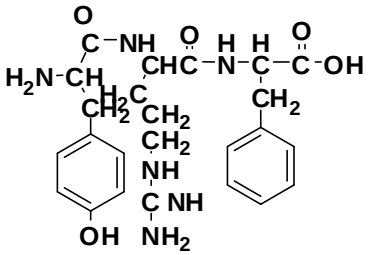


Step1

Deprotection



NH_2-NH_2/HCl



Tyr-Arg-Phe (Tripeptide)

SYNTHETIC SCHEME FOR CYCLIC PEPTIDE

Phthaloyl Tripeptide Ethyl Ester

N2H4
HCl
N2H4

$NH_2CH_2CONHCH_2CONHCH_2CONHNH_2$

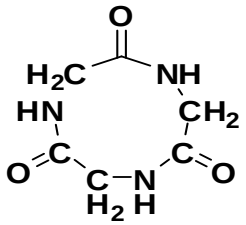
Tripeptide hydrazide

HCl
NaNO2

$NH_2CH_2CONHCH_2CONHCH_2CON_3$

Tripeptide azide

NaHCO3
0-5°C

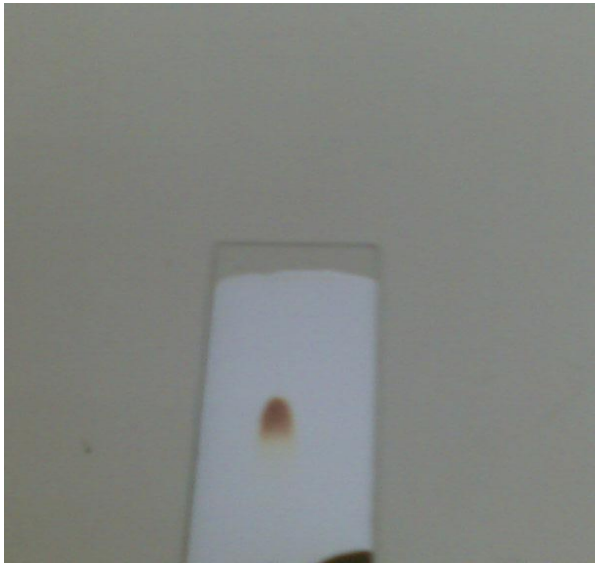


Cyclic peptide

PHYSICO- CHEMICAL CHARACTERIZATION OF SYNTHESIZED COMPOUNDS

For monitoring the chemical reactions during the course of peptide synthesis and the purity of synthesized compounds was shown by performing TLC by getting a single spot. The solvent system used for linear peptides was n-Butanol: Acetic acid: Water (BAW) = 4:1:1 and for cyclic peptides Chloroform: Methanol = 3:1. Iodine was used as detector.

TLC Chromatogram Development



TLC Chromatogram of SSLR-4

Physical properties of the synthesized compounds

Sl.No.	Name of the compound	Color & Nature	Melting point (^{0} C)	R_f (BAW*)
1.	Phthaloyl Tyrosine	Yellowish White crystalline	169	0.39
2.	Phe-OEt HCl	Dirty White crystallline	155	0.42
3.	Tyr-Pro-Phe-OEt	White crystalline	180	0.38
4.	Tyr-Arg-Phe (SSLR-1)	Yellowish White crystalline	162	0.56
5.	Tyr-Arg-Gly-Phe (SSLR-4)	White crystalline	227	0.42
6.	Tyr-Arg-Cys-Phe (SSLR-5)	White crystalline	215	0.46
7.	Tyr-Leu-Phe-OEt (SSLR-9)	White crystalline	185	0.36
8.	Cyclo-Tyr-Pro-Phe** (SSLR-11)	White crystalline	240	0.39
9.	Cyclo-Tyr-Leu-Phe** (SSLR-12)	White crystalline	252	0.32

[* n- Butanol:Acetic acid: Water (BAW)=4:1:1], [** in $CHCl_3$-CH_3OH (3:1)]

SPECTRAL DATA OF SYNTHESIZED COMPOUNDS [20,21]

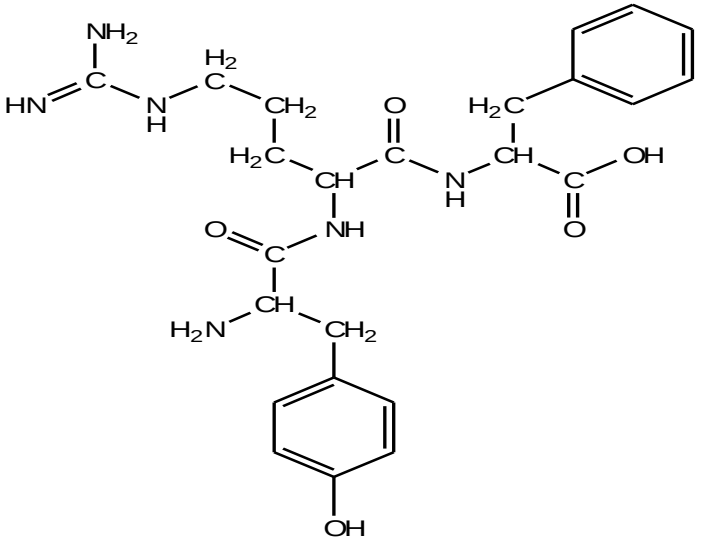
FT-IR spectral data of compound SSLR-1 (Tyr-Arg-Phe)

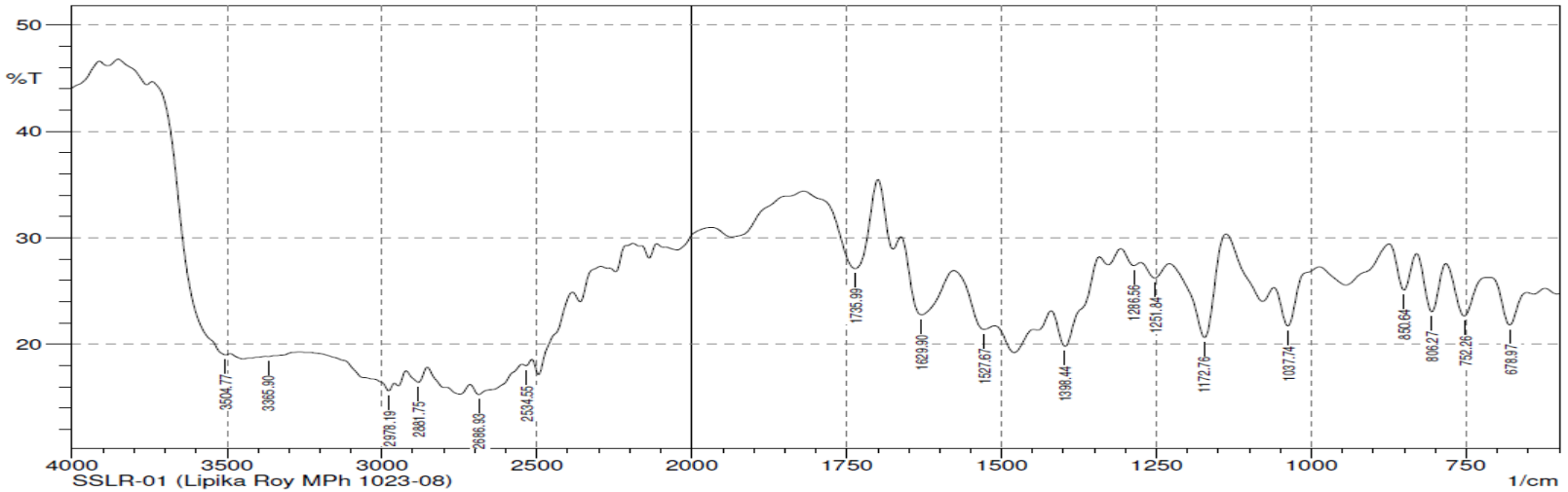
IR (KBr):

3504.77 OH stretching of Aromatic OH
3365.90 N-H stretching of secondary amide
2978.19 C-H stretching of methylene (CH_2)
2534.55 OH stretching of Carboxylic acid
1629.90 N-H bending of primary amine
1527.67 C=O stretching of secondary amide
1286.56 C-N stretching of primary amine
678.97 N-H out of plane wagging of amide

Mol. Formula: $C_{24}H_{32}N_6O_5$

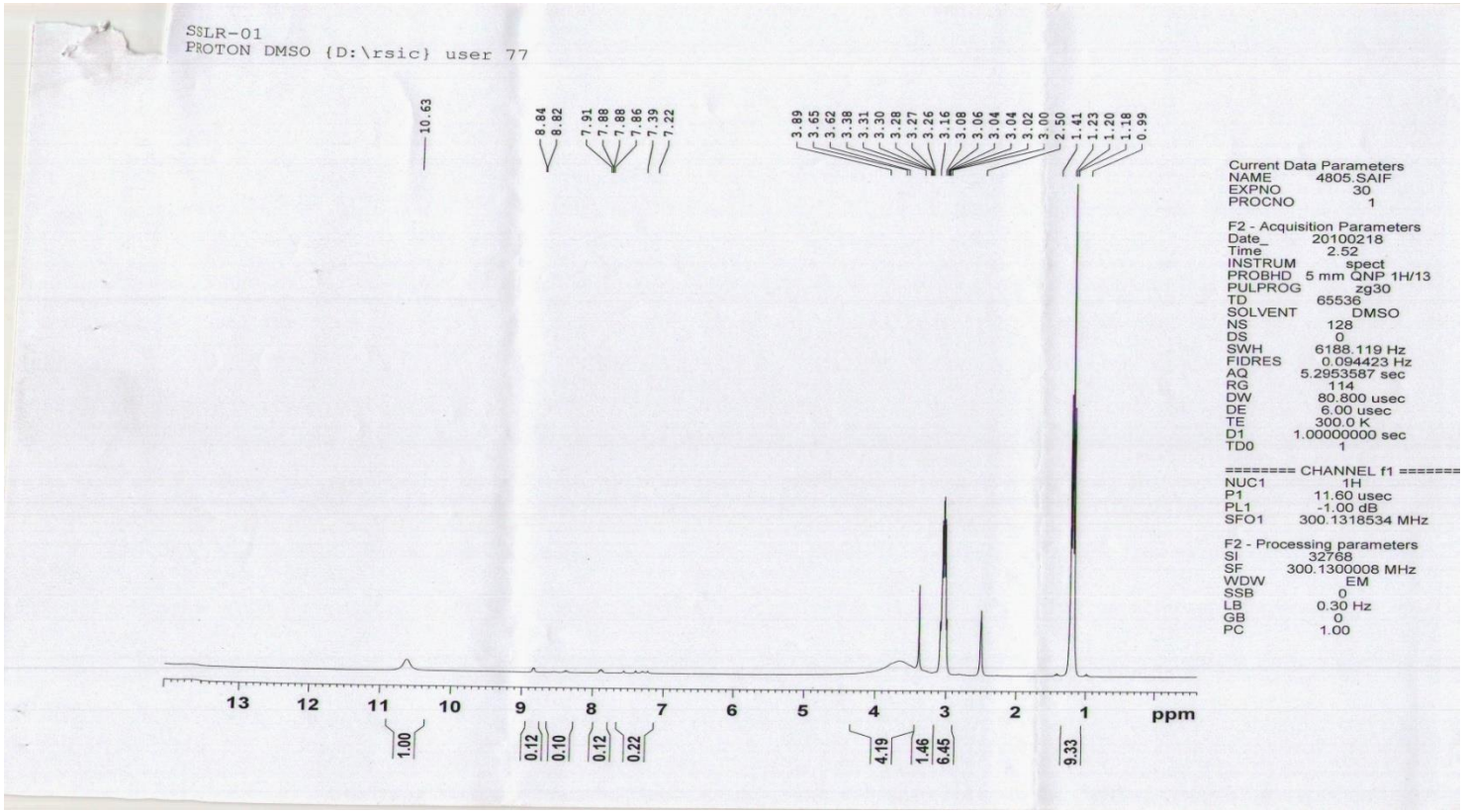
Mol. Weight : 484





^{1}H NMR spectral data (value of δ in ppm) of SSLR-1 :

H of amine ($-NH_2$) (2.50) (s), H of Methylene (CH_2) (3.00-3.08) (m), H of Methine (CH) (3.62-3.89) (t), H of Benzene (7.22, 7.39) (s), H of [–C(=O)NH] (7.86 – 7.91) (m), H of –COOH (10.63) (s).



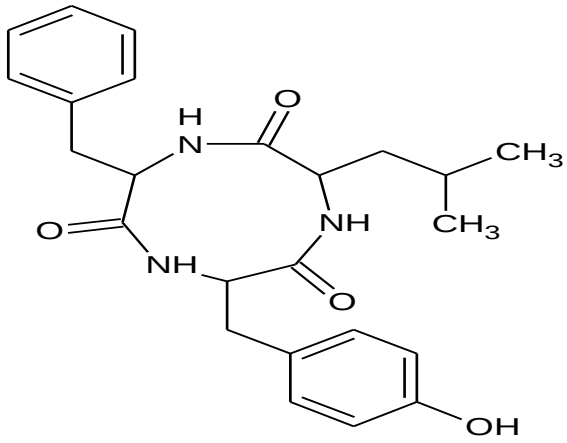
FT-IR spectral data of compound SSLR-12 [Cyclo(Tyr-Leu-Phe)]

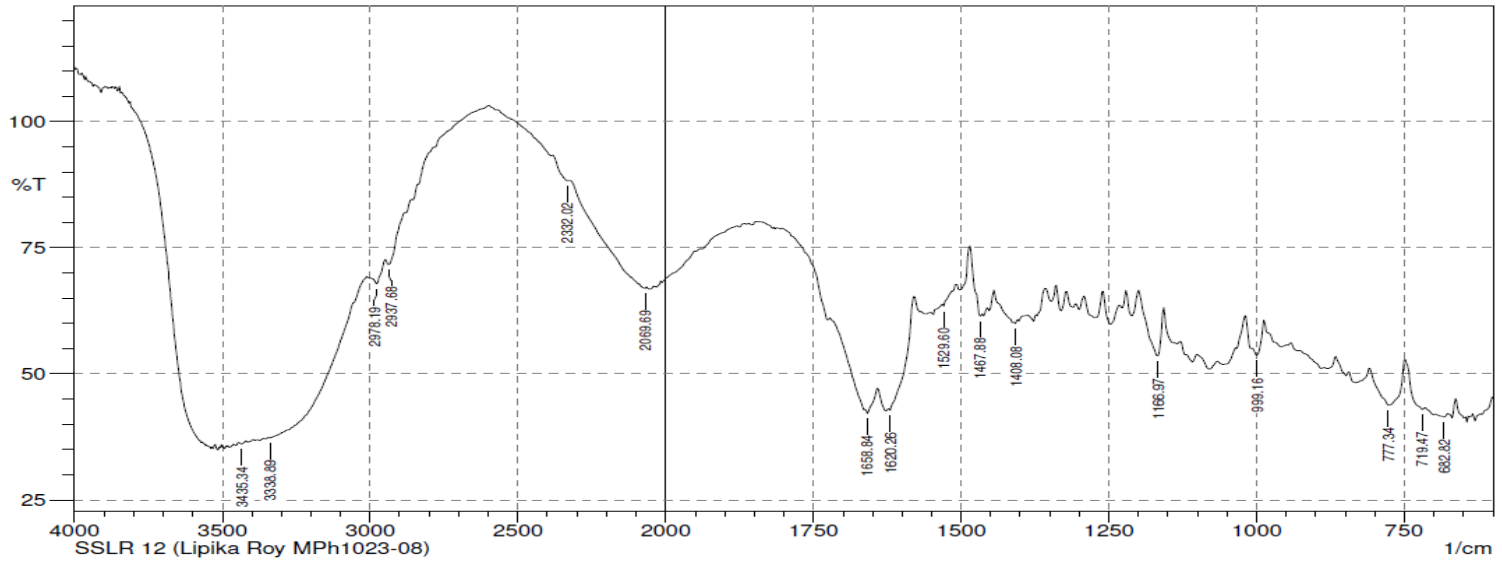
IR (KBr):

3435.34	O-H stretching of aromatic OH
3338.89	N-H stretching of secondary amide
2978.19	Aromatic C-H stretching
2937.68	C-H stretching of methylene
1529.60	C=O stretching of secondary amide
682.82	N-H out of plane wagging of amide

Mol. Formula: $C_{24}H_{29}N_3O_4$

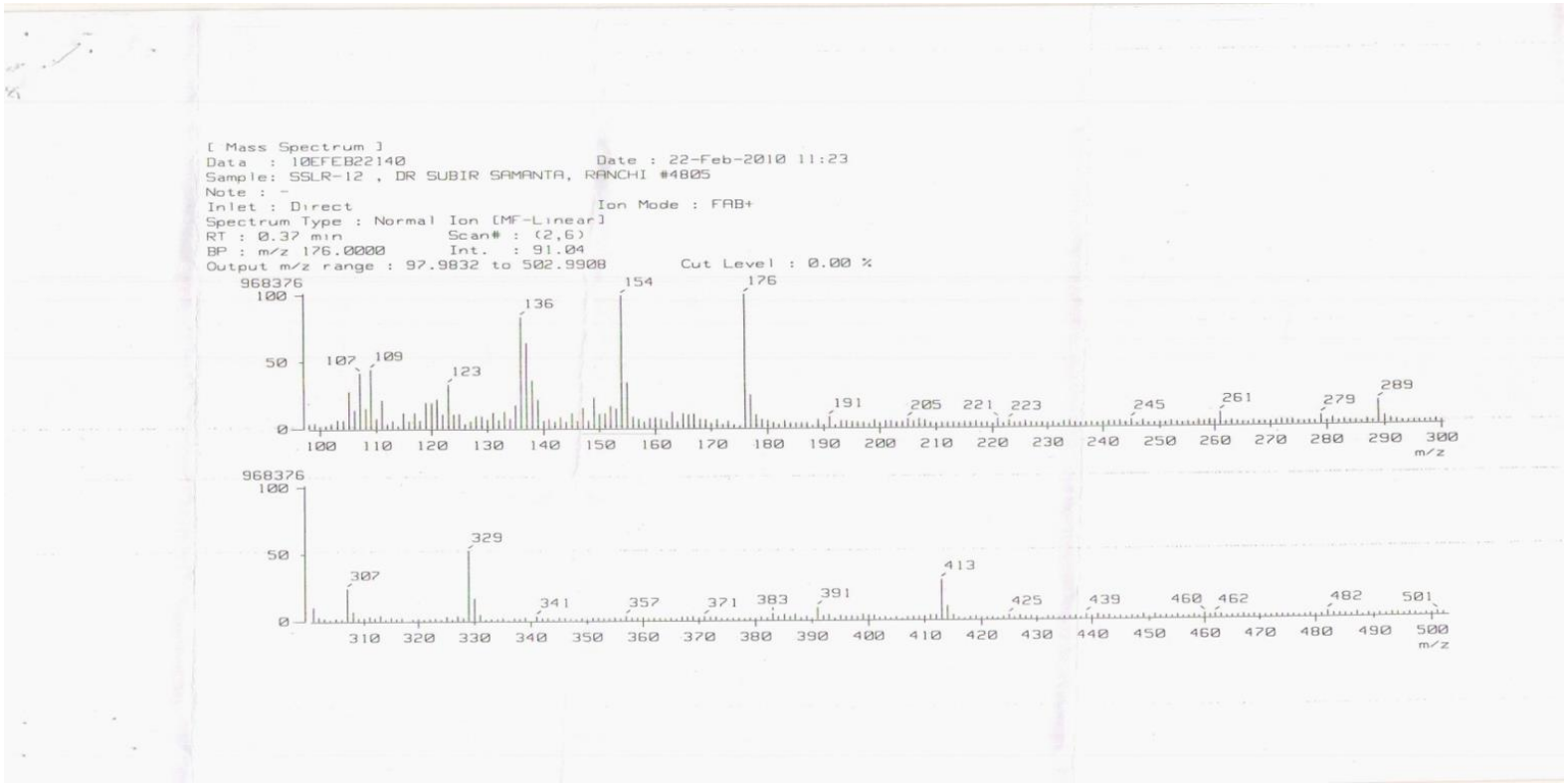
Mol. Weight : 423





FAB-Mass spectral data [m/z, relative intencity (%)] of SSLR-12

425 [M+2 peak] (5 %), 261 [M-CONHC (CH_2-PhOH)] [M-162] (15 %), 329 (55%), 307 (25%), 289 (20 %), 261 (10%), 176 [BP] (91%), 154 (90%), 136 (85%).



PHARMACOLOGICAL SCREENING [22,23]

- ***1) Analgesic activity study: Acetic acid-induced writhing response in mice***
- ***2) Anti-inflammatory activity study: Carrageenan induced hind paw edema in rats***

- ***) Analgesic activity study:*** The most commonly used method for measuring peripheral analgesic activity is writhing tests in mice induced by acetic acid.

  Materials and methods: Male albino mice, weighing 25-32 gm were used. Animals had free access to standard diet and water. Each experimental group consisted of 6 animals. The test and standard drugs given are shown in a table given below.

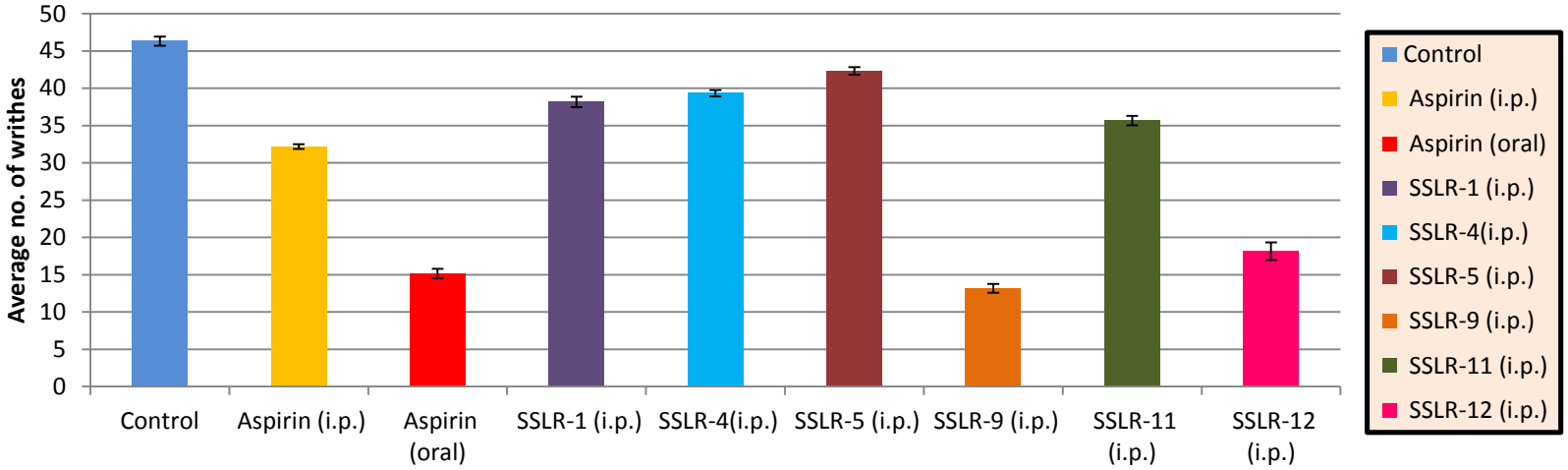
Name of Gr.	Sub Groups	Treatment 1 hour before Acetic acid induction
Control	I	Received distilled water
Standard compound	II	Received Aspirin (15 mg/kg b.w.) i.p
	III	Received Aspirin (300 mg/kg b.w.) orally
Test compounds	IV	Received SSLR-1 (15 mg/kg b.w.) i.p.
	V	Received SSLR-4 (15 mg/kg b.w.) i.p.
	VI	Received SSLR-5 (15 mg/kg b.w.) i.p.
	VII	Received SSLR-9 (15 mg/kg b.w.) i.p.
	VIII	Received SSLR-11 (15 mg/kg b.w.) i.p.
	IX	Received SSLR-12 (15 mg/kg b.w.) ip
	X	Received SSLR-11 (30 mg/kg b.w.) orally
	XI	Received SSLR-12 (30 mg/kg b.w.) orally

Each mouse was given an injection of 0.75% acetic acid aqueous solution in a volume of 0.1 ml/10 g body weight into the peritoneal cavity. The number of writhes was counted for 15 min beginning from 5 min after the acetic acid injection. Test and standard drugs were administered 1 h before the acetic acid injection. The severity of pain response (writhing) was assessed by counting number of writhes (constriction of abdomen, turning of trunk and extension of hind legs) in mice.

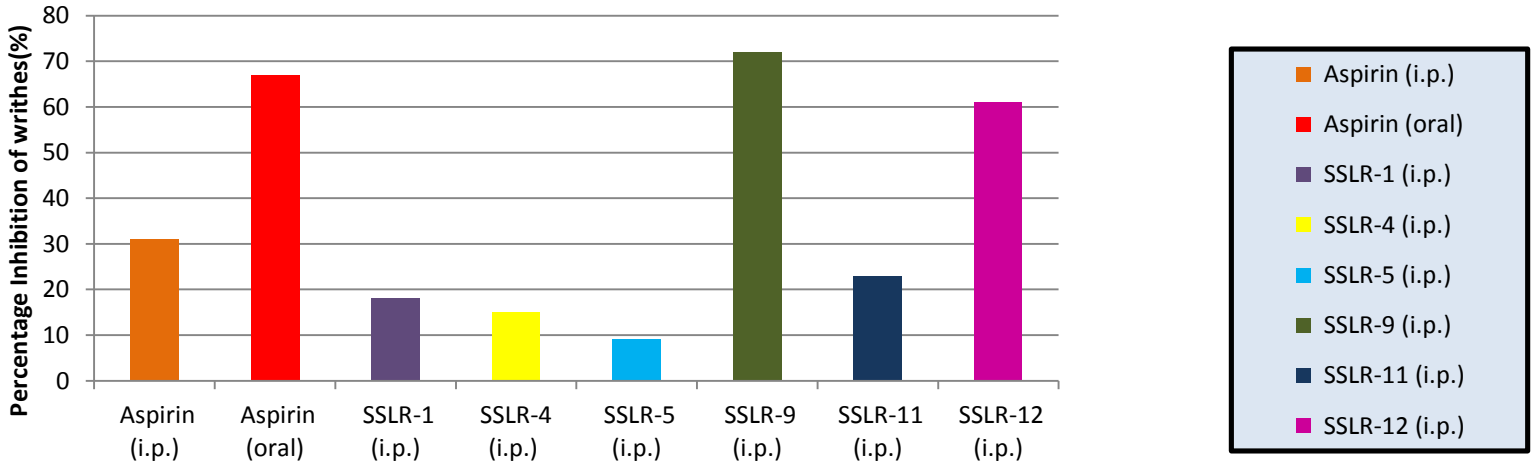
$$\%MPE = 100 \times \frac{\text{Mean of writhes in control group- mean of writhes in treated groups}}{\text{Mean of writhes in control group}}$$

The significance of results was calculated by Students “t” test.

Effect of Test compounds on writhing response in mice



Average no. of writhes on acetic acid induced writhing test in mice (Injectable compounds)

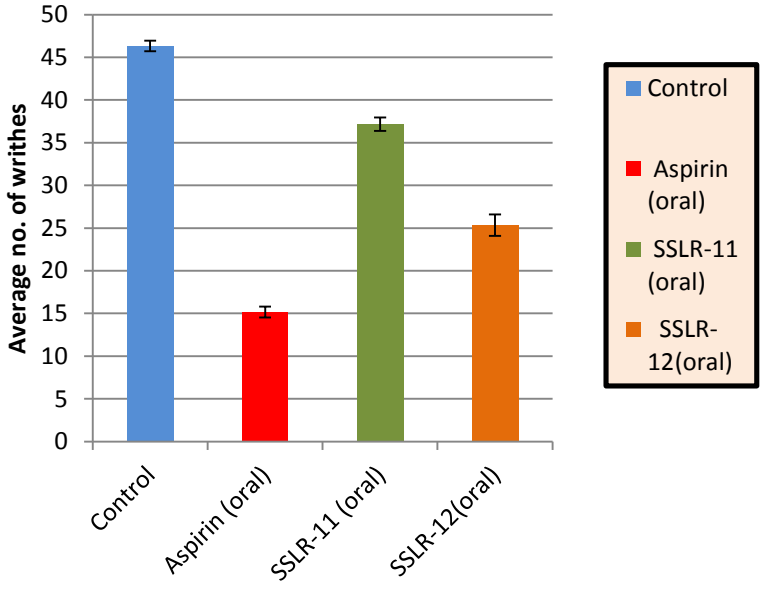


Percentage of Inhibition of writhes on acetic acid induced writhing test in mice (Injectable compounds)

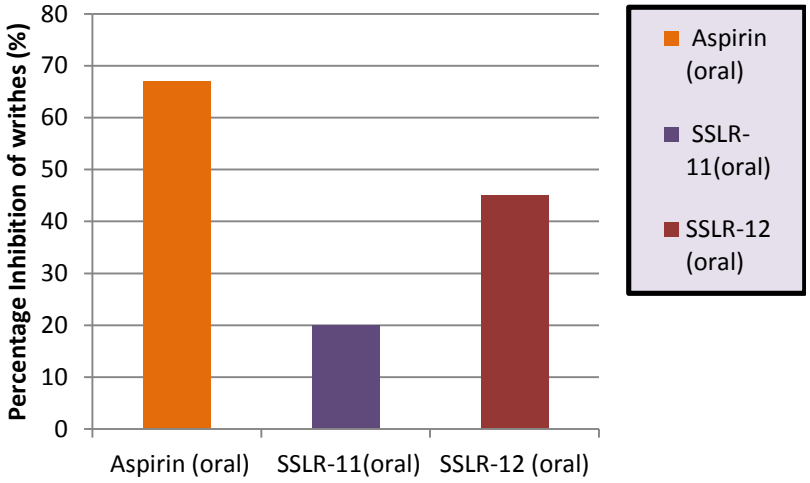
Effect of orally given test compounds on writhing response

Name of group	Name of Sub-group	Dose	No. of writhes	Inhibition of writhing response(%)
Aspirin**	I	300 mg/kg, orally	15.16 ± 0.65	67
SSLR-11*	II	30 mg/kg, orally	37.17±0.79	20
SSLR-12**	III	30 mg/kg, orally	25.33±1.26	45

[Values are expressed as mean ± S.E.M. (*N*= 6). Significantly different from the control gr. are represented as **P<0.001 significant, *p<0.05 significant and #P> 0.05 Insignificant.]



Average no of writhes in mice by orally given compounds



Percentage Inhibition of writhes by orally given compounds

3.Anti-inflammatory activity study: Carrageenan induced hind paw edema in rats

Carrageenan-induced paw edema is the simplest and most widely used model for studying the anti inflammatory activity of new compounds. The development of edema after sub plantar injection of carrageenan in the animal is attributed to the release of histamine, serotonin, kinins and prostaglandins and produce inflammation.

Materials and methods

Male rats weighing 100–130 g were used. They had free access to standard diet and water. Each experimental group consisted of 6 animals each.

PROCEDURE

Paw edema was induced in all groups by injecting 0.1 ml of 1% w/v carrageenan into the sub-plantar region of the right hind paw of the rats. The mean paw volume was measured 1 hr. prior to carrageenan injection using a plethysmometer (model 520, Almeno 2390-5, AHLBORN) and at 15, 30, 60, 120, 180 min. after carrageenan injection.

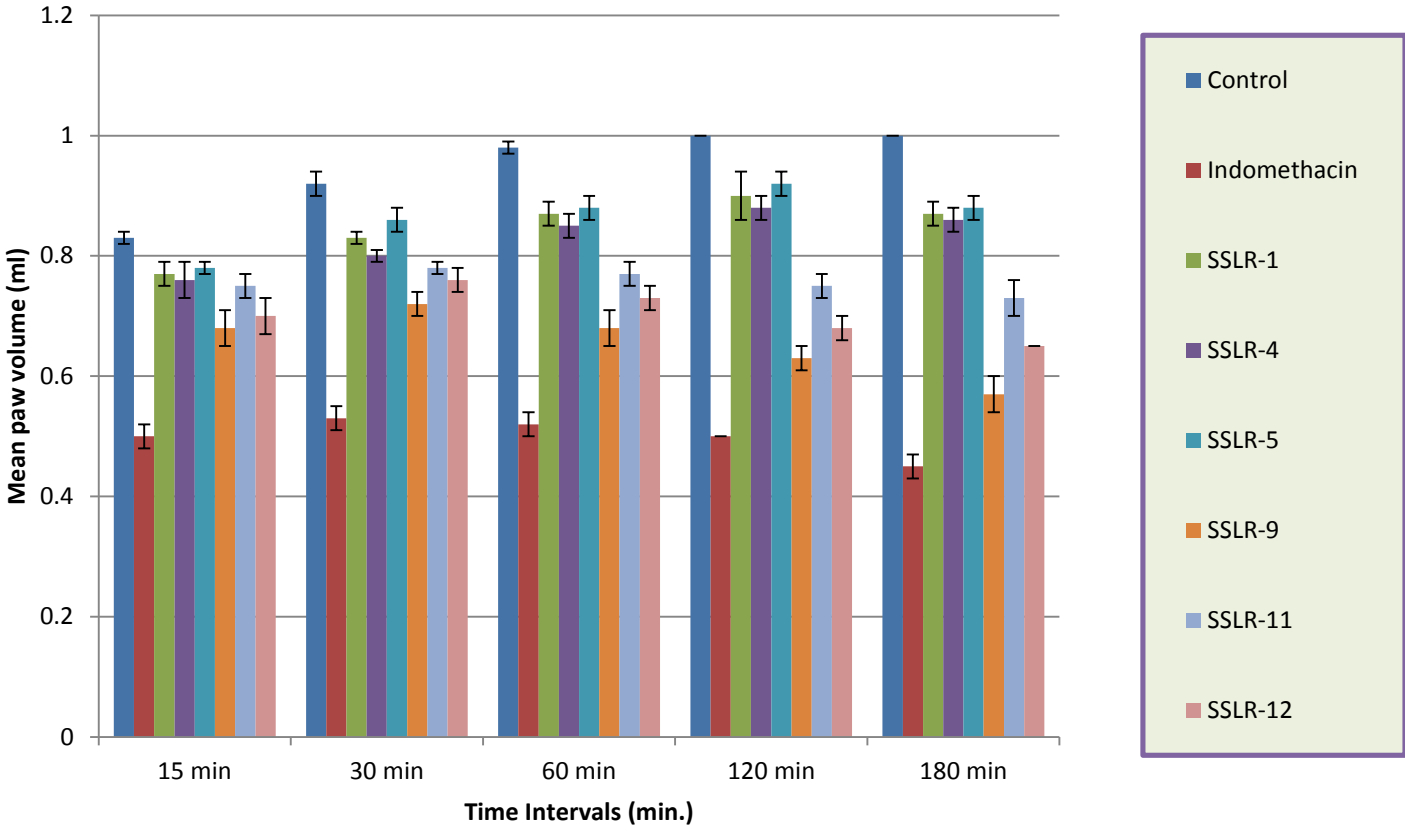
Percent inhibition (%) = 100 (1-Vt/ Vc), Where, Vc = Edema volume of control
Vt = Edema volume of test/ standard compound.

The drugs tested , their dose and % inhibition are given in the table below:

Effect of Injectable Test compounds on paw volume

Treatment	Dose mg/kg (i.p.)	Mean paw volume (ml)				
		15 min.	30 min.	60 min.	120 min.	180 min.
Control	-	0.83± 0.01	0.92±0.02	0.98± 0.01	1.0± 0.00	1.0± 0.00
Indomethacin**	10	0.50± 0.02	0.53±0.02	0.52± 0.02	0.50± 0.02	0.45± 0.02
SSLR-1 #	30	0.77± 0.02	0.83± 0.01	0.87± 0.02	0.90± 0.04	0.87± 0.02
SSLR-4*	30	0.76± 0.03	0.80± 0.01	0.85± 0.02	0.88± 0.02	0.86± 0.02
SSLR-5 #	30	0.78± 0.01	0.86± 0.02	0.88± 0.02	0.92± 0.02	0.88± 0.02
SSLR-9 **	30	0.68± 0.03	0.72± 0.02	0.68± 0.03	0.63± 0.02	0.57± 0.03
SSLR-11*	30	0.75± 0.02	0.78± 0.01	0.77± 0.02	0.75± 0.02	0.73±0.03
SSLR-12**	30	0.70±0.03	0.76± 0.02	0.73± 0.02	0.68± 0.02	0.65±0.00

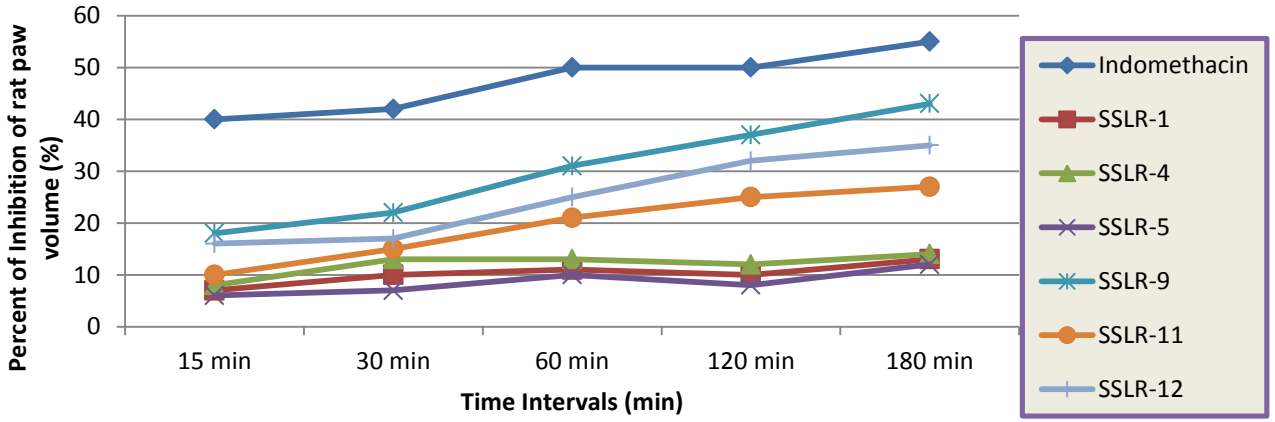
[Values are expressed as mean ± S.E.M. (*N*= 6). **P<0.001 significant, *p<0.05 significant, #P> 0.05 Insignificant]



Mean paw volume (ml.) of rat Vs Time intervals (min.) on carrageenan induced inflammation (Injectable compounds)

Percent of Inhibition of increased paw volume by injectable compounds

Groups	Dose (mg/kg, i.p.)	Percent of Inhibition of rat paw volume (%)				
		15 min	30 min	60 min	120 min	180 min
Indomethacin	10	40	42	50	50	55
SSLR-1	30	7	10	11	10	13
SSLR-4	30	8	13	13	12	14
SSLR-5	30	6	7	10	8	12
SSLR-9	30	18	22	31	37	43
SSLR-11	30	10	15	21	25	27
SSLR-12	30	16	17	25	32	35



Percentage inhibition of increased paw volume by injectable compounds

Effect of orally given test compounds on rat paw volume

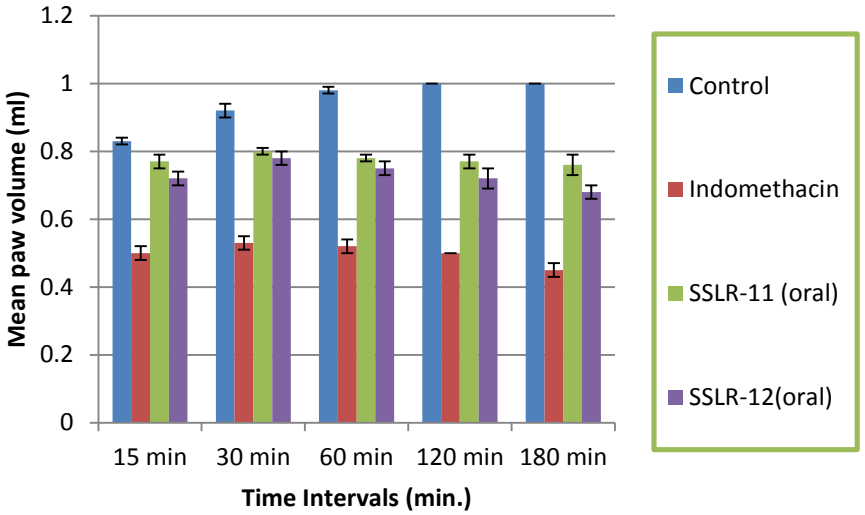
Treatment	Dose (mg/kg)	Mean paw volume (ml)				
		15 min.	30 min	60 min	120 min.	160 min
Control	-	0.83± 0.01	0.92±0.02	0.98± 0.01	1.0± 0.00	1.0± 0.00
Indomethacin**	10 mg/kg, (i.p.)	0.50± 0.02	0.53± 0.02	0.52± 0.02	0.50± 0.02	0.45± 0.01
SSLR-11*	60 mg/kg, (oral)	0.77± 0.02	0.80± 0.01	0.78± 0.01	0.77± 0.02	0.76± 0.02
SSLR-12 **	60 mg/kg, (oral)	0.72± 0.03	0.78± 0.02	0.75± 0.02	0.72± 0.03	0.68± 0.02

[Values are expressed as mean ± S.E.M. (*N*= 6). Significantly different from the control group represent as **P<0.001 significant, *p<0.05 significant and #P> 0.05 Insignificant.] [34]

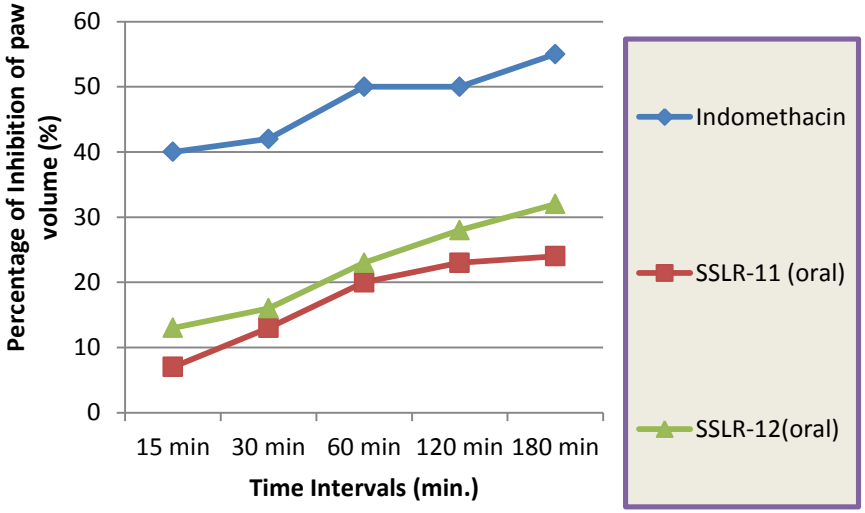
Percent of Inhibition of increased paw volume by oral compounds

Groups	Dose	Percent of Inhibition of rat paw edema (%)				
		15 min	30 min	60 min	120 min	160 min
Indomethacin (Standard)	10 mg/kg, i.p.	40	42	50	50	55
SSLR-11	60 mg/kg, orally	7	13	20	23	24
SSLR-12	60 mg/kg, orally	13	16	23	28	32

Average paw volume (ml.) of rats at different time intervals by oral compounds



Percentage inhibition of increased paw volume by oral compounds



SUMMARY AND CONCLUSION

1. **CADD APPROACH**:

Computer aided drug design using Glide 5.0 a Maestro program.The molecular modeling and docking studies revealed that amongst the 30 compounds, SSLR01,SSLR 04, SSLR05,SSLR09,SSLR11 and SSLR12 showed comparable docking scores of -8.21,-8.06,-8.54,-6.82,-6.75 respectively as compared to -8.01 of Indomethacin in 4COX and-7.19,-8.78,-6.38,-6.98,-5.17 and – 9.44 as compared to Indomethacin in OPRD_HUMAN_AD_JOM-13.(Homology model for delta opoid receptor). ADME profiles of cyclic compounds were comparable to standard clinically established drugs.Toxicity study using Osiris Toxicity Explorer showed that all compounds selected for synthesis and screening were free from toxicity.

2 . SYNTHESIS :

The small chain linear and cyclic peptides has been synthesized using liquid phase method with chlorophosphate ester as the condensing reagent. The physicochemical properties like melting point, Rf value and Spectral studies like FT-IR,NMR and FAB Mass used for characterization of all synthesized compound.

3. **PHARMACOLOGICAL SCREENING:**

Linear peptide SSLR9 showed 72% inhibition while cyclic peptide SSLR12 showed 61% inhibition of writhing response when administered intraperitoneally, thus are found to posses significant degree of peripheral analgesic activity. While orally administered cyclic peptide SSLR12 showed 45% inhibition as compared to Aspirin(67%Inhibition).

Also SSLA9 showed 43% inhibition and SSLR12 showed 35% of increased paw volume after 180 min of Carrageenan induction when administered intraperitoneally, thus are found to posses good anti-inflammatory activity while when administered orally SSLR 12 showed 32% inhibition while SSLR11 showed 24% or less anti-inflammatory activity.

SUMMARY AND CONCLUSION continued…….

Hence we draw the conclusion from the statistical analysis of the synthesized compounds that SSLR-9 (i.p.) ($p<0.001$) and SSLR-12 ($p<0.001$) (both i.p. and oral dose) are found to possess some significant degree of peripheral analgesic activity as well as anti-inflammatory activity where as SSLR-11(both i.p. and oral dose) ($p<0.05$) show minimal analgesic anti-inflammatory activity and SSLR-4 ($p<0.05$) show anti-inflammatory but not analgesic activity. This study further supports the use of small linear and cyclic peptides to be potential injectable and oral peripheral analgesic and anti- inflammatory compounds.

FUTURE SCOPE

- In near future some other suitable combinations containing Tyrosine, Phenylalanine & other aromatic amino acids will be suitable for peripheral analgesic activity whereas addition of hydrophobic amino acids such as Leucine, Proline and other moiety for establishing better anti-inflammatory agents.
- These peptide combinations can also be attached with the non peptide NSAIDs such as Indomethacin etc. and can prove to be more receptor specific, potent and bio-friendly therapeutic analgesic anti-inflammatory agents.
- This study has proved the relevance of endogenous opioid Enkephalin as natural lead and can be modified using CADD strategy .

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Department of Pharmaceutical Sciences and Technology, BIT, Mesra, INDIA……..

THANK YOU

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