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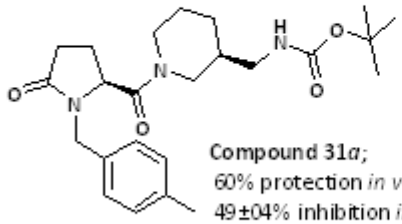
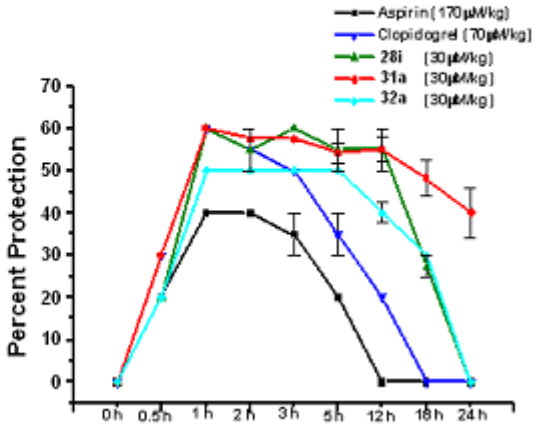
Graphical Abstract

Novel class of chiral lactam carboxamides having cyclic diamine skeleton were synthesized and explored the *in vitro* and *in vivo* antiplatelet efficacy



Compound 31*a*;
60% protection *in vivo*
49±04% inhibition *in vitro*
IC_{50} = 6.6 μM



Synthesis and Identification of Chiral Aminomethylpiperidine Carboxamides as Inhibitor of Collagen Induced Platelet Activation

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ABSTRACT

A series of chiral lactamcarboxamides of aminomethylpiperidine were synthesized and investigated for the collagen induced *in vitro* anti-platelet efficacy and collagen plus epinephrine induced *in vivo* pulmonary thromboembolism. The compound **31*a*** (30 μM/Kg) displayed a remarkable antithrombotic efficacy (60% protection) which was sustained for more than 24 hours and points to its excellent bioavailability. The compounds **31*a*** (IC_{50}= 6.6μM) and **32*a*** (IC_{50}=37μM), as well as their racemic mixture **28*i*** (IC_{50}=16μM) significantly inhibited collagen-induced human platelet aggregation *in vitro*. Compound **34*c*** displayed dual mechanism of action against both collagen (IC_{50}=3.3μM) and U46619 (IC_{50}=2.7μM) induced platelet aggregation. The pharmacokinetic study of **31*a*** indicated very faster absorption, prolonged and constant systemic exposure and thereby exhibiting better therapeutic response.

Keywords

Thrombosis, Antiplatelet, Pyroglutamic acid, Aminomethylpiperidine, Collagen, Epinephrine

1. Introduction

Arterial thrombosis is the acute complication that develops on the chronic lesions of atherosclerosis and causes heart attack and stroke, today the most common causes of mortality in the world [1]. Search of more selective and specific antithrombotic agents for the prevention and treatment of acute occlusive arterial thrombosis is, therefore, a valid approach. Platelet interaction [2-4] is the first step in the haemostatic process, where, extracellular matrix (ECM) is exposed at sites of injury. Among the macromolecular constituents of the ECM, collagen is considered to play a major role in this process, as *in vitro* it not only supports platelet adhesion through direct and indirect pathways but it also directly activates the cells in initiating aggregation and coagulant activity. The primary targets of existing anti-platelet therapy are molecules involved in platelet activation and aggregation. At present, there are no drugs in clinical use that block the initial tethering and adhesion of platelets to collagen and von Willebrand factor and hence their arrest on the blood vessel wall. The inhibition of this early step in thrombus formation is more likely to reduce or prevent the incidence of arterial thrombosis in patients of cardiovascular diseases [5]. Though several reports of peptide and proteins inhibitors of platelet aggregation by inhibiting platelet activation by collagen and other endothelial derived activating molecules [6-10], only a few small molecules inhibitors of collagen mediated platelet aggregation have been reported [11-13]

The present study originates in the serendipitous observation of collagen induced platelet aggregation inhibition by pyroglutamylpiperazines **1** and **2** in a random screening of CDRI library of compounds. Observation of antithrombotic potential in an *in vivo* model and their effect on bleeding time prompted an old fashioned systemic search for more efficacious compounds. We also took note of the published anti-adhesive property of nipecotamide derivatives, **3** [14-25] and $\alpha_4\beta_1$-integrin (VLA-4) inhibitors possessing a pyroglutamyl moiety **4** [26] and antiplatelet activity of various five and six membered heterocycles [27, 28] to arrive at possible modifications (Fig. 1) and also to make use of the combined the therapeutic benefit [29].

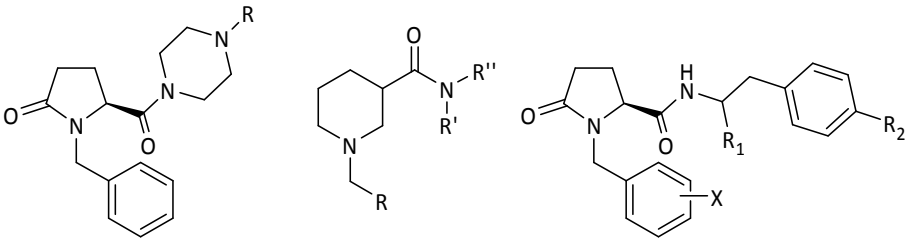


Fig. 1. Structure of pyroglutamylpiperazines (**1 & 2)**, nipecotamides (**3**) possessing anti-platelet activity and N-substituted pyroglutamic acid derivatives (**4**) as $\alpha_4\beta_1$-integrin inhibitors.

The planned exploratory sequential modification of three sub-structural components of the molecule was planned as shown in Fig.2.

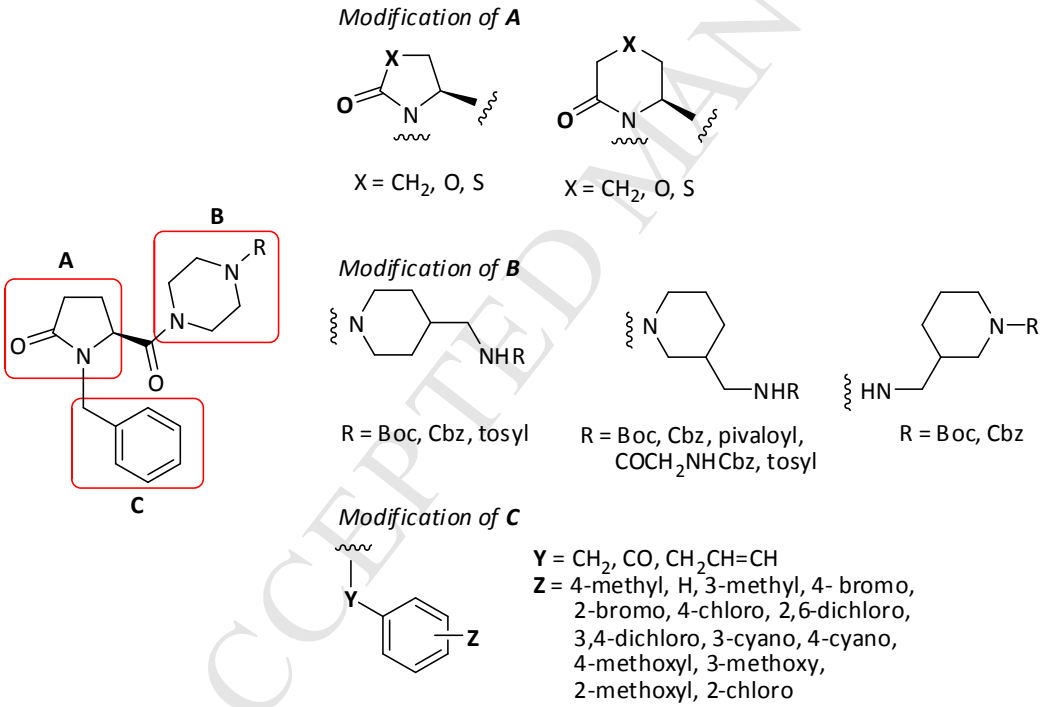


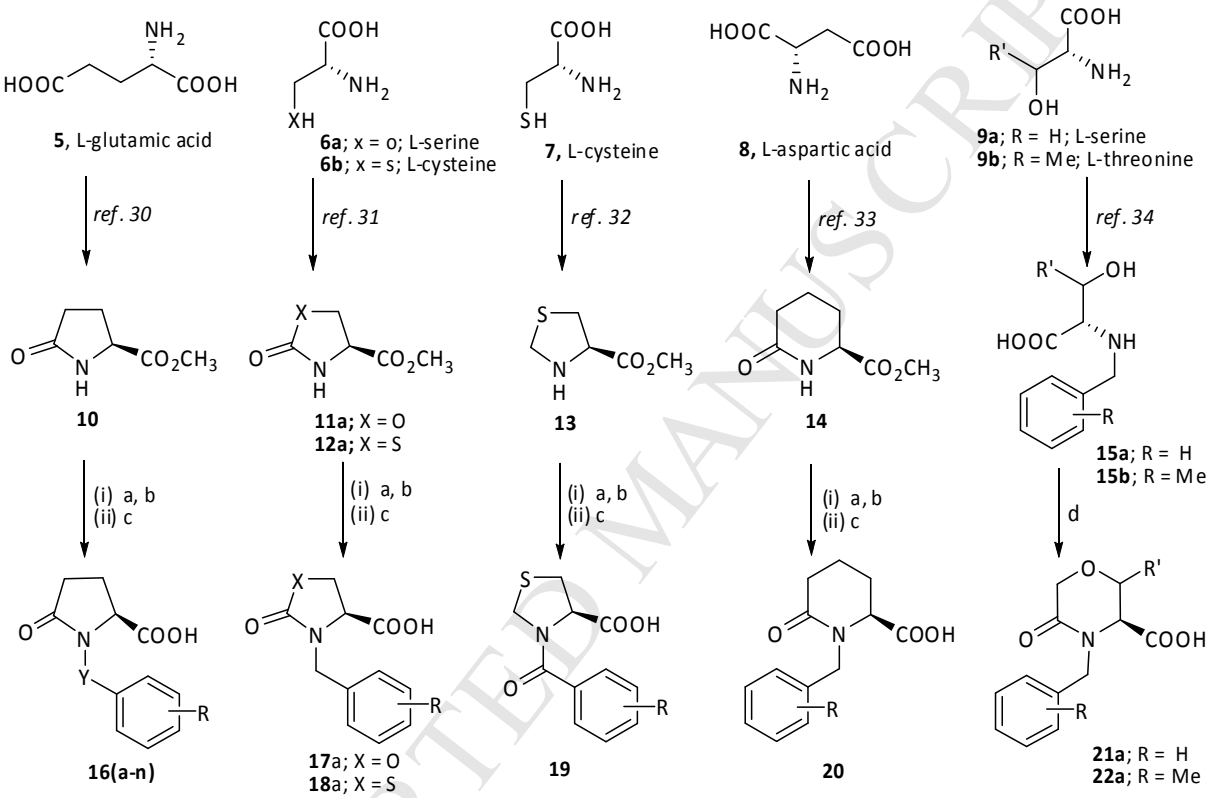
Fig. 2. Proposed structural modifications

2. Results and discussion

2.1. Chemistry

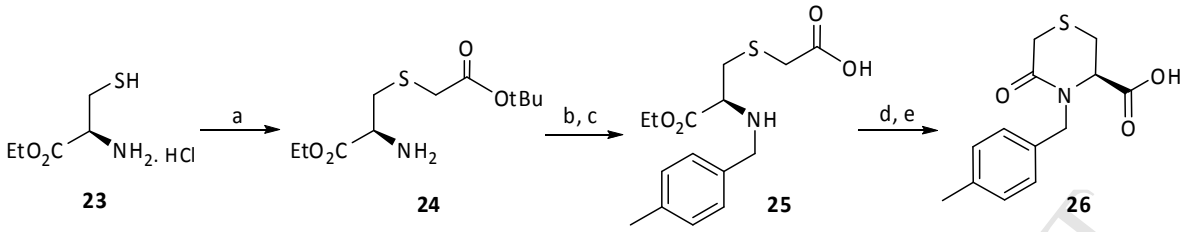
Various lactamcarboxylate esters **10-14** were prepared by reported methodologies [30-33] starting from corresponding of L-amino acids **5-9**. LiHMDS mediated N-deprotonation of these

esters and subsequent alkylation with different arylalkylhalides gave the corresponding N-substituted lactam esters which were hydrolysed to yield the corresponding lactam carboxylic acids **16-20**. (Scheme 1). N-benzylserine **15a** and N-benzylthreonine **15b** were cyclized with chloroacetyl chloride in aqueous alkali solution [34] to the corresponding 5-oxomorpholine-3-carboxylic acids **21a** and **22a** respectively (Scheme 1).



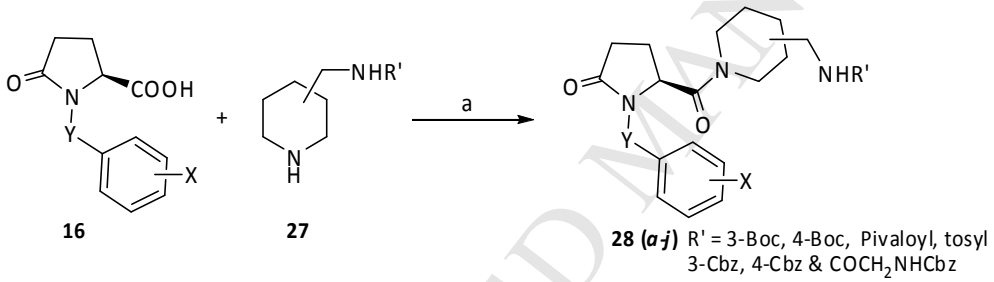
Scheme 1. Synthesis of N-arylalkyl acids. Reagents and conditions: (a) LiHMDS, Dry THF, -15 ^{0}C, 1h; (b) R-Br, dry THF, 4h, 0^{0}C to room temperature; (c) 20 % aq. Na_2CO_3 solution, 4 hrs, room temperature; (d) 2.5 N NaOH, chloroacetyl chloride.

N-substituted oxothiomorpholine carboxylic acid, **26** was prepared in a multistep reaction sequence (Scheme 2). A solution of L-cysteine **23** in dry DMF was treated with *t*-butyl-2-bromoacetate in the presence of DIPEA to provide (*S*)-*t*-butoxycarbonylmethyl cysteine ethyl ester **24.** Reaction of **24** with *p*-methyl benzaldehyde under reductive amination condition gave **25** which was converted to corresponding carboxylic acid, cyclised intramolecularly using DIC-HOBt and the resultant cyclized ester was hydrolysed to give thiomorpholinone carboxylic acid **26**.



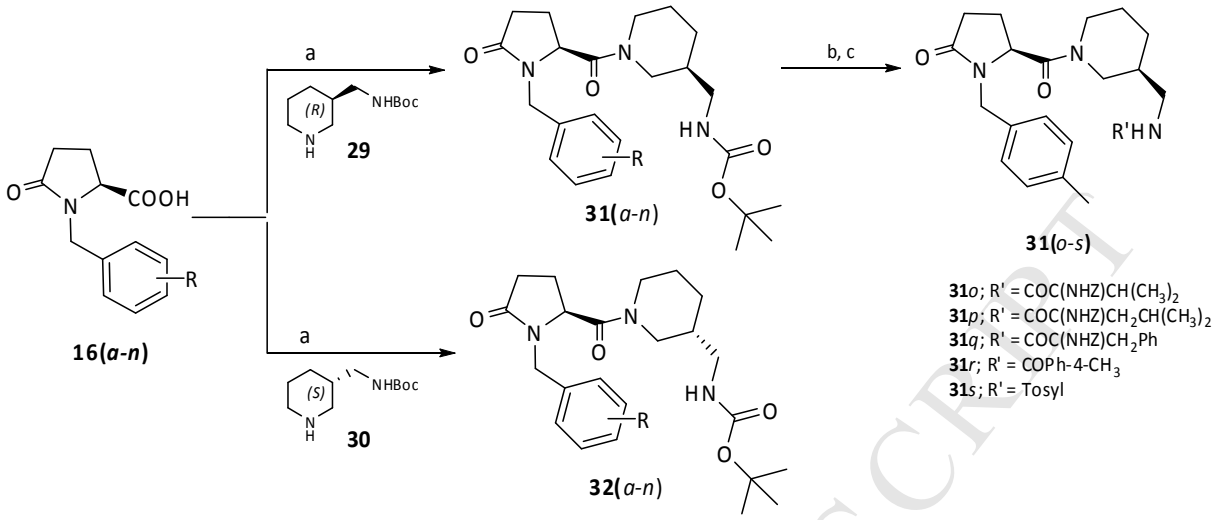
Scheme 2. Synthesis of thiomorpholinone carboxylic acid. Reagents and conditions: (a) DIPEA, dry DMF, $BrCH_2COOtBu$, -10 ^{0}C; (b) NaOAc, *p*-tolualdehyde, $NaCNBH_3$, dry methanol; (c) 40% TFA, dry DCM; (d) DIC, HOBt, Et_3N, dry DCM; (e) 15 % aq.K_2CO_3, 4h

Initially only a few differentially N-substituted pyroglutamic acids were condensed with various 3- or 4-aminomethylpiperidines to give corresponding pyroglutamides which were evaluated for *in vitro* (inducer, collagen), *in vivo* (inducer, collagen plus epinephrine) antithrombotic properties and their effect on bleeding time in mice (Scheme 3, Table-1).



Scheme 3. Synthesis of diamine coupled recemic pyroglutamides. Reagents and conditions: (a) DCC, HOBt, dry DCM, 0^{0}C, 3h

It became evident that the carboxamides having substituted 3-aminomethylpiperidine had far superior bioactivity profile taking account both *ex vivo* platelet aggregation and *in vivo* protection against thromboembolism (Table-1). Therefore our attention was directed to the most promising compound **28*i*** which despite being a diastereomeric mixture was not resolvable chromatographically even by HPLC. This prompted us to synthesize both the diastereomers in pure form using chiral 3-N-Boc-aminomethylpiperidine (**29**, **30**) which were prepared using literature procedures [35]. Condensation of several substituted N-benzylpyroglutamic acids **16**(***a-n***) individually with (3*R*)-N-Boc-3-aminomethylpiperidine, **29** and (3*S*)-N-Boc-3-aminomethylpiperidine, **30** gave **31**(***a-n***) and **32**(***a-n***) respectively (Scheme 4, Table-2). Further modification of 3-aminomethyl substituent was carried out as shown in **Fig. 6** to give **31**(***o-s***) having substitutions other than *t*-butyloxycarbonyl (Boc) group.



Scheme 4. Synthesis of chiral pyroglutamides. Reagents and conditions: (a) Coupling agent; (b) TFA, dry DCM, room temperature, 5h.; (c). R'-Cl, TEA, dry DCM, 2h

Employing similar methodology, various lactam carboxylic acids **17-22** and **26** were also condensed with **29** and **30** to provide another set of carboxamides **33**-**36** (Fig. 3, Table 2 & 3).

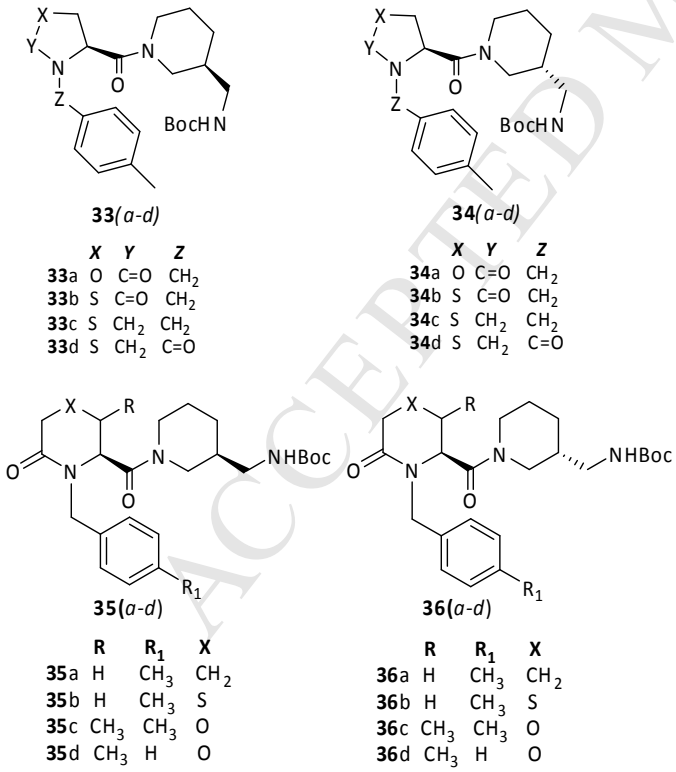


Fig. 3. N-Substituted oxazolidine and thiazolidine carboxamides and higher homologs.

All the compounds were isolated in good yields and were characterized on the basis of their ^{1}H and ^{13}C NMR, IR and mass spectra. The purity and diastereomeric composition were determined by Merck HPLC using Chiralpak 1A column.

2.2 Biology

The *in vitro* anti-platelet efficacy was investigated on the human platelet aggregation in platelet-rich plasma induced by collagen (Born's method) and *in vivo* by collagen-epinephrine induced pulmonary thromboembolism in mice (Table 1). Compounds **28*a*, 28*b*, 28*c*, 28*d*, 28*e*, 28*f*, 28*g*, 28*i*** and **28*j*** exhibited considerably high potency in inhibiting collagen induced platelet aggregation than aspirin, *in vivo*. Furthermore, compounds **28*b*, 28*c*** and **28*i*** exhibited excellent inhibitory activity against collagen induced platelet aggregation, *in vitro*. Among them, compounds with 3-substituion in the piperidine ring were the most potential leads in this series with a range of 67-70 % protection *in vivo* at 30μM concentration and above 60 % inhibition of platelet aggregation, *in vitro* at 30μM concentration. Compound **28*i*** was found to be the extremely promising among this series both *in vivo* (60%) and *in vitro* (77.3 ± 10%). Moreover, this molecule offered moderately less impact on bleeding tendency (50-60%) (Table 1) and therefore it merits comprehensive study.

Table 1

In vivo (inducer, collagen+epinephrine) *in vitro* (inducer, collagen) antiplatelet and antithrombotic properties of compounds **28(*a-j*)**.

No	Compound	X	Y	R'	% protection[a]	% inhibition[b]	% increase in bleeding time
1	28*a*	2-Cl	CH_2	3-Boc	70	15 ± 8	60-70
2	28*b*	2,6-Cl	CH_2	4-Boc	70	80 ± 9	50
3	28*c*	4-Br	CH_2	3-Boc	67	66.4 ± 5	60-70
4	28*d*	4-CN	CH_2	4-Boc	40	34 ± 8	0
5	28*e*	H	CH_2-CH=CH	3-Boc	30	42 ± 3	0
6	28*f*	H	CH_2	3-Pivaloyl	50	2.4 ± 2.4	41
7	28*g*	H	CH_2	3-$COCH_2NHCbz$	40	12 ± 5	13
8	28*h*	H	CH_2	3-Tosyl	25	72 ± 9	0
9	28*i*	4-CH_3	CH_2	3-Boc	60	77.3 ± 10	50-60
10	28*j*	4-Br	CH_2	4-Cbz	70	11±9	25

[a] Collagen-epinephrine induced pulmonary thromboembolism (*in vivo*) in mice; Compound conc. = 30 μM/kg
[b] Inhibition of collagen induced platelet aggregation (*in vitro*) in human platelets; Compound conc. = 30 μM; *n* (number of experiment) = 3

Carboxamides were prepared in chirally pure form and compound with a para-substituted methyl group at the phenyl ring showed 60 % and 50% protection in diastereomers **31*a*** and **32a** respectively, induced by collagen plus epinephrine at 30μM concentration *in vivo*. Compound **32*e*** with meta-substituted methyl group also showed significant antithrombotic activity by 70% protection *in vivo*. Moreover, compounds with 4-cyano (**31*n***, 60%), 4-chloro (**31*h***, 50%), 2-bromo (**31*i*** and **32*i***, 50%) and 4-bromo (**32*j***, 50%) substituted molecules also exhibited crucial anti-platelet activity *in vivo*. Modification of the piperidine 3-substituent of the most promising analog, **31*a*** was executed to give **31*r*** (N-acyl), **31*s*** (N-sulfonyl) and **31(*o*-*q*)** (N-substituted hydrophobic amino acids) and observed a decline in the *in vivo* activity at the same concentrations and therefore further modification of 3-piperidine substituent of ring-B was established insignificant.

All the compounds were evaluated for their ability to inhibit human platelet aggregation *in vitro* using Born's method. Compound **31*a*** showed significant activity with a percent inhibition of platelet aggregation value 49±4 % even at 10μM concentration and highly promising compared to its diastereomer, **32*a*** (30±09 %). Meanwhile, **32*e*** showed very low inhibition of percent aggregation *in vitro*. The series **31(*a*-*n*)** were found to be more dynamic than the series **32(*a*-*n*)** to the extent that *in vitro* collagen induced percentage inhibition was concerned (Table 2). Therefore diastereomers of **28*i***, that is, **31*a*** and **32*a*** were taken as the lead molecule and preceded for further studies.

Table 2

In vivo (inducer, collagen + epinephrine) *in vivo* (inducer, collagen) anti-platelet and anti-thrombotic properties of (*S*,*R*) diastereomers **31(*a*-*s*)** and (*S*,*S*) diastereomers **32(*a*-*n*)** of N-Boc-3-aminomethylpiperidine derivatives of N-substituted pyroglutamic acid and higher homologs.

				(*S*,*S*) diastereomers, **31**(*a*-*s*)		(*S*,*R*) diastereomers, **32**(*a*-*n*)	
No	compound	X	R	% protection[a]	% inhibition[b]	% protection[a]	% inhibition[b]
1	*a*	4-CH_3	$COOC(CH_3)_3$	60	49±04	50	30±09
2	*b*	4-OCH_3	$COOC(CH_3)_3$	20	07±05	20	25±05
3	*c*	3-OCH_3	$COOC(CH_3)_3$	ns	34±08	22.3	06±04
4	*d*	2-OCH_3	$COOC(CH_3)_3$	40	32±11	50	22±05
5	*e*	3-CH_3	$COOC(CH_3)_3$	30	28±08	70	13±04
6	*f*	H	$COOC(CH_3)_3$	20	26±08	50	17±08
7	*g*	Naphthyl	$COOC(CH_3)_3$	30	29±09	22.3	22±09

8	*h*	4-Cl	$COOC(CH_3)_3$	50	35±07	15	21±04
9	*i*	2-Br	$COOC(CH_3)_3$	50	27±11	50	06±05
10	*j*	4-Br	$COOC(CH_3)_3$	40	20±06	50	04±02
11	*k*	3-4-Cl	$COOC(CH_3)_3$	20	27±12	30	24±07
12	*l*	2,6-Cl	$COOC(CH_3)_3$	20	24±14	10	22±05
13	*m*	3-CN	$COOC(CH_3)_3$	30	06±05	20	19±09
14	*n*	4-CN	$COOC(CH_3)_3$	60	08±04	10	25±05
15	*o*	4-CH_3	$COC(NHZ)CH(CH_3)_2$	10	nd	nd	nd
16	*p*	4-CH_3	$COC(NHZ)CH_2CH(CH_3)_2$	30	nd	nd	nd
17	*q*	4-CH_3	$COC(NHZ)CH_2Ph$	30	nd	nd	nd
18	*r*	4-CH_3	COPh-p-CH_3	30	nd	nd	nd
19	*s*	4-CH_3	SO_2Ph-p-CH_3	40	nd	nd	nd

[a] Collagen-epinephrine induced pulmonary thromboembolism (*in vivo*) in mice; Compound conc. = 30 µM/kg; nd, not done
[b] Inhibition of collagen induced platelet aggregation (*in vitro*) in human platelets; Compound conc. = 10 µM; *n* = 3

Among all the eight N-substituted oxazolidine and thiazolidine carboxamides, **33*c*** and exhibited maximum antithrombotic protection (50% and 60%, respectively). Other compounds including **33*b*** and **34*b*** also exhibited significant protection (40% and 50%, respectively) while the remaining compounds either remained inactive or offered weaker antithrombotic activity compared to aspirin. All the four active compounds **33*b***, **34*b***, **33*c*** and **34*c*** at 30µM/kg had relatively less impact on bleeding tendency (43%, 50%, 50% and 72% respectively) than aspirin. The results indicate that these compounds satisfactorily preserve the haemostasis with minor alterations in the pre-clinical mice model and hence, they might promise some improvisation with respect to the critical disadvantage of bleeding risk endured by the current anti-platelet strategies. Therefore, all the four compounds were further pursued for their probable mode of action in platelet aggregation assay (*in vitro*).

The active compounds were evaluated for their ability to inhibit human platelet aggregation *in vitro* induced by collagen. As can be seen from Table 3, all the four active compounds **33*b***, **34*b***, **33*c*** and **34*c*** exhibited significantly high potency in inhibiting collagen induced platelet aggregation than aspirin (**34*c***>**34*b***>**33*b***>**33*c***), without affecting ADP, TRAP (thrombin receptor activated peptide) or ristocetin induced platelet aggregation. Moreover, the order of their *in vitro* potency was identical to the observations in mice model of pulmonary thromboembolism (Table 3 and 4). Among all, **34*c*** displayed the best anti-platelet potential against collagen with an IC_{50} of 3.3µM. Nevertheless, the anti-platelet activity found for **34*c***, when the platelets were induced either by arachidonic acid (AA) or U46619, indicated that **34*c*** might be operating by a dual

mechanism of action and showed a better anti-platelet potential than aspirin against both collagen and U46619 induced platelet aggregation. Moreover, it's IC_{50} regarding arachidonic acid induced platelet aggregation was almost comparable to that of aspirin (6.2 µM *vs* 5µM). Thus **34c** might offer better protection against thrombotic complications than aspirin and hence should be further pursued for its mechanism of action and binding site.

Table 3

In vivo antithrombotic properties and bleeding tendency of N-substituted oxazolidine and thiazolidine carboxamides **33(*a*-*d*)** and **34(*a*-*d*)**.

No	Compound	X	Y	Z	% protection[a]	Bleeding Time % increase[b]
1	**33***a*	O	C=O	CH_2	30	54
2	**33***b*	S	C=O	CH_2	40	43
3	**33***c*	S	CH_2	CH_2	50	50
4	**33***d*	S	CH_2	C=O	10	8
5	**34***a*	O	C=O	CH_2	20	10
6	**34***b*	S	C=O	CH_2	50	50
7	**34***c*	S	CH_2	CH_2	60	72
8	**34***d*	S	CH_2	C=O	40	50

[a] collagen-epinephrine induced pulmonary thromboembolism in mice (*in vivo*); compound dose = 30 µM/kg
[b] bleeding time by tail excision in mice; compound dose. = 30 µM/kg; $n = 3$

Table 4

Effect of active N-substituted oxazolidine and thiazolidine carboxamides on *in vitro* aggregation of human platelet rich plasma induced by Collagen (1µg/ml), Arachidonic acid (0.5mM) and U46619 (2.5 µg/ml).

Compound	Collagen		Arachidonic Acid		U46619	
	% Inhibition	IC_{50}	% Inhibition	IC_{50}	% Inhibition	IC_{50}
33*b*	81±5	6.4	15±6	>100	75±3	6.4
33*c*	74±4	8.0	25±4	>100	96±8	4.7
34*b*	83±2	6.0	8±5	>100	64±9	10.2
34*c*	87±5	3.3	93±8	6	100±7	2.7

Although the other three compounds **33*b***, **34*b*** and **33*c*** exhibited almost negligible inhibitory effect on AA induced platelet aggregation, but all three selectively attenuated collagen and U46619 induced platelet activation in a concentration dependent manner, thereby indicating towards the structurally defined anti-platelet activity. The present study therefore might provide

a basis for the development of new candidates with potent anti-platelet and antithrombotic activities.

Compounds **35*a*** and **36*a*** may be considered as six membered analogs of the pyroglutamate based amide derivatives **31*a*** and **32*a***, respectively. Results revealed the fact that both diastereomers could be a comparable antithrombotic agent, although with a slightly decreased activity in terms of percent protection *in vivo* (40 and 50 %, respectively). However there is much considerable reduction in the *in vitro* activity in comparison to their lower homologs. Similarly, activity of thiomoprpholinone **35(*b*-*d*)** and oxomorpholinone carboxamides **36(*b*-*d*)** were also displayed a considerable drop. Thus, expansion of ring size of the lactam scaffold seems to have least effect as far as *in vitro* and *in vivo* anti-platelet activity results were concerned (Table 5).

Table 5

In vivo and *in vitro* antiplatelet and antithrombotic properties of six-membered analogs **35(*a*-*d*)** and **36(*a*-*d*)**

No	Compound	R	R_1	X	% protection[a]	% inhibition[b]
1	**35*a***	H	CH_3	CH_2	40	18±9
2	**35*b***	H	CH_3	S	30	15±0
3	**35*c***	CH_3	CH_3	O	20	nd
4	**35*d***	CH_3	H	O	20	nd
5	**36*a***	H	CH_3	CH_2	50	22±10
6	**36*b***	H	CH_3	S	30	18±0
7	**36*c***	CH_3	CH_3	O	30	nd
8	**36*d***	CH_3	H	O	20	nd

[a] collagen-epinephrine induced pulmonary thromboembolism in mice (*in vivo*); compound conc. = 30 μm
[b] inhibition of collagen induced platelet aggregation (*in vitro*) in human platelets; compound conc. = 10 μm; *n* = 3; nd = not done

*2.2.1. Antithrombotic efficacy of **31a** in collagen-epinephrine induced thrombosis in mice (in vivo)*

31*a* and **32*a*** are the diastereomers while **28*i*** is the racemic mixture of the two. After one hour of administration by oral route at a dose of 30 μM/Kg, the three compounds viz. **28*i***, **31*a*** and **32*a*** offered significant protection in mice against thrombotic challenge.. All the three compounds exhibited better antithrombotic efficacy than standard drug Aspirin (40% protection at 170 μm), therefore, all of them were further tested in order to identify the best among them. The

compound **31*a*** (30 µM/Kg) displayed a remarkable antithrombotic efficacy (60% protection) which was sustained for more than 24 hours and thus highlights its excellent bioavailability. The compound **28*i*** exhibited almost similar efficacy like compound **31*a*** but only upto 18 hours after which it became ineffective, while compound **32*a*** displayed relatively weaker antithrombotic potential throughout and remained effective only upto 12 hours, when compared against compound **31*a***. However, the standard anti-platelet drugs Aspirin (170µM/kg, 40% protection) and Clopidogrel (70 µM/kg, 60% protection) were effective only upto 5 hours after which their effect was perished and that too at a very high dose sufficient enough to cause bleeding complications (Fig. 4).

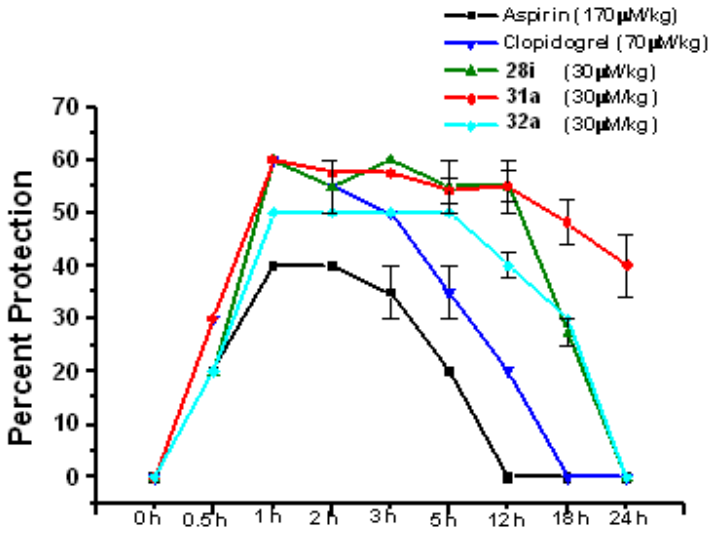


Fig. 4. Time dependent effect of compounds (**28*i***, **31*a*** and **32*a***) and standard antithrombotic drugs (Aspirin and Clopidogrel) after single dose administration (30µM/kg, p.o.) on collagen-epinephrine induced pulmonary thromboembolism in mice. Results are expressed as Mean±SEM (n=5, 10 animals/group/ experiment).

2.2.2. Effect on Bleeding Time in mice

Tail bleeding time was significantly prolonged in standard anti-platelet drugs, Aspirin (2.2 fold) and Clopidogrel (2.3 fold) treated mice as compared to control. The diastereomers **31*a*** and **32*a*** as well as their mixture **28*i*** (30µM/kg) although exhibited a mild prolongation in bleeding time (~1.5 fold) but it was considerably less in comparison to both the standard anti-platelet drugs, Aspirin and Clopidogrel (Fig. 5).

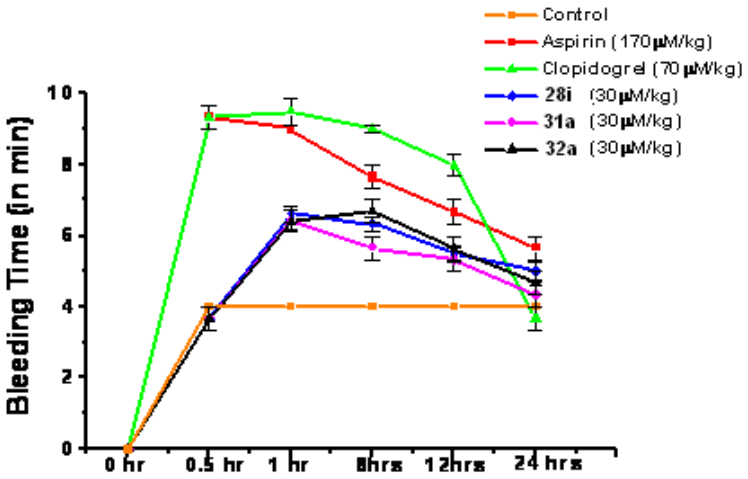


Fig. 5. Time dependent effect of CDRI candidate compounds (**28*i***, **31*a***, **32*a***) and standard antithrombotic drugs (Aspirin and Clopidogrel) after single dose administration (30µM/kg, p.o.) on tail bleeding time in mice. Results are expressed as Mean±SEM (n=5, 10 animals/group/experiment).

2.2.3. Aggregation in human platelet rich plasma (in vitro)

Various platelet agonists were used in order to identify their anti-platelet action and also the selectivity of their pharmacological target over platelet surface. The compounds **31*a*** (IC_{50}= 6.6µM) and **32*a*** (IC_{50}=37µM) as well as their racemic mixture **28*i*** (IC_{50}=16µM) significantly inhibited collagen induced human platelet aggregation but had no effect on ADP, TRAP and Arachidonic acid induced platelet aggregation. The involvement of platelet GP 1b-IX-V was also ruled out as the compound **31*a*** did not show any inhibitory effect on Ristocetin induced platelet aggregation. The aggregation observed with type 1 fibrillar collagen is primarily mediated by the functional GPVI receptor on the surface of platelets. Therefore, specific agonists, Convulxin (snake venom protein) and CRP-XL (cross linked collagen related peptide) were used to examine the interaction between compound and GPVI, which activate platelets selectively via GPVI. The compound **31*a*** had no effect on Convulxin induced platelet aggregation but at higher concentrations, it attenuated the CRP-XL induced platelet aggregation at concentrations up to 500µM. This suggests that the compound **31*a*** inhibits collagen induced platelet aggregation with a better affinity over CRP-XL and convulxin (Fig. 6).

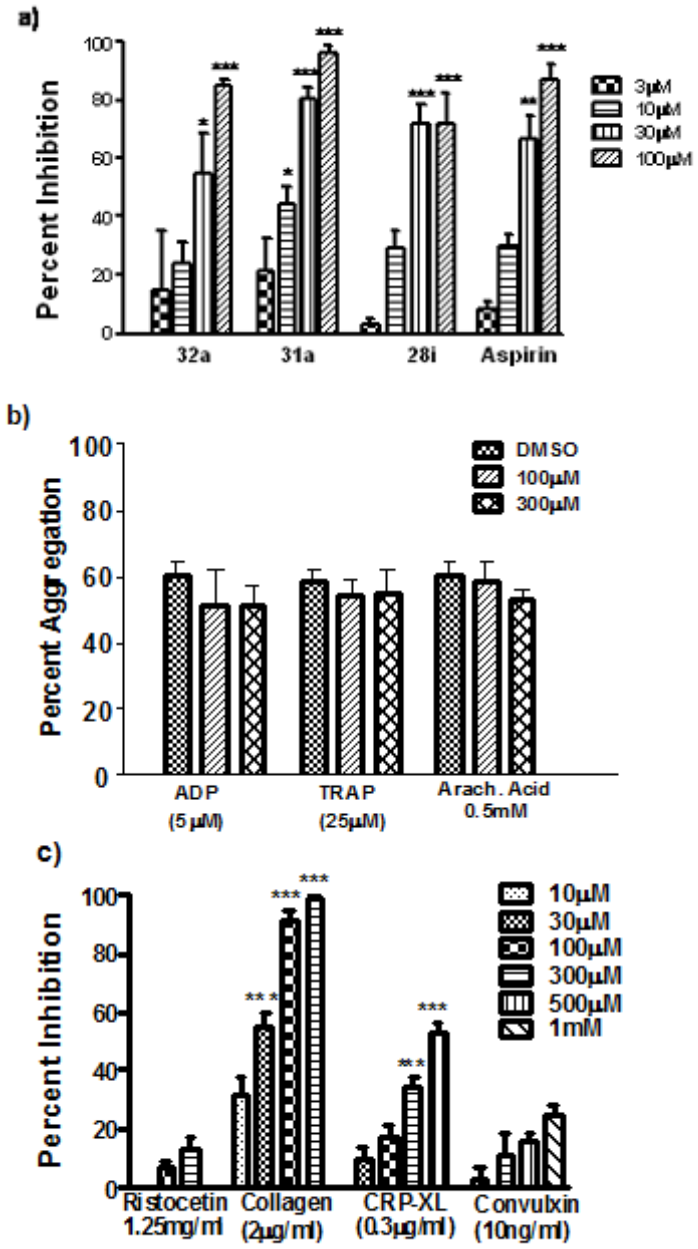


Fig. 6. (a) Effect of Aspirin, compound **28*i***, **31*a*** and **32*a*** on aggregation of human platelet rich plasma (*in vitro*) induced by Collagen, (b) effect of compound **31*a*** on aggregation of human platelet rich plasma (*in vitro*) induced by ADP, TRAP and Arachidonic acid, (c) effect of compound **31*a*** on aggregation of human platelet rich plasma (in vitro) induced by ristocetin, collagen, Convulxin and CRP-XL (n=3) Data shown as Mean ± SEM. ***p<0.001,**p<0.01 vs control.

2.3 Oral pharmacokinetic study in NZ rabbits

The oral pharmacokinetic study of **31*a*** was carried out in healthy male *New Zealand* (NZ) rabbits (~3kg) at 20 mg/kg dose. The animals were obtained from Laboratory Animal Services Division of CDRI, and housed in cages in standard laboratory conditions with a regular 12 h day-night cycle. The prescribed diet was given and water *ad libitum*. The animals were acclimatized for two days before carrying out the pharmacokinetic study. The **31*a*** suspension formulation was prepared using 0.5 % w/v carboxymethyl cellulose in a mortar and pestle. Blood samples from marginal ear vein were collected at 0.25 to 48 hr post oral dosing. Plasma was separated and stored at –80°C until analysis by HPLC-UV method. The pharmacokinetic parameters C_{max} , t_{max} clearance (CL/F), volume of distribution (V_d/F), MRT and area under curve (AUC) for all the analytes were determined using non-compartmental analysis (Fig. 7, Table 6).

The pharmacokinetic data of the compound **28*i*** indicate very fast absorption leading to C_{max} of 947.02 ± 237.4 ng/ml which appears to be a very high concentration therefore on repeated dosing for prolonged durations may lead to plasma and peripheral accumulation and toxicity. On the other hand compound **31*a***, owing to its higher solubility (416.41 ± 62.35 µg/ml) than compound **32*a*** (71.75 ± 13.45 µg/ml) exhibits comparatively faster absorption (C_{max} 149.49 ± 53.12 at t_{max} 0.75 ± 0.144 hrs) and prolonged and constant systemic exposure and more likely maintaining the desired therapeutic levels, for more than 24 hours than compound **32*a*** (t_{max} 8.67 ± 0.66 hrs) thereby exhibiting better therapeutic response.

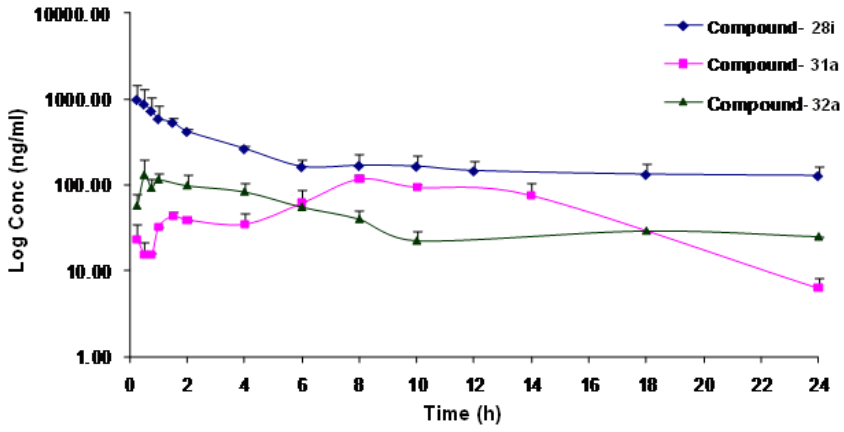


Fig. 7. Plasma concentration time profiles of compounds **28*i***, **31*a*** and **32*a***

Table 6: Estimates of pharmacokinetic parameters of compounds **28*i***, **31*a*** and **32*a*** in male NZ rabbits at a dose of 20 mg/kg body weight.

PK Parameters	Compound 28*i*	Compound 31*a*	Compound 32*a*
C_{max} (ng/ml)	947.02 ± 237.4	149.49 ± 53.12	112.40 ± 10.25
T_{max} (h)	0.68 ± 0.29	0.75 ± 0.144	8.67 ± 0.66
AUC (ng.h/ml)	6524.15 ± 1502.7	874.8 ± 232.01	1112.81 ± 169.77
MRT (h)	16.20 ± 1.46	5.25 ± 1.75	16.20 ± 1.46

3. Conclusion

In the present study, a novel class of various amino acids derived, constrained lactam carboxamides having chirally pure cyclic diamines were synthesized. The entire series of compounds were subjected to a preliminary screening for their antithrombotic activity in mice model of collagen epinephrine induced pulmonary thromboembolism. This is a simple preclinical test model based upon the synergistic effect of collagen and epinephrine to induce thrombosis, and is of great value for studying antithrombotic agents which work primarily by inhibiting pulmonary thromboembolism through their effect on platelet aggregation. The compound **28*i*** and its diastereomers **31*a*** and **32*a*** exhibited remarkable antithrombotic efficacy in mice model of collagen epinephrine induced pulmonary thromboembolism, which was better than standard drug Aspirin and almost comparable to Clopidogrel. After one hour of oral administration, **28*i*** and one of its diastereomer **31*a*** exhibited almost identical efficacy, while **32*a*** showed relatively less antithrombotic potential. However, the compound **31*a*** offered significant protection for a longer duration, i.e., upto 24 hours, while the activity of **28*i*** gradually declined after 18 hours. This observation was also in complete agreement with the pharmacokinetic properties of these compounds, which indicates that unlike **28*i*** and **32*a***, the compound **31*a*** not only exhibits faster absorption but also maintains a prolonged and constant systemic exposure thereby maintaining the desired therapeutic levels for more than 24 hours. Moreover, its anti-platelet action of these compounds was specific as it did not inhibit other platelet activation pathways as evidenced by its ineffectiveness to alter ADP, arachidonic acid or thombin mimetic TRAP induced platelet aggregation. Further, compound **34*c*** displayed promising anti-platelet potential via dual mechanism of action against both collagen (IC_{50}=3.3μM) and U46619 (IC_{50}=2.7μM) induced platelet aggregation.

The existing anti-thrombotic therapy suffers from a major drawback of bleeding risk that further leads to haemorrhage. Thus the objective of the ideal anti-thrombotic therapy is to prevent thrombosis but to leave haemostasis sufficiently intact to prevent bleeding complications. The three active compounds were therefore compared to the standard drugs aspirin and clopidogrel regarding bleeding complications in mice. Both aspirin and clopidogrel at their respective efficacy dose, showed a >2 fold prolongation in bleeding time in mice, while all the three test compounds exhibited a relatively milder increase (1.3 fold) in bleeding. Therefore, these compounds satisfactorily preserve the haemostasis with minor alterations in the pre-clinical mice model and hence they might promise some improvisation with respect to the critical disadvantage of bleeding risk endured by the current anti-platelet strategies.

4. Experimental

4.1. Chemistry

4.1.1. General

Reagents and other chemicals were used as purchased without further purification. All reactions with moisture/air sensitive reactants and solvents were carried out under nitrogen atmosphere using flame dried apparatus and all the solvents were distilled prior to use. THF was distilled under N_2 over potassium benzophenoneketyl radical prior to use. Dichloromethane was dried over P_2O_5 and methanol using magnesium cake. All the compounds were vacuum dried in Abderhalden. Reactions were monitored by thin layer chromatography over pre-coated silica gel plates ($60F_{254}$, E. Merck, Germany), using UV, iodine vapour, acidic and basic $KMnO_4$ or Dragendorff's reagent spray as the developing agents. Chromatographic separations were performed on flash column using silica gel (60-120 and 230-400 mesh). All melting points were taken in open tubes and are uncorrected. IR spectra were recorded on a Perkin-Elmer 881 and FTIR 8201 PC Shimadzu spectrophotometers and values are expressed in cm^{-1}. ^{1}HNMR and ^{13}CNMR spectra were recorded on Bruker WM-200, WM-300 or WM-400 Spectrometer. The chemical shifts are expressed in δ using TMS as internal standard. Mass spectra were recorded on a JEOL JMS-D-3000 spectrometer with an ionization potential of ~70 eV and and FAB on SX 102 instrument. Purity and diastereomeric ratios were determined by HPLC using Chiralpak-1A (Diacel) column with gradient elution (acetonitrile/2-propanol).

4.1.2. Synthesis of (2S)-N-arylalkyl acids.

*4.1.2.1. (2S)-N-(4-methylbenzyl) pyroglutamic acid, (**16a**)*

A solution of methyl pyroglutamate, 10 (2.0 gm, 1 eq, 13.9 mmol) and THF (100 ml) was taken in a three necked RBF fitted with rubber septa, N_2 inlet and cooled to -20 ^{0}C. LiHMDS (14 ml, 1.2 eq, 16.7 mmol) was added through a syringe to that solution and allowed to stir for 1h. Substituted benzylbromide (1.1 eq) was added and stirring was continued for 4h. from 0^{o}C to room temperature. The reaction was quenched by addition of 1N HCl (10 ml) and extracted with ethyl acetate (3 x 25 ml). The organic layer was washed with brine (2 x 25 ml), dried over Na_2SO_4 and concentrated under reduced pressure to give an oily ester. The ester was then re-dissolved in methanol (10 ml) and cooled to 0 ^{0}C. 20% sodium carbonate solution was then added to the reaction mixture portion wise. The reaction mixture was then stirred at room temperature for 5 hours. Methanol was then distilled off and the reduced reaction mixture was then extracted with ether (1 x 25 ml). The mixture was acidified with conc. HCl and extracted with ethyl acetate (3 x 30 ml). The organic layer was dried and concentrated. Yield: 90%; $[\alpha]_D^{27\,^0C}$: - 3.97 (c = 0.24 ; Methanol); IR (KBr): 3408.3, 2366.5, 1647.2, 1456.2, 1220.3 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.05-2.18 (m, 1H, 3-H_a); 2.20-2.27 (m, 1H, 3-H_b); 2.50- 2.60 (m, 2H, 4-H); 3.93-3.98 (d, 1H, -NC<u>H</u>Ph); 4.02-4.04 (m, 1H, 2-H); 5.09-5.17 (d, 1H, -NC<u>H</u>Ph); 7.12 (s, 5H, Ph-H); ^{13}C NMR (50 MHz, $CDCl_3$): 21.52, 23.26, 30.10, 45.83, 59.01, 61.00, 128.95, 129.92, 132.62, 138.11, 174.74, 176.90; FAB MS (m/z): 234 $(M+H)^+$; HRMS: (m/z) calc. for $C_{13}H_{15}NO_3$: 234.1052. Found: 234.1085

*4.1.2.2 (S)-3-(4-methylbenzyl)-2-oxooxazolidine-4-carboxylic acid, [**17a**]:* Prepared from **11a**. Yield: 58 %; IR (KBr): 3469, 3019, 2926, 2601, 1912, 1759, 1645, 1515, 1420, 1226, 1086, 1049 cm^{-1}; ^{1}HNMR ($CDCl_3$, 300 MHz): δ 2.35 (s, 3H, CH_3); 4.09-4.12 (t, 1H, 5H); 4.18-4.23 (d, 1H, NCHPh); 4.39-4.42 (m, 2H, 5H); 4.92-4.97 (d, 1H, NCHPh); 7.17 (s, 4H, Ph-H); FABMS: m/z : 236 $[M+1]^+$; HRMS: (m/z) calc. for $C_{12}H_{13}NO_4$: 236.0845. Found: 235.0851

*4.1.2.3. (R)-3-(4-methylbenzyl)-2-oxothiazolidine-4-carboxylic acid, [**18a**]:* Prepared from **12a**. Yield: 70 %; IR (KBr): 3015, 2930, 2504, 2504, 1925, 1731, 1666, 1513, 1440, 1387, 1297, 1231, 1160, 1018 cm^{-1}; ^{1}HNMR ($CDCl_3$, 300 MHz): δ 2.34 (S, 3H, -CH_3); 3.43-3.50 (m, 2H, 5-H); 4.00-4.05 (d, 1H, NCHPh); 4.13-4.17(dd, 1H, 4H); 5.15-5.20 (d, 1H, NCHPh); 7.15 (s, 4H, Ph-H); ^{13}CNMR ($CDCl_3$, 75 MHz): 21.09, 29.14, 31.80, 36.92, 47.43, 59.11, 128.40, 129.51,

132.74, 137.71, 163.44, 171.94; FABMS: m/z : 252 $[M+1]^+$; HRMS: (m/z) calc. for $C_{12}H_{13}NO_3S$: 252.3015. Found: 252.3045

*4.1.2.4. (R)-3-(4-methylbenzoyl)thiazolidine-4-carboxylic acid, (**19**): Step-1*: (R)-methyl thiazolidine-4-carboxylate, 13 (0.50 g, 1 eq, 3.40 mmol) was dissolved in dry DCM (10 ml) and cooled to 0^0C in an ice bath. TEA (1.1 ml, 2.3 eq, 7.82 mmol) was added drop wise to the reaction mixture and further stirred for 15 min. 4-methylbenzoyl chloride (0.60 g, 1.1 eq, 3.74 mmol) dissolved in dry DCM (10 ml) was added to the reaction mixture maintaining the temperature at 0^0C. The stirring was continued for 3 hrs at room temperature. The reaction mixture was then washed with dilute citric acid 1 x 25 ml), saturated aq.$NaHCO_3$ solution (1 x 25 ml) and then with brine. The organic layer was separated, dried over Na_2SO_4, filtered and concentrated. *Step-2*: The sticky oil obtained from the above step was dissolved in minimum quantity of methanol (5 ml) and cooled to 0^0C. 20 % aq.Na_2CO_3 solution (25 ml) was then added portion wise and the resulting solution was stirred for 4hrs at room temperature. Methanol present in the reaction mixture was evaporated and aqueous solution was then acidified with con.HCl. The turbid solution thus obtained was extracted with ethyl acetate (3 x 25 ml). The organic layer was separated, dried over Na_2SO_4, filtered and concentrated. *2.1.2.4. (R)-3-(4-methylbenzyl)thiazolidine-4-carboxylic acid, (19a):* Prepared from 13: Yield: 82 %; IR (KBr): 3013, 2928, 2854, 1908, 1720, 1621, 1514, 1448, 1379, 1218, 1137, 1049, 949 cm^{-1}; ^{1}HNMR ($CDCl_3$, 300 MHz): δ 2.37 (s, 3H, CH_3); 3.26-3.29 (m, H, 5-H_a); 3.46-3.50 (m, H, 5-H_b); 3.73-3.77 (d, 1H, 2-H_a); 3.84-3.88 (d, 1H, NCH_aPh); 4.02-4.03 (t, 1H, 4H); 4.05 (d, 1H, 2-H_b); 4.11-4.14 (d, 1H, NCH_bPh); 7.21-7.28 (m, 4H, Ph-H); ; HRMS: (m/z) calc. for $C_{12}H_{13}NO_3S$: 252.3015. Found: 252.3032

*4.1.2.5. (S)-1-(4-methylbenzyl)-6-oxopiperidine-2-carboxylic acid, [**20**]:* Prepared from **14**. Yield: 90.3 %; MP: 140-142 ^{0}C; $[\alpha]_D^{27\,^0C}$: +40.80 (c = 0.10; Chloroform); IR (KBr): 3449, 2964, 2925, 2534, 1952, 1710, 1594, 1499, 1445, 1411, 1333, 1267, 1181, 1004 cm^{-1}; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.85 (m, 2H, 4-H); 1.90 (m, 1H, 3-H_a); 2.12 (m, 1H, 3-H_b); 2.34 (s, 3H, Ph-CH_3); 2.51-2.69 (m, 2H, 3-H); 3.63-3.68 (d, 1H, Ph-C<u>H</u>); 4.06 (t, 1H, 3-H); 5.59-5.64 (d, 1H, Ph-C<u>H</u>); 7.13 (s, 4H, Ph-H); FAB-MS: (m/z): 248, $[M+H]^+$; HRMS: (m/z) calc. for $C_{14}H_{17}NO_3$: 247.1208. Found: 234.1025

*4.1.2.6. (3S)-4-benzyl-2-methyl-5-oxomorpholine-3-carboxylic acid, [**21a**]*

N-benzyl serine, 15 (5g) was stirred with 35 ml aq. NaOH (2.5M) at -10°C for 10-15 min. Chloro acetyl chloride (4.5 ml) was added drop wise, after 10-15 min stirring at 0^oC the pH was adjusted to 8.5 using NaOH (7.5M) aq. solution. The reaction was then carried out at 30^oC for 2-3 hrs and then cooled to 0^oC and added HCl (conc.) for precipitation. The precipitate was filtered and washed with hexane and ether and dried in Abdel-Halden under vacuum. (same procedure was followed for the preparation of 21*b*, starting from N-benzyl threonine) Yield: 60%; MP: 127^oC; IR (KBr): 3427, 3020, 2360, 1719, 1638, 1515, 1439, 1216, 1049, 928, 761, 670 cm^{-1}; ^{1}HNMR ($CDCl_3$, 300 MHz): δ 1.24 (d, 3H, CH_3), 3.78-3.90 (m, 2H, H-2), 4.20-4.26 (m, 2H, H-6), 4.80 (m, 1H, H-3), 5.61-5.65 (d, 2H, $NC\underline{H}_2Ar$), 7.27-7.35 (m, 5H, Ph); FABMS m/z : 250 $[M+1]^+$; HRMS: (m/z) calc. for $C_{13}H_{15}NO_4$: 250.1001. Found: 234.1038

*4.1.2.7. (S)-tert butoxy carbonyl methyl cysteine ethyl ester, [**24**]:* L-cysteine ethyl ester hydrochloride (11 gm, 0.059 mol) was taken in dry DMF (30 ml) and at -10^oC DIPEA (26 ml, 0.148 mol) was added; when half of the DIPEA added the reaction mixture turned into yellow slurry and after the complete addition $BrCH_2COOtBu$ (10 ml, 0.065 mol) was added at 0^oC slowly through a dropping funnel. Reaction mixture turned gradually within 3-4 hr as white slurry. Reaction mixture was poured into a large excess of (250 ml) of chilled ice water with stirring and the aqueous layer then extracted with ethyl acetate (3 x 150 mL). All the organic layers were separately washed with brine water and then combined and dried over activated Na_2SO_4. Concentrated at reduced pressure gave a crude oil which was purified using flash chromatography with 28% ethyl acetate in hexane as an eluant. Yield: 70 %; IR (neat): 3432, 3330, 1976, 1735, 1623, 1498, 1408, 1289, 1206, 1153, 909 cm^{-1}; ^{1}HNMR ($CDCl_3$, 300 MHz): δ 1.25-1.32 (t, 3H, $CH_2C\underline{H}_3$), 1.45 (s, 9H, *t*-Butyl-H), 2.91-2.94 (d, 2H, α-$CH\underline{C}H_2S$), 3.17 (s, 2H, $SC\underline{H}_2COOtBu$), 3.6 (m, 1H, α-$C\underline{H}CH_2S$), 4.2 (q, 2H, $COOC\underline{H}_2CH_3$), 2.1 (br, 2H, NH); FABMS (m/z): 264 $[M+1]^+$, 208 $[(M+1)-57]^+$

*2.1.2.8. N-p-Methyl benzyl (S)-tert butoxy carbonyl methyl cysteine ethyl ester, [**25**]:* Compound (3 gm, 0.011 mol) was taken in dry methanol and under N_2 environment added baked NaOAc (2 gm, 0.025 mol) followed by tolualdehyde (1.4 ml, 0.013 mol) and the slurry was stirred at 0^oC for 10 min after which $NaCNBH_3$ (1.6 gm, 0.025 mol) was gradually added in portions and the reaction mixture was allowed to stir over night. Reaction mixture was quenched with dil. citric

acid and the reaction contents were concentrated prior to washing in a biphasic system of water-ethyl acetate. Organic layer was again washed with brine and dried over activated sodium sulfate and flash chromatographed using 12% ethyl acetate in hexane as an eluent gave the pure oily product. Yield: 40%; IR (neat): 3432, 3330, 1976, 1735, 1623, 1498, 1408, 1289, 1206, 1180 cm^{-1} ; ^{1}HNMR ($CDCl_3$, 300 MHz): δ 1.25-1.32 (t, 3H, $COOCH_2C\underline{H}_3$), 1.48 (s, 9H, COOtBu), 2.34 (s, 3H, $C\underline{H}_3$), 2.91-2.94 (d, 2H, α-$CHC\underline{H}_2S$), 3.19 (s, 2H, $SC\underline{H}_2COOtBu$), 3.43 (m, 2H, $NH\underline{CH}_2Ar$), 3.82 (m, 1H, α-$C\underline{H}CH_2S$), 4.22 (q, 2H, $COOC\underline{H}_2CH_3$), 7.11-7.24 (dd, 4H, Ar), 2.00 (br, 1H, NH).; FABMS m/z: 368 $[M+1]^+$

*4.1.2.9. N-p-methyl benzyl thiomorpholinone carboxylic acid, (**26**):* Compound (0.4 gm, 1.1 mmol) was dissolved in dry DCM (12 ml), and treated with 40% TFA in DCM (5 ml) at 40^{o}C with stirring for about 3-4 h until the TLC indicated the complete removal of *t*-butyl ester group. Reaction mixture was then concentrated at ambient temperature under vacuum to remove the solvent and traces of unreacted acid. The crude (without purification) was again dissolved in dry DCM and added Et_3N (0.27 ml, 1.94 mmol), followed by HOBt (0.17 gm, 1.34 mmol)) and then through syringe DIC (0.24 ml, 1.52 mmol) was added under nitrogen atmosphere. Reaction was stirred over night and quenched with saturated ammonium chloride solution. The compound was taken in a THF: Methanol mixture and cooled to 0^{o}C. A methanolic solution of K_2CO_3 (15%) and few pellets of KOH at 0°C was added to the above solution and made it to stir at the same temperature for about 5-6 hrs. The reaction mixture was then concentrated and washed twice with ether. The aqueous layer was made acidic and then extracted with ethyl acetate. The combined organic layers were washed with brine and dried over sodium sulfate and concentrated under reduced pressure. Yield: 50%; MP: 128-130 ^{0}C; IR (KBr): 3432, 3330, 1976, 1750, 1623, 1498, 1408, 1289, 1190, 1180 cm^{-1}; ^{1}HNMR ($CDCl_3$): δ 2.35 (s, 3H, $C\underline{H}_3$), 3.10-3.20 (m, 2H, α-$CHC\underline{H}_2S$), 3.53-3.61 (m, 1H, α-$C\underline{H}CH_2S$), 3.42 (d, 2H, $SC\underline{H}_2CO$), 4.33 (s, 2H, $NC\underline{H}_2Ar$), 7.11-7.24 (dd, 4H, Ar); HRMS: (m/z) calc. for $C_{13}H_{15}NO_3S$: 266.0773. Found: 234.0745

4.1.3. Synthesis of chiral carboxamide derivatives

*4.1.3.1. tert-butyl ((S)-1-((S)-1-(4-methylbenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate (**31a**):* Step-1: (2*S*)-N-(4-methylbenzyl)-pyroglutamic acid (3.50 g, 1 eq, 15 mmol) was dissolved in dry DCM (50 ml) and cooled to 0 ^{0}C. Oxalyl chloride (1.3 ml, 1.2 eq, 18 mmol) was then added drop wise at 0 ^{0}C and the mixture was allowed to warm up to room

temperature and stirred for 12 hours. The reaction mixture was then concentrated over reduced pressure. Step-2: (3*R*)-N-Boc-3-aminomethyl-piperidine (3.53 g, 1.1 eq, 16 mmol) was dissolved in dry DCM (50 ml) and cooled to 0 ^{0}C. TEA (4.82 ml, 2.3 eq, 35 mmol) was then added to the reaction mixture and stirred for 10 min. The acid chloride (from step-1) dissolved in dry DCM (25 ml) was then added drop wise at 0 ^{0}C and stirred at room temperature for 3 hrs. The reaction mixture was then washed with saturated sodium bicarbonate (1 x 50 ml), ice cold 1N HCl (1 x 50 ml) and then with brine. The organic layer was separated, dried and concentrated and flash chromatographed. Yield: 75 %; $[\alpha]_D^{27\,^0C}$: + 35.00 (c = 0.098; Chloroform); MP: 138-140 ^{0}C; IR (KBr): 3274, 2977, 2934, 2860, 1714, 1674, 1630, 1541, 1449, 1365, 1272, 12489, 1176, 1144, 1039, 997, 958 cm^{-1}; ^{1}H NMR ($CDCl_3$; 600MHz): δ 1.119-1.390 (m, 2H, 4'-H); 1.448 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.704 (m, 1H, 3'-H); 1.725-1.887 (m, 2H, 5'-H); 2.138-2.175 (m, 1H, 3-H$_a$); 2.305 (s, 3H, Ph-C<u>H</u>3), 2.361-2.398 (m, 1H, 3-H$_b$); 2.496-2.555 (m, 2H, 4-H); 2.646-2.757 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.833-3.090 (m, 2H, 6'-H); 3.324-3.428 (m, 1H, 2'-H$_a$) 3.731-3.769 (m, 1H, 2'-H$_b$); 4.110-4.200 (m, 2H, -C<u>H</u>$_2$-Ph & 2-H); 4.871 (s, 1H. NH); 5.133-5.138 (d, 1H, -C<u>H</u>$_2$-Ph); 7.060-7.090 (dd, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 100 MHz): 21.063, 21.086, 22.651, 24.614, 27.898, 28.287, 28.639, 29.748, 35.955, 38.330, 42.728, 43.118, 43.332, 45.065, 45.646, 49.273, 55.673, 55.841, 79.331, 128.427, 128, 733, 129.351, 133.078, 137.339, 156.028, 168.810, 169.253, 175.164; FAB MS (m/z): 430 $(M+1)^+$, 330, 374, 452 $(M+Na)^+$; HRMS: (m/z calcd. for $C_{24}H_{35}N_3O_4$, 430.2628; Found, 430.2661.

*4.1.3.2. tert-butyl ((R)-1-((S)-1-(4-methylbenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate (**32a**)*: Same as 31a. Yield: 85 %; $[\alpha]_D^{27\,^0C}$: +5.69 (c = 0.99; Chloroform); MP: 180-182 ^{0}C; IR (KBr): 3330, 2974, 2935, 2857, 1710, 1677, 1642, 1528, 1444, 1363, 1274, 1173, 1146, 1080, 997, 958 cm^{-1}; ^{1}H NMR ($CDCl_3$; 600MHz): δ 1.177-1.270 (m, 2H, 4'-H); 1.424 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.668 (m, 1H, 3'-H); 1.768-1.856 (m, 2H, 5'-H); 2.138-2.175 (m, 1H, 3-H$_a$); 2.305 (s, 3H, Ph-C<u>H</u>$_3$), 2.361-2.398 (m, 1H, 3-H$_b$); 2.496-2.555 (m, 2H, 4-H); 2.646-2.757 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.833-2.984 (m, 2H, 6'-H); 3.324-3.428 (dd, 1H, 2'-H$_a$); 3.731-3.769 (m, 1H, 2'-H$_b$); 4.160-4.180 (m, 2H, -C<u>H</u>$_2$-Ph & 2-H); 4.871 (s, 1H. NH); 5.133-5.138 (m, 1H, -C<u>H</u>$_2$-Ph); 7.060-7.090 (dd, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 100 MHz): 21.086, 22.728, 23.866, 24.614, 28.096, 28.303, 28.700, 29.784, 36.405, 37.322, 42.828, 43.011, 43.263, 45.042, 45.852, 48.441, 55.986, 56.047, 79.316, 128.389, 128.710, 129.336, 133.048, 137.339, 156.080, 168.810,

169.001, 175.072; FABMS (m/z): 429[M^+],330, 452 $(M+Na)^+$; HRMS: (m/z) calcd. for $C_{24}H_{35}N_3O_4$) 430.2628; Found, 430.2684.

*4.1.3.3. tert-butyl ((S)-1-((S)-1-(4-methoxybenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**31b**]*: Same as 31a. Yield: 84 %; $[\alpha]_D^{27\,^0C}$: + 32.50 (c = 0.24; Chloroform); IR (KBr): 3349, 3008, 2934, 2361, 1684, 1513, 1455, 1365, 1250, 1174, 1035, 963 cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 1.16-1.29 (m, 2H, 4'-H); 1.43 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.63 (m, 1H, 3'-H); 1.76-1.87 (m, 2H, 5'-H); 2.15-2.18 (m, 1H, 3-H_a); 2.36-2.39 (m, 1H, 3-H_b); 2.52-2.57 (m, 2H, 4-H); 2.61-2.65 (m, 2H, -$C\underline{H}_2$-NH-Boc); 2.88-3.00 (m, 2H, 6'-H); 3.34-3.43 (m, 1H, 2'-H_a); 3.73-3.86 (m, 1H, 2'-H_b); 3.78 (s, 3H, -OC$\underline{H}$3); 4.15-4.22 (m, 2H, 2-H & -$C\underline{H}_2$-Ph); 4.87 (s, 1H. NH); 5.10-5.13 (d, 1H, -$C\underline{H}_2$-Ph); 6.84-6.89 (d, 2H, Ph-H); 7.06-7.09 (d, 2H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.06, 22.65, 24.61, 27.89, 28.28, 29.74, 43.33, 55.67, 55.84, 79.33, 112.81, 126.94, 126.53, 128.86, 155.02, 168.78, 175.14; FAB MS (m/z): 446 $(M+H)^+$; HRMS: (m/z) calc. for $C_{13}H_{15}NO_4$: 446.1252. Found: 446.1285

*4.1.3.4. tert-butyl (R-1-((S)-1-(4-methoxybenzyl)-5-oxopyrrolidine-2 carbonyl) piperidine-3-yl)methylcarbamate [**32b**]*: Same as 31a. Yield: 75 %; $[\alpha]_D^{27\,^0C}$: - 15.00 (c = 0.10; Chloroform); IR (KBr): 3328, 2975, 2937, 2362, 1685, 1652, 1516, 1451, 1366, 1251, 1175, 1026, 961 cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 1.25 (m, 2H, 4'-H); 1.46 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.60 (m, 1H, 3'-H); 1.82-1.92 (m, 2H, 5'-H); 2.19-2.23 (m, 1H, 3-H_a); 2.40-2.47 (m, 1H, 3-H_b); 2.52-2.58 (m, 2H, 4-H); 2.64-2.79 (m, 2H, -$C\underline{H}_2$-NH-Boc); 2.91-3.01 (m, 2H, 6'-H); 3.36-3.45 (m, 1H, 2'-H_a); 3.74-3.83 (m, 1H, 2'-H_b); 3.81 (s, 3H, -OCH_3); 4.15-4.27 (m, 2H, 2-H & -$C\underline{H}_2$-Ph); 4.83 (s, 1H. NH); 5.12-5.17 (m, 1H, -$C\underline{H}_2$-Ph); 6.84-7.15 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 22.78, 23,98, 24.61, 28.13, 29.73, 42.87, 45.31, 48.58, 55.27, 56.11, 113.07, 114.42, 120.61, 129.72, 137.68, 156.10, 159.92, 169.01, 175.24; ESI MS (m/z): 446 $(M+H)^+$, 345 (M-Boc), 390 (M-t_butyl), 486 $(M+Na)^+$; HRMS: (m/z) calc. for $C_{13}H_{15}NO_4$: 446.1252. Found: 446.1258

*4.1.3.5. tert-butyl ((S)-1-((S)-1-(3-methoxybenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**31c**]:* Same as 31a. Yield: 82 %; $[\alpha]_D^{27\,^0C}$: + 47.37 (c = 0.19; Chloroform); IR (KBr): 3451, 3010, 2937, 2361, 1684, 1647, 1512, 1456, 1365, 1256, 1218, 1169, 1044 cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 1.16-1.38 (m, 2H, 4'-H); 1.45 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.62 (m, 1H, 3'-H); 1.76-1.89 (m, 2H, 5'-H); 2.17-2.24 (m, 1H, 3-H_a); 2.37-2.47 (m, 1H, 3-H_b); 2.52-2.66 (m, 2H, 4-H); 2.80-3.02 (m, 2H, -$C\underline{H}_2$-NH-Boc); 2.88-3.04 (m, 2H, 6'-H); 3.34-3.49 (m, 1H, 2'-H_a); 3.78-

3.81 (m, 1H, 2'-H_b); 3.80 (S, 3H, -OC<u>H</u>3); 4.08-4.25 (m, 2H, 2-H & -C<u>H</u>$_2$-Ph); 4.89 (s, 1H. NH); 5.13-5.18 (m, 1H, -C<u>H</u>$_2$-Ph); 6.74-6.84 (m, 3H, Ph-H); 7.20-7.28(m, 1H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.42, 21,81, 27.01, 27.11, 44.10, 44.40, 53.98, 54.36, 112.69, 113.42, 119.42, 128.43, 128.54, 136.52, 158.68, 168.00, 173.95; ESI MS (m/z): 446 $(M+H)^+$, 345 (M-Boc), 390 (M-t_butyl), 486 $(M+Na)^+$; HRMS: (m/z) calc. for $C_{13}H_{15}NO_4$: 446.1252. Found: 446.1261

*4.1.3.6. tert-butyl((R)-1-((S)-1-(3-methoxybenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**32c**]:* Same as 31a. Yield: 78 %; $[\alpha]_D^{27\,^oC}$: -12.00 (c = 0.15; Chloroform); IR (KBr): 3330, 2974, 2935, 2857, 1710, 1677, 1642, 1528, 1444, 1363, 1274, 1173, 1146, 1080, 997, 958 cm^{-1}; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.24 (m, 2H, 4'-H); 1.43 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.67 (m, 1H, 3'-H); 1.77-1.86 (m, 2H, 5'-H); 2.15-2.19 (m, 1H, 3-H_a); 2.40 (m, 1H, 3-H_b); 2.49-2.57 (m, 2H, 4-H); 2.60-2.79 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.82-3.09 (m, 2H, 6'-H); 3.34-3.48 (dd, 1H, 2'-H_a); 3.73 (m, 1H, 2'-H_b); 3.76(s, 3H, -OC<u>H</u>$_3$) 4.14-4.23 (m, 2H, 2-H & -C<u>H</u>$_2$-Ph); 4.90 (s, 1H. NH); 5.10-5.15 (m, 1H, -C<u>H</u>$_2$-Ph); 6.72-6.81 (m, 3H, Ph-H); 7.18-7.24 (m, 1H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 22.75, 24.61, 28.38, 29.64, 36.60, 43.08, 45.39, 55.23, 56.11, 79.37, 113.06, 114.41, 120.58, 129.72, 137.60, 159.89, 168.98, 175.29; ESI MS (m/z): 446 $(M+H)^+$, 345 (M-Boc), 390 (M-t_butyl), 486 $(M+Na)^+$; HRMS: (m/z) calc. for $C_{13}H_{15}NO_4$: 446.1252. Found: 446.1221

*4.1.3.7. tert-butyl ((S)-1-((S)-1-(2-methoxybenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**31d**]:* Same as 31a. Yield: 80 %; $[\alpha]_D^{27\,^oC}$: +8.15 (c = 0.26; Chloroform); IR (KBr): 3454, 3342, 3008, 1235, 2361, 1681, 1498, 1461, 1249, 1171, 1030 cm^{-1}; ^{1}H NMR ($CDCl_3$; 300 MHz): δ 1.26-1.33 (m, 2H, 4'-H); 1.43 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.60-1.66 (m, 1H, 3'-H); 1.71-1.91 (m, 2H, 5'-H); 2.16-2.175 (m, 1H, 3-H_a); 2.40-2.42 (m, 1H, 3-H_b); 2.50-2.60 (m, 2H, 4-H); 2.84-2.90 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.95-3.00 (m, 2H, 6'-H); 3.32-3.58 (m, 1H, 2'-H_a); 3.74-3.79 (m, 1H, 2'-H_b); 3.80 (s, 3H, -OC<u>H</u>$_3$); 4.02-4.07 (d, 1H, -C<u>H</u>$_2$-Ph); 4.24-4.31 (m, 1H, 2-H); 4.91 (s, 1H. NH); 4.96-5.04 (d, 1H, -C<u>H</u>$_2$-Ph); 6.85-6.94 (m, 3H, Ph-H); 7.19-7.28 (m, 1H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.42, 21.81, 27.01, 27.11, 44.10, 44.40, 53.98, 54.36, 109.14, 113.42, 119.46, 127.78, 129.36, 136.52, 158.68, 168.00, 173.95; FAB MS (m/z): ESI MS (m/z): 446 $(M+H)^+$, 345 (M-Boc), 390 (M-t_butyl), 486 $(M+Na)^+$; HRMS: (m/z) calc. for $C_{13}H_{15}NO_4$: 446.1252. Found: 446.1233

*4.1.3.8. tert-butyl((R)-1-((S)-1-(2-methoxybenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**32d**]:* Same as 31a. Yield: 85 %; $[\alpha]_D^{27\,^{\circ}C}$: - 6.25 (c = 0.24; Chloroform); IR (KBr) 3337, 3005, 2930, 2361, 1684, 1496, 1460, 1365, 1249, 1172, 1119, 1029 cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 1.17-1.26 (m, 2H, 4'-H); 1.45 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.66-1.73 (m, 1H, 3'-H); 1.81-1.88 (m, 2H, 5'-H); 2.15-2.18 (m, 1H, 3-H_a); 2.32-2.42 (m, 1H, 3-H_b); 2.47-2.56 (m, 2H, 4-H); 2.60-2.67 (m, 2H, -$C\underline{H}_2$-NH-Boc); 2.85-3.01 (m, 2H, 6'-H); 3.39 (dd, 1H, 2'-H_a); 3.45-3.80 (m, 1H, 2'-H_b); 3.78 (s, 3H, -OCH_3); 4.02-4.06 (d, 1H, -$C\underline{H}_2$-Ph); 4.23-4.29 (m, 1H, 2-H); 4.90 (s, 1H. NH); 4.95-5.03 (d, 1H, -$C\underline{H}_2$-Ph); 6.84-6.93 (m, 2H, Ph-H); 7.20-7.28 (m, 2H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 22.78, 23.98, 28.13, 29.73, 42.87, 45.41, 45.93, 48.58, 55.27, 56.11, 79.31, 113.07, 113.99, 121.00, 129.72, 137.68, 156.10, 159.92, 169.01, 175.24; FAB MS (m/z): 446 $(M+H)^+$; HRMS: (m/z) calc. for $C_{13}H_{15}NO_4$: 446.1252. Found: 446.1227

*4.1.3.9. tert-butyl ((S)-1-((S)-1-(3-methylbenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**31e**]:* Same as 31a. Yield: 80 %; $[\alpha]_D^{27\,^{\circ}C}$: +23.00 (c = 0.20; Chloroform); IR (KBr): 3451, 3012, 2933, 2360, 1688, 1512, 1455, 1251, 1219, 1170 cm^{-1}; ^1H NMR ($CDCl_3$; 300 MHz): δ 1.17-1.25 (m, 2H, 4'-H); 1.42 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.66 (m, 1H, 3'-H); 1.76-1.86 (m, 2H, 5'-H); 2.13-2.17 (m, 1H, 3-H_a); 2.30 (s, 3H, Ph-C$\underline{H}$3), 2.36-2.39 (m, 1H, 3-H_b); 2.49-2.55 (m, 2H, 4-H); 2.64-2.75 (m, 2H, -$C\underline{H}_2$-NH-Boc); 2.83-3.00 (m, 2H, 6'-H); 3.32-3.42 (m, 1H, 2'-H_a); 3.73-3.76 (m, 1H, 2'-H_b); 4.14-4.16 (m, 2H, 2-H &-$C\underline{H}_2$-Ph); 4.85 (s, 1H. NH); 5.13-5.20 (m, 1H, -$C\underline{H}_2$-Ph); 6.89-7.26 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.10, 22.67, 23.07, 24.71, 27.91, 28.40, 29.77, 35.98, 38.32, 43.12, 45.08, 45.66, 49.29, 55.70, 79.32, 128.44, 128.74, 129.37, 133.10, 137.38, 156.11, 168.83, 175.32; FAB MS (m/z): 429 (M^+), 330, 374, 452 $(M+Na)^+$; HRMS: (m/z) calcd. for $C_{24}H_{35}N_3O_4$) 430.2628; Found, 430.2672

*4.1.3.10. tert-butyl ((R)-1-((S)-1-(3-methylbenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**32e**]:* Same as 31a. Yield: 85 %; $[\alpha]_D^{27\,^{\circ}C}$: - 7.14 (c = 0.34; Chloroform); IR (KBr): 3453, 3349, 3010, 2932, 2862, 2362, 1695, 1658, 1511, 1450, 1394, 1366, 1253, 1170, 1042, 964 cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 1.13-1.25 (m, 2H, 4'-H); 1.42 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.68 (m, 1H, 3'-H); 1.76-1.85 (m, 2H, 5'-H); 2.14-2.19 (m, 1H, 3-H_a); 2.35 (s, 3H, Ph-C$\underline{H}$3), 2.33-2.39 (m, 1H, 3-H_b); 2.47-2.59 (m, 2H, 4-H); 2.62-2.77 (m, 2H, -$C\underline{H}_2$-NH-Boc); 2.80-2.98 (m, 2H, 6'-H); 3.31-3.40 (m, 1H, 2'-H_a); 3.72-3.77 (m, 1H, 2'-H_b); 4.12-4.20 (m, 2H, 2-H &-$C\underline{H}_2$-Ph); 4.93 (s, 1H. NH); 5.09-5.14 (m, 1H, -$C\underline{H}_2$-Ph); 6.93-7.28 (dd, 4H, Ph-H); ^{13}C NMR

($CDCl_3$; 75 MHz): 21.10, 22.75, 23.89, 24.64, 28.32, 29.80, 36.43, 37.36, 42.85, 45.88, 48.47, 56.01, 79.33, 128.72, 129.37, 133.05, 137.44, 156.10, 169.01, 175.11; FAB MS (m/z): 429 (M^+), 330, 374, 452 $(M+Na)^+$; HRMS: (m/z) calcd. for $C_{24}H_{35}N_3O_4$) 430.2628; Found, 430.2684.

*4.1.3.11. tert-butyl ((S)-1-((S)-1-benzyl-5-oxopyrrolidine-2-carbonyl)piperidin-3-yl)methyl carbamate [**31f**]:* (2S)-N-(phenylmethyl)-pyroglutamic acid (0.1 g, 1 eq, 0.46 mmol) and HOBt (0.13 g, 1.5 eq, 0.68 mmol) were dissolved in dry DCM (25 ml) in a three necked round bottom flask fitted with N_2 inlet. The reaction mixture was cooled to 0^0C in an ice-salt bath. DCC (0.13 g, 1.2 eq, 0.55 mmol) dissolved in dry DCM (10 ml) was added to it and stirred while being at 0^0C for 15 min. (3R)-N-Boc-3-aminomethylpiperidine (0.098 g, 1 eq, 0.46 mmol) dissolved in dry DCM (10 ml) was added drop wise to the reaction mixture and stirring was continued for 3 hrs at 0^0C. The reaction mixture was then brought to room temperature and concentrated under reduced pressure. The residue was dissolved in ethyl acetate, filtered and the filtrate was washed successively with dil. citric acid (3 x 25 ml), dil. Na_2CO_3 solution (3 x 25 ml) and brine (1 x 25 ml). The organic layer was separated, dried and concentrated and the crude material was flash chromatographed to get pure compound. Yield: 80 %; $[\alpha]_D^{27\,^0C}$: + 27.85 (c = 0.28; Chloroform); IR (KBr): 3353, 2932, 2360, 1687, 1517, 1454, 1364, 1255, 1171 cm^{-1}; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.17-1.38 (m, 2H, 4'-H); 1.44 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.65 (m, 1H, 3'-H); 1.72-1.88 (m, 2H, 5'-H); 2.12-2.21 (m, 1H, 3-H_a); 2.40-2.47 (m, 1H, 3-H_b); 2.51-2.63 (m, 2H, 4-H); 2.70-2.84 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.88-2.93 (m, 2H, 6'-H); 3.29-3.45 (m, 1H, 2'-H_a); 3.72-3.93 (m, 1H, 2'-H_b); 4.10-4.21 (m, 2H, 2-H & -C<u>H</u>$_2$-Ph); 4.92 (s, 1H. NH); 5.13-5.27 (d, 1H, -C<u>H</u>$_2$-Ph); 7.14-7.32 (m, 5H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.06, 21.06, 22.65, 24.61, 27.88, 28.28, 28.69, 29.74, 35.95, 38.33, 42.72, 43.11, 43.33, 45.06, 45.64, 49.27, 55.67, 55.84, 79.31, 128.47, 128.73, 129.35, 133.07, 137.33, 156.02, 168.81, 169.25, 175.16; ESI MS (m/z): 416 $(M+H)^+$, 316 (M-Boc), 439 $(M+Na)^+$; HRMS: (m/z) calc. for $C_{23}H_{33}N_3O_4$: 416.2471. Found: 416.2485

*4.1.3.12. tert-butyl ((R)-1-((S)-1-benzyl-5-oxopyrrolidine-2-carbonyl)piperidin-3-yl)methyl carbamate [**32f**]:* Same as 31*f*. Yield: 80 %; $[\alpha]_D^{27\,^0C}$: - 9.35 (c = 0.32; Chloroform); IR (KBr): 3353, 2932, 2360, 1687, 1517, 1454, 1364, 1255, 1171 cm^{-1}; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.17-1.38 (m, 2H, 4'-H); 1.44 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.65 (m, 1H, 3'-H); 1.72-1.88 (m, 2H, 5'-H); 2.12-2.21 (m, 1H, 3-H_a); 2.40-2.47 (m, 1H, 3-H_b); 2.51-2.63 (m, 2H, 4-H); 2.70-2.84 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.88-3.01 (m, 2H, 6'-H); 3.29-3.45 (m, 1H, 2'-H_a); 3.72-3.93 (m, 1H, 2'-H_b); (m, 1H, -

C<u>H</u>$_2$-Ph); 4.10-4.21 (m, 1H, 2-H); 4.92 (s, 1H. NH); 5.13-5.27 (m, 1H, -C<u>H</u>$_2$-Ph); 7.14-7.32 (m, 5H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.06, 21.06, 22.65, 24.61, 27.88, 28.28, 28.69, 29.74, 35.95, 38.33, 42.72, 43.11, 43.33, 45.06, 45.64, 49.27, 55.67, 55.84, 79.31, 128.47, 128.73, 129.35, 133.07, 137.33, 156.02, 168.81, 169.25, 175.16; ESI MS (m/z): 416 $(M+H)^+$, 316 (M-Boc), 439 $(M+Na)^+$; HRMS: (m/z) calc. for $C_{23}H_{33}N_3O_4$: 416.2471. Found: 416.2485

*4.1.3.13. tert-butyl ((S)-1-((S)-1-(naphthalen-2-ylmethyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**31g**]:* Same as 31*f*. Yield: 90 %; $[\alpha]_D^{27\,^0C}$: + 8.95 (c = 0.19; Chloroform); IR (KBr): 3344, 3088, 2979, 2931, 2859, 2361, 1682, 1512, 1459, 1365, 1253, 1170, 1082, 1047, 1016, 962 cm^{-1}; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.12-1.25 (m, 2H, 4'-H); 1.45 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.66 (m, 1H, 3'-H); 1.73-1.79 (m, 2H, 5'-H); 1.99-2.10 (m, 1H, 3-H$_a$); 2.37-2.41 (m, 1H, 3-H$_b$); 2.49-2.55 (m, 2H, 4-H); 2.62-2.77 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.86-2.90 (m, 2H, 6'-H); 3.08-3.14 (m, 1H, 2'-H$_a$); 3.79-3.93 (m, 1H, 2'-H$_b$); 4.05-4.15 (dd, 1H, -C<u>H</u>$_2$-Ph); 4.20-4.27 (m, 1H, 2-H); 4.83 (s, 1H. NH); 5.62-5.72 (dd, 1H, -C<u>H</u>$_2$-Ph); 7.26-8.01 (m, 7H, Naph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 23.05, 28.28, 28,63, 28.84, 30.10, 30.25, 45.01, 56.26, 56.32, 124.25, 126.60, 126.84, 127.29, 127.56, 128.95, 129.08, 129.34, 123.28, 155.17, 167.56, 173.89; ESI MS (m/z): 466 $(M+H)^+$, 366 $(M-Boc)^+$, 410 $(M-t\_butyl)^+$, 488 $(M+Na)^+$; HRMS: (m/z) calc. for $C_{27}H_{35}N_3O_4$: 466.2628. Found: 466.2620

*4.1.3.14. tert-butyl ((R)-1-((S)-1-(naphthalen-2-ylmethyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**32g**]:* Same as 31*f*. Yield: 86 %; $[\alpha]_D^{27\,^0C}$: + 26.32 (c = 0.19; Chloroform); IR (KBr): 3343, 3049, 2930, 2859, 2364, 1685, 1655, 1514, 1481, 1259, 1169, 1023, 962 cm^{-1}; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.23 (m, 2H, 4'-H); 1.45 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.668 (m, 1H, 3'-H); 1.71-1.77 (m, 2H, 5'-H); 1.90-2.10 (m, 1H, 3-H$_a$); 2.38 (m, 1H, 3-H$_b$); 2.43-2.53 (m, 2H, 4-H); 2.64-2.75 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.83-2.98 (m, 2H, 6'-H); 3.32-3.48 (dd, 1H, 2'-H$_a$); 3.90 (m, 1H, 2'-H$_b$); 4.22-4.29 (m, 1H, 2-H); 4.22-4.29 (d, 1H, -C<u>H</u>$_2$-Naphthyl); 4.90 (s, 1H. NH); 5.64-5.71 (d, 1H, -C<u>H</u>$_2$-Naphthyl); 7.26-7.87 (m, 7H, Naphthyl-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.57, 22.96, 23.50, 27.42, 28.82, 35.50, 36.45, 42.30, 44.97, 47.40, 55.12, 78.31, 122.73, 124.11, 125.90, 126.73, 127.30, 127.95, 130.79, 132.76, 155.17, 167.87, 173.92; ESI MS (m/z): 488 $(M+Na)^+$, 466 $(M+H)^+$, 366 (M-Boc) ; HRMS: (m/z) calc. for $C_{27}H_{35}N_3O_4$: 466.2628. Found: 466.2605

*4.1.3.15. tert-butyl ((S)-1-((S)-1-(4-chlorobenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**31h**]:* Same as 31*f*. Yield: 80 %; $[\alpha]_D^{27^0C}$: + 12.00 (c = 0.30; Chloroform); IR (KBr): 3452, 3348, 3010, 2979, 2931, 2860, 2361, 1689, 1646, 1511, 1450, 1406, 1365, 1253, 1218, 1170, 1092, 1015, cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 1.21 (m, 2H, 4'-H); 1.44 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.60 (m, 1H, 3'-H); 1.67-1.90 (m, 2H, 5'-H); 2.13-2.17 (m, 1H, 3-H_a); 2.39-2.48 (m, 1H, 3-H_b); 2.52-2.61 (m, 2H, 4-H); 2.66-2.84 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.88-2.95 (m, 2H, 6'-H); 3.36-3.43 (m, 1H, 2'-H_a); 3.69-3.85 (m, 1H, 2'-H_b); 4.11-4.19 (m, 2H, 2-H & -C<u>H</u>$_2$-Ph); 4.90 (s, 1H. NH); 5.03-5.13 (d, 1H, -C<u>H</u>$_2$-Ph); 7.08-7.26 (dd, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.06, 21.08, 22.65, 24.61, 27.89, 28.28, 28.63, 29.74, 35.95, 38.33, 42.72, 43.11, 43.332, 45.06, 45.64, 49.27, 55.67, 55.84, 79.33, 128.42, 128.73, 129.35, 133.07, 137.33, 156.02, 168.81, 169.25, 175.16; FAB MS (m/z): 473 $(M+Na)^+$, 349 $(M\text{-}Boc)^+$; HRMS: (m/z) calc. for $C_{23}H_{32}ClN_3O_4$: 450.2081. Found: 450.2021

*4.1.3.16. tert-butyl ((R)-1-((S)-1-(4-chlorobenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**32h**]:* Same as 31*f*. Yield: 80 %; $[\alpha]_D^{27^0C}$: - 10.30 (c = 0.28; Chloroform); IR (KBr): 3372, 2929, 2858, 2361, 1684, 1520, 1455, 1406, 1365, 1254, 1172, 1015, 759, 666; ^1H NMR ($CDCl_3$; 300 MHz): δ 1.30 (m, 2H, 4'-H); 1.44 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.55-1.70 (m, 1H, 3'-H); 1.76-1.86 (m, 2H, 5'-H); 2.15-2.18 (m, 1H, 3-H_a); 2.30-2.38 (m, 1H, 3-H_b); 2.40-2.52 (m, 2H, 4-H); 2.55-2.75 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.80-2.99 (m, 2H, 6'-H); 3.33-3.51 (m, 1H, 2'-H_a); 3.73-3.80 (m, 1H, 2'-H_b); 4.12-4.18 (m, 2H, 2-H & -C<u>H</u>$_2$-Ph); 4.87 (s, 1H. NH); 5.03-5.11 (d, 1H, -C<u>H</u>$_2$-Ph); 7.10-7.12 (d, 2H, Ph-H); 7.25-7.28 (d, 2H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 22.74, 24.04, 28.37, 27.54, 36.05, 43.01, 44.71, 45.86, 56.03, 79.31, 128.83, 129.74, 133.48, 134.80, 156.08, 168.59, 175.27; FAB MS (m/z): 473 $(M+23)^+$, 349 $(M\text{-}Boc)^+$; HRMS: (m/z) calc. for $C_{23}H_{32}ClN_3O_4$: 450.2081. Found: 450.2067

*4.1.3.17. tert-butyl ((S)-1-((S)-1-(2-bromobenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**31i**]:* (2S)-N-(2-bromophenylmethyl)-pyroglutamic acid (0.10 g, 1 eq, 0.34 mmol), (3R)-N-Boc-3-aminomethyl-piperidine (0.072 g, 1 eq, 0.34 mmol) and DIPEA (0.117 ml, 2 eq, 0.67 mmol) were dissolved in dry DCM (25 ml) in a three necked round bottom flask fitted with N_2 inlet and rubber septa. The reaction mixture was cooled to 0^0C in an ice-salt bath and stirred for 10 min. A solution of PyBOP (0.175 g, 1 eq, 0.34 mmol) dissolved in dry DCM (10 ml) was added drop wise to the reaction mixture and stirring was continued for 3 hours at 0^0C.

The reaction mixture was then brought to room temperature and concentrated under reduced pressure. The residue was dissolved in ethyl acetate, washed successively with dil. citric acid (3 x 25 ml), dil. Na_2CO_3 (3 x 25 ml) and brine (1 x 25 ml). The organic layer was separated, dried and concentrated under reduced pressure. The crude material was flash chromatographed to get pure compound. Yield: 85 %; $[\alpha]_D^{27\,^0C}$: + 9.35 (c = 0.16; Chloroform); IR (KBr): 3453, 3013, 2930, 2361, 1688, 1648, 1510, 1444, 1366, 1252, 1217, 1170, 1027 cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 1.17-1.23 (m, 2H, 4'-H); 1.42 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.69 (m, 1H, 3'-H); 1.74-1.88 (m, 2H, 5'-H); 2.17-2.23 (m, 1H, 3-H_a); 2.37-2.44 (m, 1H, 3-H_b); 2.50-2.59 (m, 2H, 4-H); 2.63-2.77 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.89-2.91 (m, 2H, 6'-H); 3.12 (m, 1H, 2'-H_a); 3.37-3.52 (m, 1H, 2'-H_b); 4.09-4.28 (m, 2H, 2-H & -C<u>H</u>$_2$-Ph); 4.88 (s, 1H. NH); 5.07-5.14 (m, 1H, -C<u>H</u>$_2$-Ph); 7.13-7.20 (dd, H, Ph-H); 7.25-7.33 (m, 2H, Ph-H); 7.52-7.57 (d, 1H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 22.97, 24.49, 28.37, 29.37, 42.76, 45.69, 56.50, 79.26, 123.98, 127.81, 129.41, 132.88, 135.57, 156.09, 168.88, 175.38; ESI MS (m/z): 495 $(M+H)^+$, 496 $(M+2)^+$, 394 (M-Boc) ; HRMS: (m/z) calc. for $C_{23}H_{32}BrN_3O_4$: 495.1576. Found: 495.1580

*4.1.3.18. tert-butyl ((R)-1-((S)-1-(2-bromobenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**32i**]:* Same as 31*i*. Yield: 88 %; $[\alpha]_D^{27\,^0C}$: - 15.00 (c = 0.20; Chloroform); IR (KBr): 3367, 3008, 2978, 2933, 2361, 1689, 1647, 1252, 1445, 1365, 1254, 1171, 1027 cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 1.18-1.22 (m, 2H, 4'-H); 1.41 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.58-1.62 (m, 1H, 3'-H); 1.71-1.89 (m, 2H, 5'-H); 2.20-2.24 (m, 1H, 3-H_a); 2.38-2.41 (m, 1H, 3-H_b); 2.48-2.54 (m, 2H, 4-H); 2.64 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.87-2.97 (m, 2H, 6'-H); 3.10 (dd, 1H, 2'-H_a); 3.34-3.49 (m, 1H, 2'-H_b); 4.05-4.09 (m, 2H, 2-H & -C<u>H</u>$_2$-Ph); 4.92 (s, 1H. NH); 5.03-5.10 (m, 1H, -C<u>H</u>$_2$-Ph); 7.12-7.51 (dd, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 22.87, 23.21, 28.41, 29.42, 45.57, 56.45, 79.32, 84.23, 112.86, 124.02, 127.85, 129.47, 131.53, 132.91, 135.59, 156.14, 169.21, 175.54; ESI MS (m/z): 495 $(M+H)^+$, 496 $(M+2)^+$, 394 (M-Boc); HRMS: (m/z) calc. for $C_{23}H_{32}BrN_3O_4$: 495.1576. Found: 495.1556

*4.1.3.19. tert-butyl ((S)-1-((S)-1-(4-bromobenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**31j**]:* Same as 31*i*. Yield: 88 %; $[\alpha]_D^{27\,^0C}$: +13.33 (c = 0.15; Chloroform); IR (KBr): 3344, 2931, 2361, 1688, 1647, 1571, 1454, 1253, 1172 cm^{-1}; ^1H NMR ($CDCl_3$; 200 MHz): δ 1.16-1.21 (m, 2H, 4'-H); 1.40 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.65 (m, 1H, 3'-H); 1.76-1.82 (m, 2H, 5'-H); 2.13-2.17 (m, 1H, 3-H_a); 2.36-2.39 (m, 1H, 3-H_b); 2,49-2.55 (m, 2H, 4-H); 2.64-2.75 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.83-2.98 (m, 2H, 6'-H); 3.32-3.42 (m, 1H, 2'-H_a); 3.73-3.76 (m, 1H, 2'-

H_b); (m, 1H,); 4.14-4.16 (m, 2H, 2-H & -C<u>H</u>$_2$-Ph); 4.871 (s, 1H, NH); 5.133-5.138 (d, 1H, -C<u>H</u>$_2$-Ph); 6.95-7.10 (d, 2H, Ph-H); 7.40-7.45 (d, 2H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 22.83, 24.74, 28.38, 29.53, 42.88, 44.80, 45.88, 56.02, 79.39, 121.61, 130.11, 130.36, 131.82, 135.36, 156.08, 168.94, 175.25; ESI MS (m/z): 495 $(M+H)^+$, 394 (M-Boc) ; HRMS: (m/z) calc. for $C_{23}H_{32}BrN_3O_4$: 495.1576. Found: 495.1534

*4.1.3.20. tert-butyl ((R)-1-((S)-1-(4-bromobenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate **[32j]:*** Same as 31*i.* Yield: 77 %; $[\alpha]_D^{27\,^0C}$: - 11.00 (c = 0.36; Chloroform); IR (KBr): 3347, 2976, 2930, 2859, 2361, 1688, 1652, 1517, 1488, 1450, 1402, 1365, 1254, 1172, 1071, 1011, 964 cm^{-1}; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.25 (m, 2H, 4'-H); 1.44 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.65 (m, 1H, 3'-H); 1.65-1.82 (m, 2H, 5'-H); 2.07-2.17 (m, 1H, 3-H_a); 2.20-2.36 (m, 1H, 3-H_b); 2.36-2.49 (m, 2H, 4-H); 2.52-2.79 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.81-2.90 (m, 2H, 6'-H); 3.35-3.50 (m, 1H, 2'-H_a); 3.73-3.78 (m, 1H, 2'-H_b); 4.13-4.17 (m, 2H, 2-H & -C<u>H</u>$_2$-Ph); 4.87 (s, 1H. NH); 5.07-5.12 (m, 1H, -C<u>H</u>$_2$-Ph); 7.06-7.09 (d, 2H, Ph-H), 7.42-7.45 (d, 2H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 23.21, 24.20, 28.41, 29.42, 36.33, 45.75, 56.66, 79.32, 124.02, 127.85, 129.47, 131.53, 135.59, 156.14, 165.13, 175.38; ESI MS (m/z): 495 $(M+H)^+$, 496 $(M+2)^+$, 394 (M-Boc) ; HRMS: (m/z) calc. for $C_{23}H_{32}BrN_3O_4$: 495.1576. Found: 495.1565

*4.1.3.21. tert-butyl ((S)-1-((S)-1-(3,4-dichlorobenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate, **[31k]:*** (2S)-N-(3,4-dichloro-phenylmethyl)-pyroglutamic acid (100 mg, 1 eq, 0.30 mmol) and triethyl amine (0.10 ml, 2 eq, 0.60 mmol) were dissolved in dry THF (50 ml) in a three necked round bottom flask fitted with dry N_2 inlet and rubber septa. The reaction mixture was cooled to -20 ^{0}C and stirred at this temperature for 10 min. Isobutylchloroformate (0.045 ml, 1 eq, 0.3 mmol) was added drop wise to the reaction mixture at -20 ^{0}C and stirring was continued for 15 min. (3R)-N-Boc-3-aminomethyl-piperidine (75 mg, 1 eq, 0.30 mmol), dissolved in dry DCM (10 ml) was then added drop wise in to the reaction mixture in 10 min at -20 ^{0}C. The reaction mixture was stirred for one hour at 0^{0}C to room temperature. The reaction was quenched with NH_4Cl solution and evaporated under reduced pressure. The residue was dissolved in ethyl acetate and washed successively with dil. citric acid (3 x 25 ml), dil.$NaHCO_3$ (3 x 25 ml) and then with brine. The organic layer was separated, dried and concentrated under reduced pressure. The crude material thus obtained was purified by flash chromatography. Yield: 82 %; $[\alpha]_D^{27\,^0C}$: + 27.50 (c = 0.20; Chloroform); IR (KBr): 3451, 3009, 2933, 2360, 1689, 1647,

1513, 1463, 1252, 1171 cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 1.19-1.35 (m, 2H, 4'-H); 1.44 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.67 (m, 1H, 3'-H); 1.78-1.90 (m, 2H, 5'-H); 2.17-2.26 (m, 1H, 3-H_a); 2.35-2.40 (m, 1H, 3-H_b); 2.45-2.54 (m, 2H, 4-H); 2.65-2.78 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.78-3.91 (m, 2H, 6'-H); 3.39-3.53 (m, 1H, 2'-H_a); 3.73-3.86 (m, 1H, 2'-H_b); 4.13-4.21 (m, 2H, 2-H & C<u>H</u>$_2$-Ph); 4.90 (s, 1H. NH); 5.03-5.10 (d, 1H, -C<u>H</u>$_2$-Ph); 7.03-7.41 (m, 3H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 22.80, 22.96, 24.80, 28.39, 29.65, 36.52, 43.01, 44.43, 45.94, 48.76, 56.52, 79.34, 128.04, 130.75, 131.83, 132.70, 136.59, 156.10, 168.00, 175.32; ESI MS (m/z): 483 $(M)^+$, 428 (M-t_butyl), 383 $(M\text{-}Boc)^+$; HRMS: (m/z) calc. for $C_{23}H_{31}C_{l2}N_3O_4$: 483.1629. Found: 483.1646

*4.1.3.22. tert-butyl ((R)-1-((S)-1-(3,4-dichlorobenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**32k**]:* Same as 31*k*. Yield: 75 %; $[\alpha]_D^{27\,^oC}$: - 6.25 (c = 0.29; Chloroform); IR (KBr): 3385, 3007, 2978, 2933, 2860, 2361, 1692, 1645, 1513, 1439, 1365, 1254, 1171, 962 cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 1.21-1.26 (m, 2H, 4'-H); 1.42 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.67 (m, 1H, 3'-H); 1.72-1.88 (m, 2H, 5'-H); 2.20 (m, 1H, 3-H_a); 2.40 (m, 1H, 3-H_b); 2.51-2.55 (m, 2H, 4-H); 2.64-2.75 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.83-2.95 (m, 2H, 6'-H); 3.35-3.50 (dd, 1H, 2'-H_a); 3.77-3.82 (m, 1H, 2'-H_b); 4.10-4.14 (m, 2H, -C<u>H</u>$_2$-Ph & 2-H); 4.80 (s, 1H. NH); 5.06-5.10 (m, 1H, -C<u>H</u>$_2$-Ph); 7.03-7.38 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 22.82, 23.13, 24.0624.73, 27.89, 28.04, 28.26, 28.38, 29.38, 36.51, 43.09, 44.44, 45.91, 56.23, 79.45, 113.18, 114.71, 117.62, 119.25, 127.79, 128.06, 130.20, 130.72, 1341.79, 132.74, 168.80, 175.28; ESI MS (m/z): 483 $(M)^+$, 428 (M-t_butyl), 383 $(M\text{-}Boc)^+$; HRMS: (m/z) calc. for $C_{23}H_{31}C_{l2}N_3O_4$: 483.1629. Found: 483.1901

*4.1.3.23. tert-butyl ((S)-1-((S)-1-(2,6-dichlorobenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**31l**]:* Same as 31*k*. Yield: 85 %; $[\alpha]_D^{27\,^oC}$: + 51.65 (c = 0.480; Chloroform); IR (KBr): 3451, 3341, 3006, 2933, 2860, 2361, 1696, 1513, 1439, 1364, 1254, 1171, 961 cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 1.20 (m, 2H, 4'-H); 1.39 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.65 (m, 1H, 3'-H); 1.78-1.81 (m, 2H, 5'-H); 2.11-2.20 (m, 1H, 3-H_a); 2.27-2.36 (m, 1H, 3-H_b); 2.41-2.47 (m, 2H, 4-H); 2.47-2.61 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.89 (m, 2H, 6'-H); 3.03-3.09 (m, 1H, 2'-H_a); 3.36-3.47 (m, 1H, 2'-H_b); 3.98-4.01 (m, 1H, 2-H); 3.98-4.08 (dd, 1H, -C<u>H</u>$_2$-Ph); 4.82 (s, 1H. NH); 5.12-5.17 (d, 1H, -C<u>H</u>$_2$-Ph); 7.15-7.30 (m, 3H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 23.02, 24.24, 24.90, 28.34, 29.14, 36.60, 37.60, 40.71, 42.81, 43.25, 45.38, 48.79, 55.83, 79.06, 128.51,

129.86, 131.30, 136.65, 156.11, 168.45, 174.86; ESI MS (m/z): 506 $(M+Na)^+$, 428 (M-t_butyl), 384 $(M-Boc)^+$; HRMS: (m/z) calc. for $C_{23}H_{31}C_{l2}N_3O_4$: 483.1629. Found: 483.1606

*4.1.3.24. tert-butyl ((R)-1-((S)-1-(2,6-dichlorobenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin -3-yl)methylcarbamate [**32l**]*: Same as 31*k*. Yield: 80 %; $[\alpha]_D^{27\,^oC}$: + 34.20 (c = 0.50; Chloroform); IR (KBr): 3362, 3009, 2978, 2931, 2361, 1689, 1648, 1512, 1469, 1395, 1365, 1253, 1171, 1033 cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 1.21-1.26 (m, 2H, 4'-H); 1.39 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.60 (m, 1H, 3'-H); 1.76-1.85 (m, 2H, 5'-H); 2.11-2.15 (m, 1H, 3-H_a); 2.30-2.43 (m, 1H, 3-H_b); 2.53-2.69 (m, 2H, 4-H); 2.79-2.86 (m, 2H, -$C\underline{H}_2$-NH-Boc); 2.88-2.99 (m, 2H, 6'-H); 3.07-3.09 (dd, 1H, 2'-H_a); 3.34-3.47 (m, 1H, 2'-H_b); 3.99-4.04 (t, 1H, 2-H); 4.32-4.43 (m, 1H, -$C\underline{H}_2$-Ph); 4.91 (s, 1H. NH); 5.14-5.19 (d, 1H, -$C\underline{H}_2$-Ph); 7.15-7.28 (m, 3H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 22.82, 23.13, 24.06, 24.73, 27.89, 28.38, 29.38, 36.51, 43.09, 44.44, 45.91, 56.23, 79.45, 127.79, 130.20, 131.79, 132.74, 156.05, 168.80, 175.28; ESI MS (m/z): 343.9 $(M)^+$, 428 (M-t_butyl)$^+$, 384 $(M-Boc)^+$; HRMS: (m/z) calc. for $C_{23}H_{31}C_{l2}N_3O_4$: 483.1629. Found: 483.1634

*4.1.3.25. tert-butyl ((S)-1-((S)-1-(3-cyanobenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**31m**]:* (2S)-N-(3-cyanophenylmethyl)-pyroglutamic acid (0.50 gm, 1 eq, 1.9 mmol) and dry triethyl amine (0.79 ml, 3 eq, 5.7 mmol) were dissolved in dry DCM (50 ml) in an RBF. 2-chloro-1-methyl-pyridinium iodide (0.58 gm, 1.2 eq, 2.2 mmol) was added to it and the reaction mixture was refluxed for 2-3h during which a clear solution was obtained. The reaction mixture was cooled and (3R)-N-Boc-3-aminomethylpiperidine (0.42 gm, 1 eq, 1.9 mmol) dissolved in dry dichloromethane (20 ml) was added drop wise to it and again refluxed for 5-6 hrs. The reaction mixture was then brought to 25^{o}C and concentrated under reduced pressure. The concentrate was dissolved in ethyl acetate and was washed successively with dil. citric acid (3 X 50 ml), saturated sodium bicarbonate solution (3 X 50 ml) and brine (1 X 50 ml). The organic layer was separated, dried over an.Na_2SO_4 and concentrated in vacuum, purified by column chromatography. Yield: 76 %; $[\alpha]_D^{27\,^oC}$: + 12.70 (c = 0.26; Chloroform); IR (KBr): 3453, 3365, 3013, 2933, 2361, 2232, 1691, 1647, 1512, 1450, 1365, 1253, 1219, 1170, 965 cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 1.22 (m, 2H, 4'-H); 1.45 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.69 (m, 1H, 3'-H); 1.72-1.96 (m, 2H, 5'-H); 2.17-2.27 (m, 1H, 3-H_a); 2.32-2.36 (m, 1H, 3-H_b); 2.47-2.59 (m, 2H, 4-H); 2.64-2.78 (m, 2H, -$C\underline{H}_2$-NH-Boc); 2.83-2.93 (m, 2H, 6'-H); 3.39-3.49 (m, 1H, 2'-H_a); 3.81-3.95 (m, 1H, 2'-H_b); 4.14-4.25 (m, 2H, 2-H & -$C\underline{H}_2$-Ph); 4.83 (s, 1H. NH); 5.06-5.17 (d, 1H, -

C<u>H</u>$_2$-Ph); 7.46-7.55 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 22.91, 23.74, 27.93, 28.53, 29.65, 42,90, 45.89, 49.10, 56.57, 79.34, 111.52, 118.57, 128.85, 132.50, 142.12, 156.08, 168.66; 175.53; ESI MS (m/z): 441 (M+H), 463 $(M+Na)^+$; HRMS: (m/z) calc. for $C_{24}H_{32}N_4O_4$: 441.2424. Found: 441.2396

*4.1.3.26. tert-butyl ((R)-1-((S)-1-(3-cyanobenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**32m**]:* Same as 31*m*. Yield: 85 %; $[\alpha]_D^{27.9^\circ C}$: - 15.71 (c = 0.21 ; Chloroform); IR (KBr): 3453, 3014, 2980, 2933, 2361, 2232, 1690, 1647, 1512, 1447, 1365, 1253, 1218, 1170, 1040, 964, 853 cm^{-1}; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.22 (m, 2H, 4'-H); 1.41 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.66 (m, 1H, 3'-H); 1.81-1.88 (m, 2H, 5'-H); 2.22 (m, 1H, 3-H_a); 2.34-2.49 (m, 1H, 3-H_b); 2.51-2.54 (m, 2H, 4-H); 2.73 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.90-2.95 (m, 2H, 6'-H); 3.40-3.50 (m, 1H, 2'-H_a); 3.83-3.95 (m, 1H, 2'-H_b); 4.14-4.24 (m, 2H, 2-H & -C<u>H</u>$_2$-Ph); 4.87 (s, 1H. NH); 5.03-5.27 (m, 1H, -C<u>H</u>$_2$-Ph); 7.42-7.54 (dd, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 22.76, 24.61, 28.37, 29.63, 36.48, 43.03, 45.95, 48.57, 55.23, 56.18, 79.34, 113.05, 113.97, 120.58, 129.71, 137.61, 156.10, 159.89, 168.98, 175.27; ESI MS (m/z): 441 $(M+H)^+$, 463 $(M+Na)^+$, 341 $(M-Boc)^+$, 385 (M-t_butyl) $^+$; HRMS: (m/z) calc. for $C_{24}H_{32}N_4O_4$: 441.2424. Found: 441.2389

*4.1.3.27. tert-butyl ((S)-1-((S)-1-(4-cyanobenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**31n**]:* Same as 31*m*. Yield: 85%; $[\alpha]_D^{27.9^\circ C}$: + 16.47 (c = 0.17; Chloroform); IR (KBr): 3331, 2977, 2932, 2858, 2362, 2340, 2231, 1710, 1679, 1643, 1530, 1451, 1365, 1258, 1173, 1080, 962, 856, 730, 647, 558; ^{1}H NMR ($CDCl_3$; 600MHz): δ 1.25-1.33 (m, 2H, 4'-H); 1.45 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.65 (m, 1H, 3'-H); 1.83-1.93 (m, 2H, 5'-H); 2.28 (m, 1H, 3-H_a); 2.41-2.46 (m, 1H, 3-H_b); 2.52-2.55 (m, 2H, 4-H); 2.63-2.79 (m, 2H, -C<u>H</u>$_2$-NH-Boc); 2.83-2.98 (m, 2H, 6'-H); 3.39-3.57 (m, 1H, 2'-H_a); 3.88-3.98 (m, 1H, 2'-H_b); 4.16-4.45 (m, 2H, 2-H & -C<u>H</u>$_2$-Ph); 4.64 (s, 1H, NH); 5.11-5.23 (m, 1H, -C<u>H</u>$_2$-Ph); 7.28-7.36 (d, 2H, Ph-H); 7.62-7.64 (d, 2H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.06, 24.614, 27.02, 27.12, 28.03, 52.18, 55.84, 110.00, 127.51, 127.59, 127.73, 131.25, 140.83, 156.02, 168.81, 175.16; ESI MS (m/z): 463 $(M+Na)^+$, 341 (M-Boc), 385 (M-*t*_butyl) ; HRMS: (m/z) calc. for $C_{24}H_{32}N_4O_4$: 441.2424. Found: 441.2435

*4.1.3.28. tert-butyl ((R)-1-((S)-1-(4-cyanobenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylcarbamate [**32n**]* Same as 31*m*. Yield: 85 %; $[\alpha]_D^{27.9^\circ C}$: - 11.58 (c = 0.19; Chloroform); IR (KBr): 3365, 3007, 2931, 2860, 2361, 2229, 1691, 1645, 1512, 1450, 1413, 1365, 1254, 1172, 853 cm^{-1}; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.23 (m, 2H, 4'-H); 1.43 (s, 9H, -O-C(C<u>H</u>$_3$)$_3$); 1.65 (m,

1H, 3'-H); 1.80-1.96 (m, 2H, 5'-H); 2.25 (m, 1H, 3-H_a); 2.49 (m, 1H, 3-H_b); 2.51-2.58 (m, 2H, 4-H); 2.64-2.757 (m, 2H, -C$\underline{H}_2$-NH-Boc); 2.89-3.02 (m, 2H, 6'-H); 3.35-3.51 (m, 1H, 2'-H_a); 3.86-3.91 (m, 1H, 2'-H_b); 4.20-4.24 (m, 2H, 2-H & -C$\underline{H}_2$-Ph); 4.85 (s, 1H. NH); 5.13-5.20 (m, 1H, -C$\underline{H}_2$-Ph); 7.28-7.62 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 22.91, 23.19, 24.74, 27.79, 28.39, 29.65, 42.90, 45.22, 45.89, 56.57, 79.30, 111.52, 118.67, 128.790, 129.00, 132.50, 142.12, 156.08, 168.66, 175.53; ESI MS (m/z): 463 $(M+Na)^+$, 341 (M-Boc), 385 (M-t_butyl) ; HRMS: (m/z) calc. for $C_{24}H_{32}N_4O_4$: 441.2424. Found: 441.2402

*4.1.3.29 benzyl (S)-1-(((S)-1-((S)-1-(4-methylbenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylamino)-1-oxo-3-phenylpropan-2-ylcarbamate [**31o**]:* (L)-Cbz-Phenyl alanine (0.30 g, 1 eq, 1 mmol) and 1-hydroxy benzotrizole (0.203 g, 1.5 eq, 1.51 mmol) were dissolved in dry DCM (20 ml) in a three necked round bottom flask fitted with N_2 inlet. The reaction mixture was cooled to 0^0C in an ice-salt bath. DCC (0.206 g, 1.2 eq, 1.20 mmol) dissolved in dry DCM (5 ml) was added to it and stirred while being at 0^0C for 15 min. The Boc-deprotected amine (S)-5-((S)-3-(aminomethyl)piperidine-1-carbonyl)-1-(4-methylbenzyl) pyrrolidin-2-one (0.364 g, 1 eq, 1.12 mmol) dissolved in dry DCM (5 ml) was added drop wise to the reaction mixture and stirring was continued for 3 hrs at 0^0C. The reaction mixture was then brought to room temperature and concentrated under reduced pressure. The residue was dissolved in ethyl acetate, filtered and the filtrate was washed successively with dil.citric acid (3 x 20 ml), dil. sodium bicarbonate solution (3 x 10 ml) and brine (1 x 10 ml). The organic layer was separated, dried and concentrated under reduced pressure. The crude material was flash chromatographed to get pure compound. Yield: 75 %; $[\alpha]_D^{27.9^0C}$: + 16.17 (c = 0.22; Chloroform); IR (KBr): 3425, 3306, 3009, 2932, 2861, 1672, 1518, 1449, 1357, 1253, 1220, 1139, 1041, 965 cm^{-1}; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.27 (m, 2H, 4'-H); 1.44 (m, 1H, 3'-H); 1.63 (m, 2H, 5'-H); 2.15-2.19 (m, 1H, 3-H_a); 2.34 (s, 3H, Ph-C$\underline{H}$3), 2.36 (m, 1H, 3-H_b); 2.42-2.53 (m, 2H, 4-H); 2.89 (m, 2H, 6'-H); 3.00 (m, 1H, 2'-H_a); 3.03-3.12 (d 2H, CH-C$\underline{H}_2$Ph); 3.24-3.28 (m, 2H, -C$\underline{H}_2$-NH-Boc); 3.67 (m, 1H, 2'-H_b); 3.75-3.80 (d, 1H, -C$\underline{H}_2$-Ph); 4.17-4.20 (t, 1H, 2-H); 4.42 (t, 1H, C$\underline{H}$CH_2Ph); 5.06-5.09 (d, 1H, C$\underline{H}_2$-Ph); 5.12 (s, 2H, C$\underline{H}_2$-Cbz group); 7.08-7.33 (m, 9H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.10, 23.02, 24.29, 28.32, 29.76, 35.25, 37.19, 41.02, 45.11, 55.87, 66.87, 126.89, 128.49, 129.34, 132.95, 136.66, 137.47, 169.34, 171.30, 175.27; HRMS: (m/z) calc. for $C_{36}H_{42}N_4O_5$: 611.3155. Found: 611.3181

*4.1.3.30 benzyl (S)-4-methyl-1-(((S)-1-((S)-1-(4-methylbenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylamino)-1-oxopentan-2-ylcarbamate [**31p**]:* Same as 31*o*. Yield: 78 %; $[\alpha]_D^{27\,^0C}$: + 8.05 (c = 0.40; Chloroform); IR (KBr): 3322, 2954, 2868, 2343, 1664, 1525, 1450, 1361, 1255, 1361, 1225, 1129, 1044, 965 cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 0.95 (d, 6H, $CHCH_2CH(C\underline{H}_3)_2$); 1.27 (m, 2H, 4'-H); 1.35 (m, 1H, $CHCH_2C\underline{H}(CH_3)_2$); 1.46 (m, 1H, 3'-H); 1.57 (m, 2H, 5'-H); 1.74 (m, 2H, $CHC\underline{H}_2CH(CH_3)_2$); 2.18 (m, 1H, 3-$H_a$); 2.34 (s, 3H, Ph-$C\underline{H}_3$), 2.36 (m, 1H, 3-$H_b$); 2.42 (m, 2H, 4-H); 2.53 (m, m, 2H, -$C\underline{H}_2$-NH-Boc); 2.98 (m, 2H, 6'-H); 3.03 (m, 1H, 2'-H_a); 3.43 (m, 1H, 2'-H_b); 3.71-3.77 (d, 1H, CH<u>H</u>-Ph) 4.17-4.20 (t, 1H, 2-H); 4.21 (m, 1H, 2H); 4.45 (m, 1H, $C\underline{H}CH_2CH(CH_3)_2$); 5.08-5.16 (d, 1H, CH<u>H</u>-Ph); 5.12 (s, 2H, $C\underline{H}_2$-Cbz group); 7.06-7.14 (m, 4H, Ph-H); 7.35 (s, 5H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.12, 23.03, 24.77, 27.91, 28.33, 28.42, 29.78, 35.53, 41.38, 43.17, 45.12, 45.88, 53.82, 55.95, 66.95, 127.78, 128.00, 128.39, 128.55, 129.44, 132.86, 133.01, 136.34, 137.37, 156.39, 169.32, 173.06, 175.50; HRMS: (m/z) calc. for $C_{33}H_{44}N_4O_5$: 577.3312. Found: 577.3329

*4.1.3.31. benzyl (S)-3-methyl-1-(((S)-1-((S)-1-(4-methylbenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methylamino)-1-oxobutan-2-ylcarbamate, [**31q**]:* Same as 31*o*. Yield: 80 %; $[\alpha]_D^{27\,^0C}$: + 13.32 (c = 0.26; $CHCl_3$); IR (KBr): 3319, 3013, 2966, 2872, 1672, 1513, 1457, 1359, 1219, 1135, 1028, cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 0.90-0.99 (d, 6H, $CHCH(C\underline{H}_3)_2$); 1.20 (m, 2H, 4'-H); 1.45 (m, 1H, 3'-H); 1.77 (m, 2H, 5'-H); 1.77 (m, 2H, $CHC\underline{H}(CH_3)_2$); 2.18 (m, 1H, 3-H_a); 2.33 (s, 3H, Ph-$C\underline{H}_3$), 2.36 (m, 1H, 3-H_b); 2.44 (m, 2H, 4-H); 2.55 (m, , 2H, -$C\underline{H}_2$-NH); 2.96 (m, 2H, 6'-H); 3.00 (m, 1H, 2'-H_a); 3.44 (m, 1H, 2'-H_b); 3.71-3.76 (d, 1H, CH<u>H</u>-Ph); 4.05 (t, 1H, 2-H); 4.22 (m, 1H, $C\underline{H}CH(CH_3)_2$); 5.08-5.14 (d, 1H, CH<u>H</u>-Ph); 5.11 (s, 2H, $C\underline{H}_2$-Cbz); 7.07-7.11 (m, 4H, Ph-H); 7.34 (s, 5H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 17.76, 19.28, 21.08, 22.63, 23.00, 24.50, 27.91, 28.39, 29.74, 30.98, 35.40, 37.22, 41.39, 43.13, 45.14, 49.16, 55.96, 66.94, 127.94, 128.06, 128.47, 129.42, 132.74, 136.30, 137.52, 156.59, 169.39, 172.02, 175.58; HRMS: (m/z) calc. for $C_{32}H_{42}N_4O_5$: 563.3155. Found: 563.3124

*4.1.3.32. 4-methyl-N-(((S)-1-((S)-1-(4-methylbenzyl)-5-oxopyrrolidine-2-carbonyl) piperidine-3-yl)methyl)benzamide [**31r**]:* (S)-5-((S)-3-(aminomethyl)piperidine-1-carbonyl)-1-(4-methylbenzyl) pyrrolidin-2-one (0.445 g, 1 eq, 1.35 mmol) was dissolved in dry DCM (10 ml) and the reaction mixture was cooled to 0^0C and TEA (0.50 ml, 1 eq, 3.10 mmol) was added. Stirring was continued for 15 min at this temperature. 4-methylbenzoyl chloride (0. g, 1.1 eq,

1.48 mmol) dissolved in dry DCM (5 ml) was added and stirred at room temperature for 3 hrs. The reaction mixture was then washed with 1N HCl, saturated (1 x 20 ml) aq. $NaHCO_3$ (1 x 20 ml) and brine. The organic layer was separated, dried over Na_2SO_4, filtered and concentrated. The compound was further purified by flash chromatography. Yield: 75 %; $[\alpha]_D^{27\,^0C}$: + 36.45 (c = 0.34; Chloroform); IR (KBr): 3345, 3007, 2929, 2860, 1679, 1646, 1543, 1505, 1447, 1357, 1285, 1253, 1218, 1133, 1024, 965 cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 1.34-1.38 (m, 2H, 4'-H); 1.62 (); 1.87-1.92 (m, 1H, 3'-H & m, 2H, 5'-H); 2.10-2.20 (m, 2H, 3-H_a, 3-H_b); 2.34 (s, 3H, Ph-C$\underline{H}_3$), 2.41 (s, 3H, Ph-C$\underline{H}_3$); 2.52-2.58 (m, 2H, 4-H); 3.08-3.12 (m, 2H, 6'-H) 2.833-2.984 (m, 2H, -C$\underline{H}_2$-NH-Boc); 3.40 (m, 1H, 2'-H_a) 3.63-3.67 (m, 1H, 2'-H_b); 3.73-3.78 (d, 1H, -C$\underline{H}_2$-Ph); 4.20-4.24 (m, 1H, 2-H); 5.13-5.18 (d, 1H, -C$\underline{H}_2$-Ph); 7.07-7.15 (m, 4H, Ph-H); 7.24-7.28 (m, 2H, Ph-H); 7.73-7.76 (m, 2H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.28, 22.81, 24.38, 28.01, 29.78, 35.83, 37.42, 41.86, 42.56, 42.93, 45.10, 46.07, 48.87, 56.05, 127.12, 128.55, 129.11, 131.53, 132.78, 137.51, 141.74, 167.89, 169.30, 175.40; HRMS: (m/z) calc. for $C_{27}H_{33}N_3O_3$: 448.2522. Found: 448.2510

*4.1.3.33. 4-methyl-N-(((R)-1-((S)-1-(4-methylbenzyl)-5-oxopyrrolidine-2-carbonyl) piperidin-3-yl)methyl)benzenesulfonamide [**31s**]:* Same as 31*r*. Yield: 72%; $[\alpha]_D^{27\,^0C}$: + 38.82 (c = 0.24; Chloroform); IR (KBr): 3183, 3011, 2928, 2862, 1672, 1514, 1451, 1328, 1286, 1255, 1159, 1095, 962, 911, 815 cm^{-1}; ^1H NMR ($CDCl_3$; 300MHz): δ 1.42 (m, 2H, 4'-H); 1.62 (); 1.72-1.81 (m, 1H, 3'-H & m, 2H, 5'-H); 2.17-2.21 (m, 2H, 3-H_a, 3-H_b); 2.33 (s, 3H, Ph-C$\underline{H}_3$), 2.38 (s, 3H, Ph-C$\underline{H}_3$); 2.55-2.66 (m, 2H, 4-H); 2.73-3.75 (m, 2H, -C$\underline{H}_2$-NH-Boc); 2.95-3.09 (m, 2H, 6'-H) 3.20-3.23 (m, 1H, 2'-H_a) 3.51-3.56 (m, 1H, 2'-H_b); 3.75-3.79 (d, 1H, -C$\underline{H}_2$-Ph); 4.15-4.26 (m, 1H, 2-H); 5.13-5.17 (d, 1H, -C$\underline{H}_2$-Ph); 6.92-7.80 (m, 8H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.09, 22.81, 24.38, 28.01, 29.78, 35.83, 37.42, 41.86, 42.56, 42.93, 45.10, 46.07, 48.87, 56.05, 126.99, 128.34, 129.41, 129.78, 132.80, 137.48, 169.43, 175.57; HRMS: (m/z) calc. for $C_{26}H_{33}N_3O_4S$: 484.2192. Found: 484.2177

*4.1.3.34. tert-butyl((S)-1-((S)-3-(4-methylbenzyl)-2-oxooxazolidine-4-carbonyl)piperidin-3-yl)methyl carbamate (**33a**):* (S)-3-(4-methylbenzyl)-2-oxooxazolidine-4-carboxylic acid (0.20 g, 1 eq, 0.85 mmol) and HOBt (0.162 g, 1.5 eq, 1.27 mmol) were dissolved in dry DCM followed by the addition of DCC (0.198 g, 1.2 eq, 1.02 mmol) and (3S)-N-(t-butoxycarbonyl)-3-aminomethyl-piperidine (0.171 g, 1 eq, 0.85 mmol). Yield: 75 %; $[\alpha]_D^{27\,^0C}$: - 24.78 (*c* = 0.16;

Chloroform); IR (KBr): 3348, 2932, 2734, 2367, 1758, 1654, 1524, 1440, 1366, 1253, 1173, 1050 cm^{-1} ; ^1H NMR ($CDCl_3$; 300MHz): δ 1.28 (m, 2H, 4'-H_a, H_b); 1.47 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.64 (m, 1H, 3'-H); 1.73-1.77 (m, 2H, 5'-H); 2.36 (s, 3H, Ph-C$\underline{H}$3); 2.64-2.80 (m, 2H, -$C\underline{H}_2$-NH-Boc); 2.91-2.93 (m, 2H, 6'-H); 3.14-3.26 (m, 2H, 2'-H_a, H_b); 4.05 (m, 1H, 5-H_a); 4.12 (m, 1H, -$C\underline{H}_2$-Ph); 4.22 (m, 1H, 5-H_a); 4.33 (m, 1H, 5-H_a); 4.39(m, 1H, 4-H); 4.81 (s, 1H. NH); 4.93-4.98 (d, 1H, -$C\underline{H}_2$-Ph); 7.15-7.14 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.08, 24.41, 24.81, 27.80, 28.39, 36.08, 38.01, 42.78, 43.32, 45.89, 46.73, 49.12, 53.49, 64.70, 79.28, 128.41, 129.51, 132.19, 137.83, 156.14, 158.02, 166.54; HRMS: (m/z) calc. for $C_{23}H_{33}N_3O_5$: 432.2420. Found: 432.2405

*4.1.3.35. tert-butyl ((R)-1-((S)-3-(4-methylbenzyl)-2-oxooxazolidine-4-carbonyl) piperidin-3-yl)methyl carbamate, (**34a**):* Same as 33*a*. Yield: 78 %; $[\alpha]_D^{27\,^0C}$: - 58.32 (*c* = 0.32; Chloroform); IR (KBr): 3356, 3931, 2362, 1761, 1652, 1445, 1365, 1253, 1171, 1077 cm^{-1} ; ^1H NMR ($CDCl_3$; 300MHz): δ 1.22-127 (m, 2H, 4'-H_a, H_b); 1.47 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.65 (m, 1H, 3'-H); 1.82 (m, 2H, 5'-H); 2.36 (s, 3H, Ph-C$\underline{H}$3); 2.67-2.77 (m, 2H, -$C\underline{H}_2$-NH-Boc); 2.94-2.99 (m, 2H, 6'-H); 3.13-3.34 (m, 1H, 2'-H_a, H_b); 4.10 (m, 1H, -$C\underline{H}_2$-Ph); 4.05 (m, 1H, 5-H_a); 4.12; 4.33 (m, 1H, 5-H_a); 4.39 (m, 1H, 4-H); 4.80 (s, 1H. NH); 4.93-4.98 (d, 1H, -$C\underline{H}_2$-Ph); 7.15-7.14 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.07, 23.95, 24.77, 27.99, 28.38, 36.43, 37.99, 43.10, 45.85, 46.19, 46.19, 48.68, 53.96, 48.68, 53.96, 64.50, 79.40, 128.41, 128.51, 132.09, 137.80, 156.13, 157.98, 166.54; FABMS m/z : 432 $[M+1]^+$, 454 $[M+Na]^+$, 332 $[M\text{-}Boc]^+$; HRMS: (m/z) calc. for $C_{23}H_{33}N_3O_5$: 432.2420. Found: 432.2409

*4.1.3.36. tert-butyl ((S)-1-((R)-3-(4-methylbenzyl)-2-oxothiazolidine-4-carbonyl) piperidin-3-yl)methyl carbamate, (**33b**):* Same as 33*a*. Yield: 75 %; $[\alpha]_D^{27\,^0C}$: - 56.45 (*c* = 0.148; Chloroform); MP: 90-92 ^0C; IR (KBr): 3350, 2931, 2861, 2367, 1673, 1516, 1448, 1392, 1364, 1249, 1173, 1003, 962 cm^{-1} ; ^1H NMR ($CDCl_3$; 300MHz): δ 1.27-1.35 (m, 2H, 4'-H_a, H_b); 1.46 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.71 (m, 1H, 3'-H); 1.78 (m, 2H, 5'-H); 2.35 (s, 3H, Ph-C$\underline{H}_3$); 2.58-2.63 (m, 1H, -$C\underline{H}_2$-NH-Boc); 2.85 (m, 1H, -$C\underline{H}_2$-NH-Boc), 2.92-2.95 (m, 2H, 6'-H); 3.12-3.17 (m, 1H, 2'-H_a, H_b); 3.37-3.41 (m, 2H, 5-H_a, H_b); 3.78-3.93 (dd, 1H, -$C\underline{H}_2$-Ph); 4.40-4.44 (t, 1H, 4-H); 4.87 (s, 1H. NH); 5.14-5.19 (d, 1H, -$C\underline{H}_2$-Ph); 7.12-7.16 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.09, 24.60, 27.78, 28.31, 28.40, 28.51, 28.65, 30.65, 38.34, 42.74, 43.39, 45.97, 47.39, 49.41,

56.96, 128.44, 129.50, 132.52, 137.77, 156.13, 166.39, 166.90, 172.10; HRMS: (m/z) calc. for $C_{23}H_{33}N_3O_4S$: 448.2192. Found: 448.2212

*4.1.3.37. tert-butyl ((R)-1-((R)-3-(4-methylbenzyl)-2-oxothiazolidine-4-carbonyl) piperidin-3-yl)methylcarbamate, (**34b**):* Same as 33*a*. Yield: 78 %; $[\alpha]_D^{27\,^0C}$: - 111.98 (*c* = 0.096; Chloroform); MP: 135-140 ^{0}C; IR (KBr): 3350, 3007, 3932, 2862, 2368, 1681, 1516, 1449, 1393, 1365, 1249, 1175, 1003, 964 cm^{-1} ; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.27 (m, 2H, 4'-H_a, H_b); 1.47 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.73 (m, 1H, 3'-H); 1.81-1.83 (m, 2H, 5'-H); 2.35 (s, 3H, Ph-$C\underline{H}_3$); 2.67-2.74 (m, 2H, -$C\underline{H}_2$-NH-Boc); 2.97 (m, 2H, 6'-H); 3.10-3.14 (m, 1H, 2'-H_a, H_b); 3.36-3.40 (m, 2H, 5-H_a, H_b); 3.84-3.89 (d, 1H, -$C\underline{H}_2$-Ph); 4.38-4.40 (t, 1H, 4-H); 4.82 (s, 1H. NH); 5.14-5.19 (d, 1H, -$C\underline{H}_2$-Ph); 7.12-7.14 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.11, 23.94, 24.76, 24.94, 28.07, 28.32, 28.39, 36.50, 37.64, 43.20, 46.01, 46.30, 47.31, 47.41, 48.66, 57.17, 126.75, 129.50, 132.42, 123.66, 137.85, 156.13, 166.48, 172.13 ; FABMS m/z : 448 $[M+1]^+$, 347 $[M\text{-Boc}]^+$; HRMS: (m/z) calc. for $C_{23}H_{33}N_3O_4S$: 448.2192. Found: 448.2185

*4.1.3.38. tert-butyl ((S)-1-((R)-3-(4-methylbenzyl)thiazolidine-4-carbonyl) piperidin-3-yl) methylcarbamate, (**33c**):* Same as 33*a*. Yield: 75 %; $[\alpha]_D^{27\,^0C}$: + 26.02 (*c* = 0.26; Chloroform); IR (KBr): 3453, 3368, 3010, 2932, 2860, 2373, 1703, 1635, 1512, 1449, 1367, 1249, 1219, 1169, 1043, 964 cm^{-1} ; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.27 (m, 2H, 4'-H_a, H_b); 1.44 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.70 (m, 1H, 3'-H); 1.74-1.77 (m, 2H, 5'-H); 2.36 (s, 3H, Ph-$C\underline{H}_3$); 2.74-2.88 (m, 2H, -$C\underline{H}_2$-NH-Boc); 3.04.08 (m, 1H, 5-H_a); 3.14-3.36 (m, 1H, 2'-H_a); 3.25 (m, 2H, 6'-H); 3.33-3.36 (m, 1H, 5H_b); 3.56 (d, 1H, -$C\underline{H}_2$-Ph); 3.56-3.59 (m, 1H, 2'-Ha); 3.59 (m, 1H, 2-H_a); 4.03-4.05 (m, 1H, 2-H_b); 4.07 (d, 1H, -$C\underline{H}_2$-Ph); 4.10 (m, 1H, 4-H); 4.89 (s, 1H. NH); 7.17-7.24 (dd, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.15, 24.41, 28.43, 31.03, 35.93, 37.69, 43.00, 45.64, 46.92, 49.43, 54.81, 56.02, 58.48, 68.73, 69.34, 128.76, 129.28, 134.73, 137.44, 156.03, 167.32; FABMS m/z : 456 $[M+23]^+$, 434 $[M+1]^+$, 378 [M-*t*_butyl], 333 $[M\text{-Boc}]^+$; HRMS: (m/z) calc. for $C_{23}H_{35}N_3O_3S$: 434.2399. Found: 434.2373

*4.1.3.39. tert-butyl ((R)-1-((R)-3-(4-methylbenzyl)thiazolidine-4-carbonyl) piperidine -3-yl)methylcarbamate, (**34c**):* (R)-3-(4-methylbenzyl)thiazolidine-4-carboxylic acid (0.237 g, 1 eq, 1 mmol) and HOBt (0.202 g, 1.5 eq, 1.5 mmol) were dissolved in dry DCM followed by the addition of DCC (0.248 g, 1.2 eq, 1.2 mmol) and (3S)-N-Boc-3-aminomethyl-piperidine (0.214

g, 1 eq, 1 mmol). Yield: 75 %; $[\alpha]_D^{27\,^0C}$: + 7.44 (*c* = 0.298; Chloroform); IR (KBr): 3346, 2932, 2858, 2369, 1902, 1703, 1640, 1516, 1449, 1366, 1251, 1171, 1042, 1006, 963 cm^{-1} ; ^1H NMR ($CDCl_3$; 300MHz): δ 1.27 (m, 2H, 4'-H_a, H_b); 1.44 (s, 9H, -O-C(C$\underline{H}_3$)$_3$); 1.66 (m, 1H, 3'-H); 1.82 (m, 2H, 5'-H); 2.36 (s, 3H, Ph-C$\underline{H}_3$); 2.72-2.85 (m, 2H, -C$\underline{H}_2$-NH-Boc); 3.02-3.05 (m, 1H, 5-H_a); 3.08 (m, 1H, 5H_b); 3.14 (m, 1H, 2'-H_a); 3.18 (m, 2H, 6'-H); 3.56 (d, 1H, -C$\underline{H}_2$-Ph); 3.56-3.58 (m, 1H, 2'-Ha); 3.61-3.62 (m, 1H, 2-H_a); 4.04-4.06 (m, 1H, 2-H_b); 4.09 (d, 1H, -C$\underline{H}_2$-Ph); 4.11 (m, 1H, 4-H); 4.78 (s, 1H. NH); 7.17-7.24 (dd, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.11, 24.29, 25.09, 28.38, 30.96, 33.95, 36.67, 37.86, 43.07, 46.07, 49.67, 55.79, 58.40, 68.96, 79.15, 129.16, 134.63, 137.36, 156.07, 167.47; FABMS m/z : 434 $[M+1]^+$, 333 $[M\text{-Boc}]^+$; HRMS: (m/z) calc. for $C_{23}H_{35}N_3O_3S$: 434.2399. Found: 434.2382

*4.1.3.40. tert-butyl ((S)-1-((R)-3-(4-methylbenzoyl)thiazolidine-4-carbonyl) piperidin-3-yl)methylcarbamate (**33d**):* Same as above. Yield: 75 %; $[\alpha]_D^{27\,^0C}$: - 158.14 (*c* = 0.12; Chloroform); IR (KBr): 3345, 2929, 2859, 2367, 1745, 1705, 1638, 1569, 1513, 1443, 1397, 1250, 1174, 1020, 960 cm^{-1} ; ^1H NMR ($CDCl_3$; 300MHz): δ 1.27 (m, 2H, 4'-H_a, H_b); 1.44 (s, 9H, -O-C(C$\underline{H}_3$)$_3$); 1.70 (m, 1H, 3'-H); 1.81 (m, 2H, 5'-H); 2.40 (s, 3H, Ph-C$\underline{H}_3$); 2.95-3.07 (m, 2H, -C$\underline{H}_2$-NH-Boc); 3.14-3.17 (m, 2H, 5-H_a, 5H_b); 3.17-3.24 (m, 2H, 6'-H); 3.63-3.66 (m, 2H, 2'-H); 4.47-4.52 (m, 2H, 2-H); 4.66-4.78 (m, 1H, 4-H); 7.22-7.48 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.43, 24.58, 27.73, 28.81, 29.64, 33.62, 35.54, 37.97, 42.07, 43.62, 45.75, 48.76, 50.39, 52.81, 58.33, 79.03, 127.35, 129.15, 132.38, 141.08, 156.16, 168.56, 169.25, 170.48; FABMS m/z : 448 $[M+1]^+$, 347 $[M\text{-Boc}]^+$; HRMS: (m/z) calc. for $C_{23}H_{33}N_3O_4S$: 448.2192. Found: 448.2181

*4.1.3.41. tert-butyl ((R)-1-((R)-3-(4-methylbenzoyl)thiazolidine-4-carbonyl) piperidin-3-yl)methylcarbamate (**34d**):* Same as above. Yield: 68 %; $[\alpha]_D^{27\,^0C}$: - 134.75 (*c* = 0.23; Chloroform); IR (KBr): 3344, 3004, 2933, 22862, 2460, 2366, 1695, 1636, 1524, 1396, 1224, 1175, 1025, 960 cm^{-1} ; ^1H NMR ($CDCl_3$; 300MHz): δ 1.27 (m, 2H, 4'-H_a, H_b); 1.44 (s, 9H, -O-C(C$\underline{H}_3$)$_3$); 1.72 (m, 1H, 3'-H); 1.80 (m, 2H, 5'-H); 2.40 (s, 3H, Ph-C$\underline{H}_3$); 2.95-3.10 (m, 2H, -C$\underline{H}_2$-NH-Boc); 3.14-3.19 (m, 2H, 5-H_a,H_b); 3.31-3.48 (m, 2H, 6'-H); 3.60-3.64 (m, 2H, 2'-H); 4.64-4.77 (m, 2H, 2-H); 4.80 (m, 1H, 4-H); 7.22-7.49 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 21.39, 24.39, 28.37, 29.59, 33.80, 36.12, 43.25, 46.44, 46.90, 48.51, 53.01, 58.29, 79.08, 127.61, 129.01,

132,33, 141.07, 156.10, 168.47, 169.23; FABMS m/z : 448 $[M+1]^+$, 347 $[M\text{-}Boc]^+$; HRMS: (m/z) calc. for $C_{23}H_{33}N_3O_4S$: 448.2192. Found: 448.2188

*4.1.3.42. tert-butyl((S)-1-(((S)-1-(4-methylbenzyl)-6-oxopiperidin-2-yl)methyl) piperid-in-3-yl) methyl carbamate [**35a**]:* (Same as *31o*). Yield: 80 %; $[\alpha]_D^{27\,^0C}$: +26. 90 (c = 0.05; Chloroform); MP: 150-152 ^{0}C; IR (KBr): 3754, 3675, 3454, 3350, 3011, 2928, 2858, 1703, 1646, 1512, 1448, 1365, 1247, 1218, 1171, 910 cm^{-1}; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.27 (m, 2H, 4'-H); 1.46 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.62-1.64 (m, 1H, 3'-H); 1.70 (m, 2H, 5'-H); 1.81 (m, 2H, 4'-H); 1.90 (m, 1H, 3-H_a); 2.05 (m, 1H, 3-H_b); 2.34 (s, 3H, -CH_3); 2.48-2.58 (m, 2H, CH_2-NH-); 2.85-2.88 (m, 1H, 6'-H); 3.02 (m, 1H; 2'-H_a); 3.17 (m, 1H; 2'-H_b); 3.42-3.49 (m, 2H, 5-H); 3.96-4.16 (m, 1H, -CH_2-Ph); 4.25 (t, 1H, 2-H); 4.85 (bs, 1H, NH); 5.56-5.60 (m, 1H, -CH_2-Ph); 7.11 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 14.14, 18.10, 21.13, 26.40, 28.41, 29.70, 31.69, 31.93, 33.83, 46.02, 48.29, 55.08, 80.00, 104.00, 113.12, 128.29, 128.49, 128.61, 128.70, 129.33, 137.23, 156.13, 171.10; FAB MS (m/z): 444 $(M+H)^+$; HRMS: (m/z) calc. for $C_{25}H_{37}N_3O_4$: 444.2784. Found: 444.2799

*4.1.3.43. tert-butyl((R)-1-(((S)-1-(4-methylbenzyl)-6-oxopiperidin-2-yl) methyl) piperidin-3-yl) methyl carbamate (**36a**):* Same as *31o*. Yield: 85 %; $[\alpha]_D^{27\,^0C}$: +29.93 (c = 0.04; Chloroform); MP: 198-200 ^{0}C; IR (KBr): 3455, 3017, 2401, 1705, 1647, 1511, 1447, 1364, 1216, 1170, 961 cm^{-1}; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.27-1.30 (m, 2H, 4'-H); 1.46 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.62-1.64 (m, 1H, 3'-H); 1.73 (m, 2H, 5'-H); 1.81 (m, 2H, 4'-H); 1.87 (m, 1H, 3-H_a); 2.06 (m, 1H, 3-H_b); 2.35 (s, 3H, -CH_3); 2.48-2.64 (m, 2H, CH_2-NH-); 2.86 (m, 1H, 6'-H); 3.02 (m, 1H; 2'-H_a); 3.15-3.18 (m, 1H; 2'-H_b); 3.41-3.46 (m, 2H, 5-H); 4.12-4.15 (m, 1H, -CH_2-Ph); 4.24 (t, 1H, 2-H); 4.85 (bs, 1H, NH); 5.57-5.62 (m, 1H, -CH_2-Ph); 7.11 (m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 14.14, 18.10, 21.13, 26.40, 28.41, 29.70, 31.69, 31.93, 33.83, 46.02, 48.29, 55.08, 80.00, 104.00, 113.12, 128.29, 128.49, 128.61, 128.70, 129.33, 137.23, 156.13, 171.10; FAB MS (m/z): 444 $(M+H)^+$; HRMS: (m/z) calc. for $C_{25}H_{37}N_3O_4$: 444.2784. Found: 444.2774

*4.1.3.44. tert-butyl ((S)-1-((S)-4-(4-methylbenzyl)-5-oxothiomorpholine-3-carbonyl) piperidin-3-yl)methylcarbamate, (**35b**):* Same as *31o*. Yield: 68 %; $[\alpha]_D^{27\,^0C}$: + 12.60 (*c* = 0.052; Chloroform); IR (KBr): 3451, 3012, 2929, 2860, 2361, 1702, 1650, 1511, 1453, 1363, 1218, 1169, 1028 cm^{-1}; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.26 (m, 2H, 4'-H); 1.45 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.60 (m, 1H, 3'-H); 1.75 (m, 2H, 5'-H); 2.34 (s, 3H, C$\underline{H}_3$); 2.65 (m, 2H, -C$\underline{H}$2-NH-Boc); 2.80 (m, 2H, 3-H); 3.00 (m, 1H, 2'-Ha); 3.05 (m, 1H, 2'-Hb); 3.50 (s, 2H, 5-H); 4.52 (d, 2H -C$\underline{H}_2$-Ph); 5.52 (m, 1H, 2-H);

7.11-7.13(m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 14.08, 21.09, 28.38, 29.67, 30.44, 46.30, 49.32, 57.24, 128.52, 129.45, 133.14, 156.10, 167.02; FABMS m/z : 462 $[M+1]^+$; HRMS: (m/z) calc. for $C_{24}H_{35}N_3O_4S$: 462.2348. Found: 462.2332

*4.1.3.45. tert-butyl ((S)-1-((R)-4-(4-methylbenzyl)-5-oxothiomorpholine-3-carbonyl) piperidin-3-yl)methylcarbamate, (**36b**):* Same as *31o*. Yield: 65 %; $[\alpha]_D^{27\,^0C}$: - 7.07 (*c* = 0.082; $CHCl_3$); IR (KBr): 3451, 3016, 2933, 2864, 2361, 1703, 1649, 1511, 1449, 1365, 1217, 1169, 1026 cm^{-1}; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.26 (m, 2H, 4'-H); 1.44 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.60 (m, 1H, 3'-H); 1.75 (m, 2H, 5'-H); 2.34 (s, 3H, $C\underline{H}_3$); 2.65 (m, 2H, -C<u>H</u>2-NH-Boc); 2.80 (m, 2H, 3-H); 3.00 (m, 1H, 2'-Ha); 3.05 (m, 1H, 2'-Hb); 3.49 (s, 2H, 5-H); 4.52-4.54 (d, 2H -$C\underline{H}_2$-Ph); 5.46-5.51 (m, 1H, 2-H); 7.11-7.13(m, 4H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 14.08, 21.08, 28.38, 29.67, 30.44, 46.29, 49.33, 57.24, 128.51, 129.45, 133.11, 137.53, 156.12, 167.08; FABMS m/z : 462 $[M+1]^+$; HRMS: (m/z) calc. for $C_{24}H_{35}N_3O_4S$: 462.2348. Found: 462.2337

*4.1.3.46. tert-butyl ((3S)-1-((3S)-4-4(methylbenzyl)-5-oxomorpholine-3-carbonyl) piperidin-3-yl)methylcarbamate, (**35d**):* Same as *31o*. Yield: 60 %; $[\alpha]_D^{27\,^0C}$: + 28.36 (*c* = 0.088; Chloroform); IR (KBr): 3453, 3360, 3014, 2980, 2930, 2858, 2362, 1765, 1702, 1655, 1511, 1449, 1367, 1253, 1449, 1376, 1253, 1217, 1169, 1112, 1080, 1045, 1007, 963, 910, 853, 757, 667; ^{1}H NMR ($CDCl_3$; 300MHz): δ; 1.26-1.29 (m, 2H, 4'-H); 1.44 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.65 (m, 1H, 3'-H); 1.77 (m, 2H, 5'-H); 2.32 (s, 3H, $C\underline{H}_3$); 2.50 (m, 2H, -C<u>H</u>2-NH-Boc); 2.82-2.98 (m, 2H, 6'-H); 3.13-3.17 (m, 1H, 2'-H_a); 3.41-3.68 (m, 1H, 2'-H_b); 4.00 (m, 1H, 3-H); 4.3 (m, 2H, 5-H); 4.40 (d, 2H -$C\underline{H}_2$-Ph); 5.63-5.68 (d, 1H, 2-H); 7.19-7.35 (m, 5H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 17.44, 17.64, 22.65, 24.62, 24.85, 28.37, 36.40, 38.44, 43.34, 46.04, 46.18, 46.58, 46.84, 49.93, 59.18, 59.37, 66.74, 72.30, 72.50, 79.46, 127.83, 128.02, 128.28, 128.83, 128.93, 129.05, 135.51, 156.08, 165.85, 166.21, 167.69, 167.87; HRMS: (m/z) calc. for $C_{24}H_{35}N_3O_5$: 446.2577. Found: 446.2561

*4.1.3.47. tert-butyl ((3S)-1-((3R)-4-4-(methylbenzyl)-5-oxomorpholine-3-carbonyl) piperidin-3-yl)methylcarbamate, (**36d**):* Same as *31o*. Yield: 60 %; $[\alpha]_D^{27\,^0C}$: + 12.94 (*c* = 0.065; Chloroform); IR (KBr): 3381, 3017, 2932, 2360, 1761, 1701, 1655, 1445, 1366, 1217, 1167, 1111; ^{1}H NMR ($CDCl_3$; 300MHz): δ 1.26 (m, 2H, 4'-H); 1.46 (s, 9H, -O-C($C\underline{H}_3$)$_3$); 1.65 (m, 1H, 3'-H); 1.77 (m, 2H, 5'-H); 2.32 (s, 3H, $C\underline{H}_3$); 2.52 (m, 2H, -$C\underline{H}_2$-NH-Boc); 2.80-2.98 (m, 2H, 6'-H); 3.15-3.20

(m, 1H, 2'-H_a); 3.40-3.65 (m, 1H, 2'-H_b); 4.00 (m, 1H, 3-H); 4.32 (d, 2H -C<u>H</u>$_2$-Ph); 4.40 (s, 2H, 5-H); 5.58-5.65 (d, 1H, 2-H); 7.20-7.30 (m, 5H, Ph-H); ^{13}C NMR ($CDCl_3$; 75 MHz): 17.39, 24.41, 29.67, 36.33, 43.03, 46.18, 46.42, 46.99, 59.37, 60.56, 66.23, 70.52, 71.95, 128.84, 135.58, 156.11, 165.89, 166.24, 167.66; HRMS: (m/z) calc. for $C_{24}H_{35}N_3O_5$: 446.2577. Found: 446.2589

4.2. Biology (materials and method)

4.2.1. Collagen epinephrine induced Pulmonary Thromboembolism in Mice:

Pulmonary thromboembolism was induced by a method as described [36, 37]. Briefly, the compounds to be tested, standard drugs or the vehicle were administered by oral route 60 minutes (or at different time points) prior to the thrombotic challenge. Ten mice were used for evaluating the effect of test compound, while a group of 5 mice was used to evaluate the effect of aspirin or vehicle. A mixture of collagen (150µg/ml) and epinephrine (50µg/ml) was injected into the tail vein to achieve final doses of collagen (1.5 mg/kg) and epinephrine (0.5 mg/kg) to induce hind limb paralysis or death. Results have been reported as percentage protection, which represents protection against collagen and epinephrine induced thrombosis and expressed as:

Percent Protection = $[1-(P_{test} /P_{control})] \times 100$

P_{test} - number of animals paralyzed/dead in test compound-treated group; $P_{control}$ - number of animals paralyzed/dead in vehicle treated group.

4.2.2. Bleeding Time in Mice:

The tip of tail (approximately 2mm) of mice was incised with a sharp razor blade. The time elapsed from the tip incision to the stoppage of bleeding was determined as the bleeding time. The change in bleeding time was compared to that of vehicle treated mice and results have been depicted as percent increase from control.

4.2.3. Aggregation in Human platelet rich plasma (in vitro):

Human platelets were isolated from whole blood as described by Prakash *et al* [37]. The platelet-rich plasma (PRP) was separated by centrifugation of the citrated blood at 200 g for 20 min. A turbidimetric method was applied to measure platelet aggregation [37, 38] using a dual channel-aggregometer (560 Ca, 230 VAC Chronolog-corp, Havertown, USA). Platelet rich plasma (1×10^8 platelets/ml, 0.45 ml) was pre-warmed to 37°C for 2 minutes, then incubated with test

compound (3–100 μM) or an isovolumetric solvent control (0.5% DMSO) for 5 minutes before addition of the agonists (2μg/ml Collagen (Chrono-log, Corp.),10μM ADP (Sigma), 15ng/ml Convulxin (obtained as kind gift),25 μM thrombin receptor activated peptide (TRAP) 2.5 μM U46619 or 1.5mg/ml Ristocetin (Chrono-log Corp)). The reactions were allowed to proceed for at least 5 minutes, and the extent of aggregation was expressed in light-transmission units. The percentage of aggregation was calculated by using following formula:

Percent Aggregation = A/B x 100

Where, A is the number of division traversed by the recorder pen on chart paper in the presence of Inducer and B is the total number of divisions. In the subsequent studies the percent aggregation was also calculated by using Aggrolink software.

4.2.4. Statistical analysis:

Data are represented as Mean ± SEM, of at least 3-5 independent experiments and were analyzed by one way ANOVA test followed by Dunnett's test. Data were considered significant at $p<0.05$.

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HIGHLIGHTS

- Novel chiral carboxamides synthesized and explored the antiplatelet efficacy.
- Compound **31*a*** **(**IC_{50}= 6.6µM) exhibited remarkable antithrombotic activity.
- Compound **34*c*** displayed antiplatelet potential and dual mechanism of action.
- Pharmacokinetic study of **31*a*** showed faster absorption and better therapeutic response.
- Compounds satisfactorily preserve the haemostasis with less adverse effect on BT

Supplementary Information

Synthesis and Identification of Chiral Aminomethylpiperidine Carboxamides as Inhibitor of Collagen Induced Platelet Activation.

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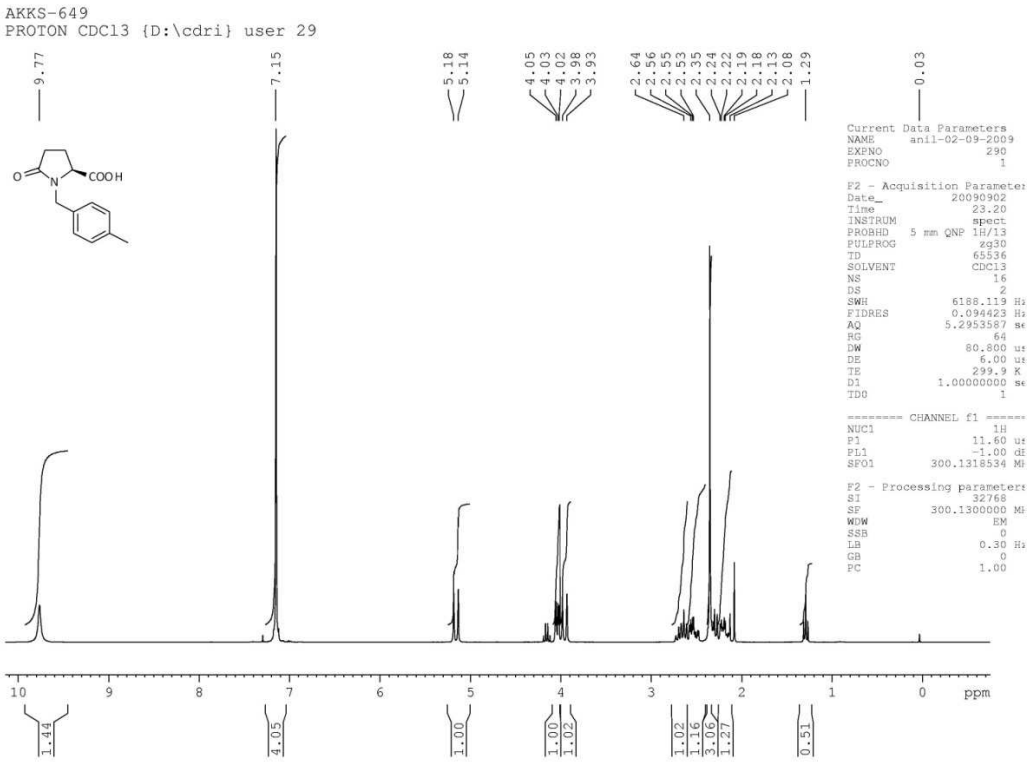


Fig. 1S. ^{1}H NMR ($CDCl_3$; 400 MHz) spectra **of Compound-16a**

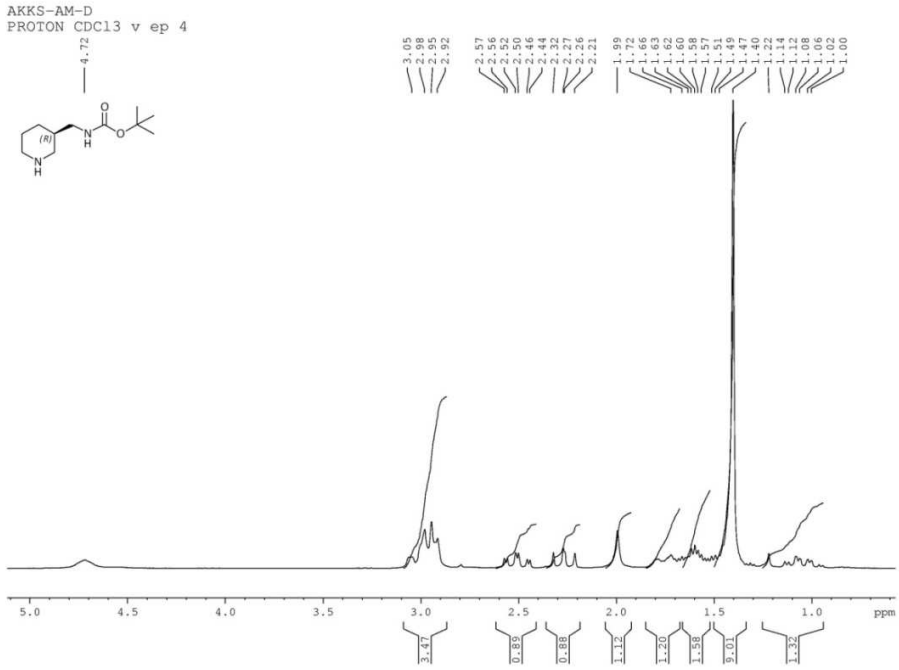


Fig. 2S.^{1}H NMR ($CDCl_3$; 400 MHz) spectra of compound-**29**

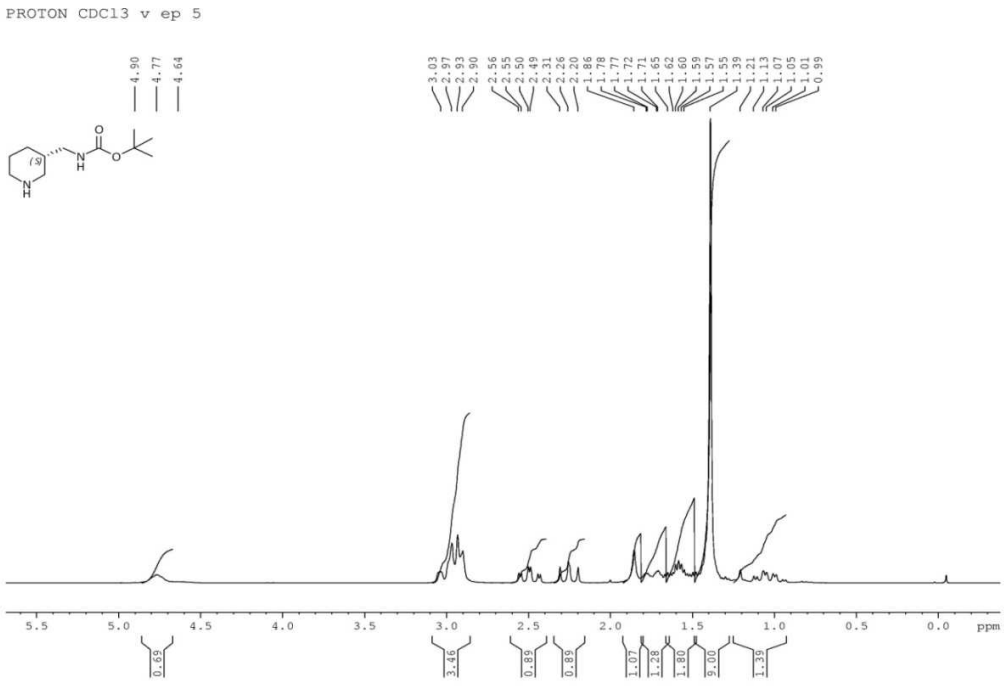


Fig. 3S.^{1}H NMR ($CDCl_3$; 400 MHz) spectra **of** Compound-**30**

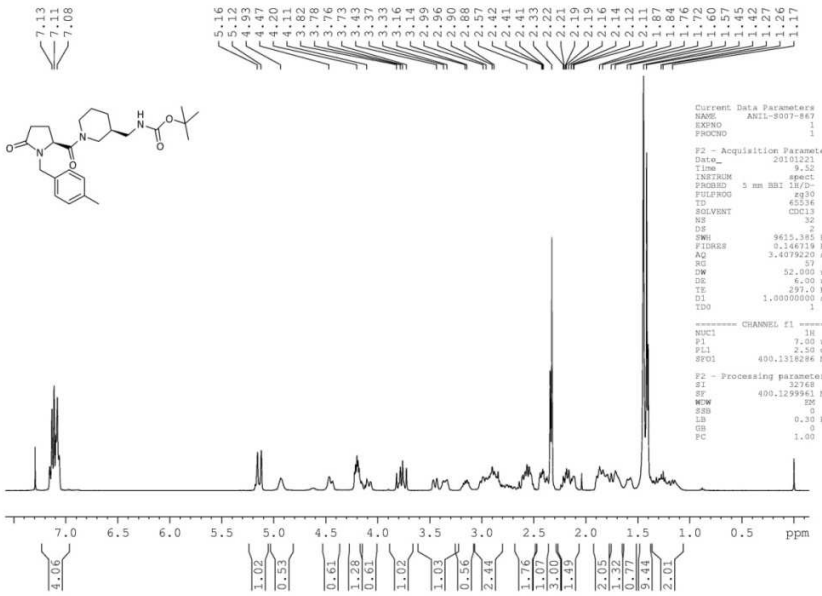


Fig. 4S.^{1}H NMR ($CDCl_3$; 400 MHz) spectra of compound **31a**

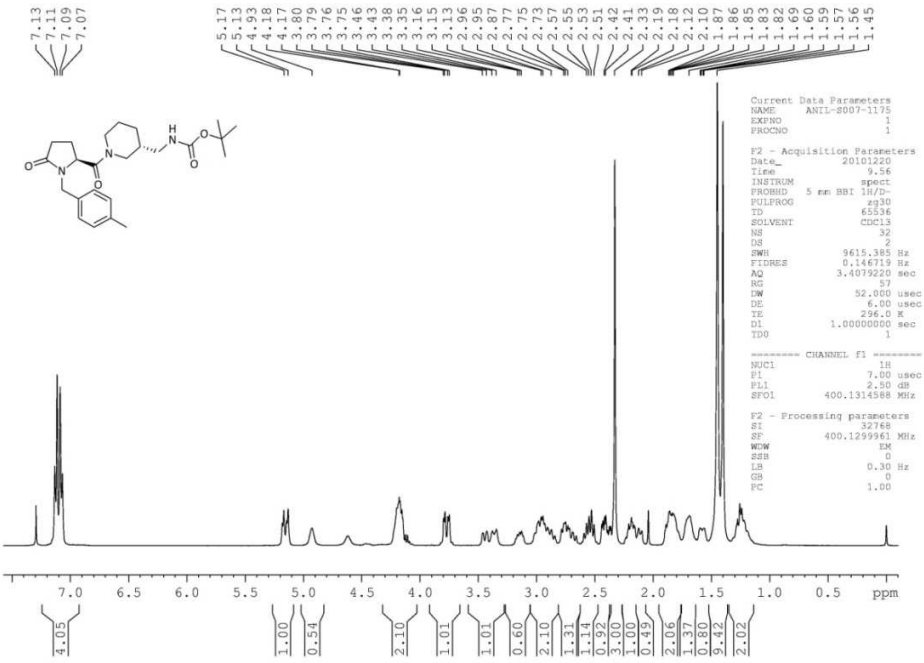


Fig. 5S.^{1}H NMR ($CDCl_3$; 400 MHz) spectra of compound **32a**

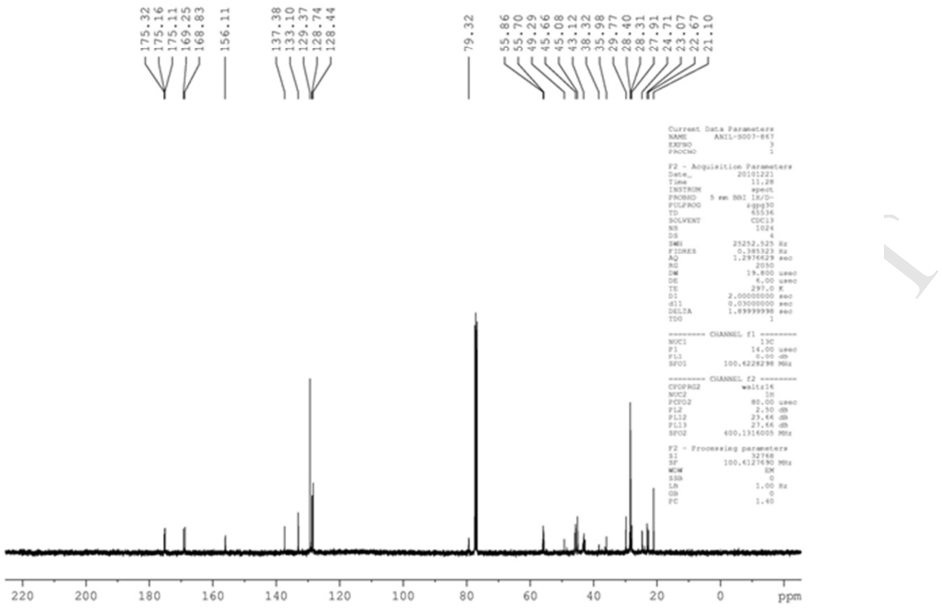


Fig. 6S.[13]C NMR ($CDCl_3$; 400 MHz) spectra of compound **31a**

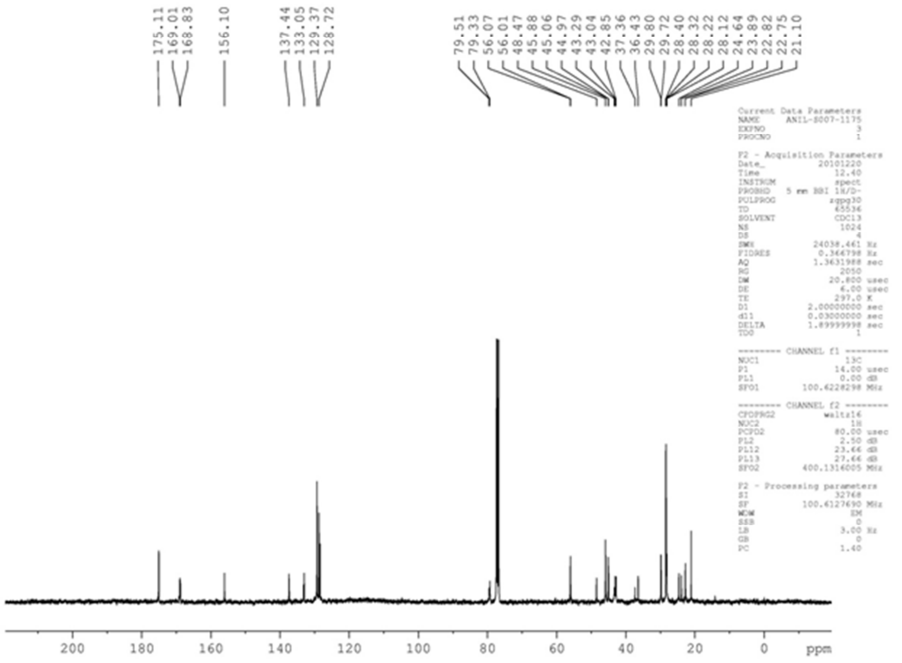


Fig. 7S.[13]C NMR ($CDCl_3$; 400 MHz) spectra of compound **32a**

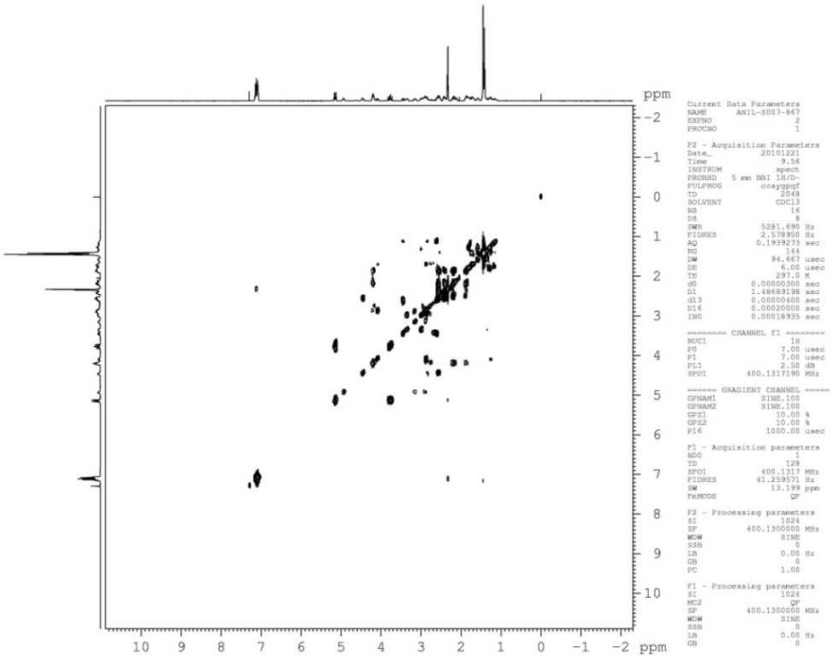


Fig. 8S.[1]H COSY ($CDCl_3$; 400 MHz) spectra of **31a**

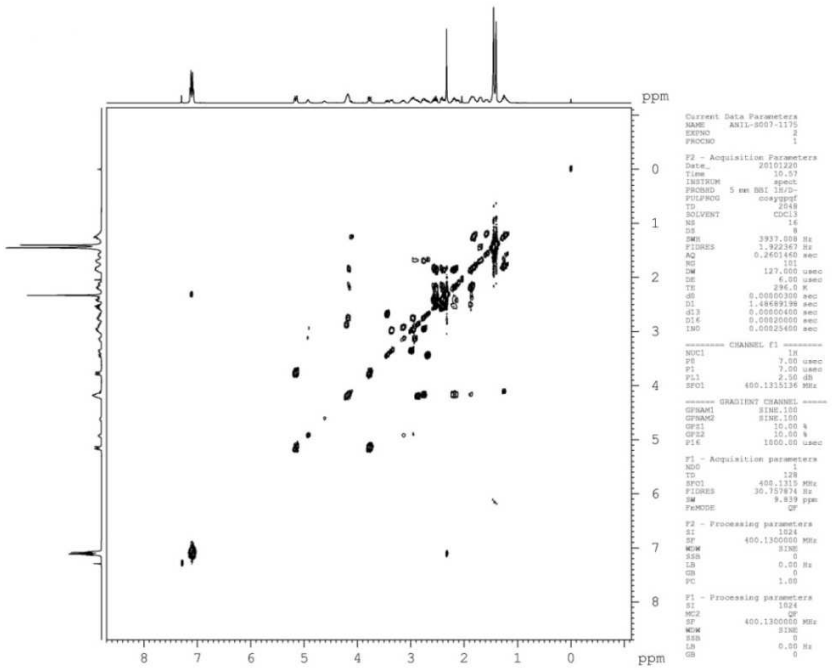
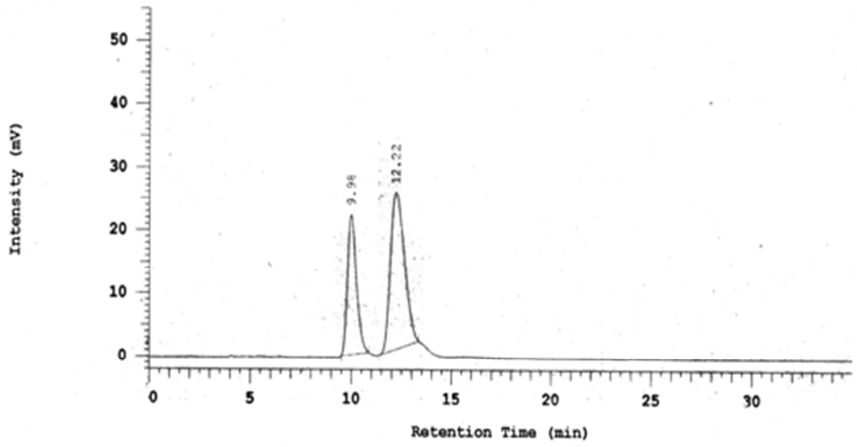
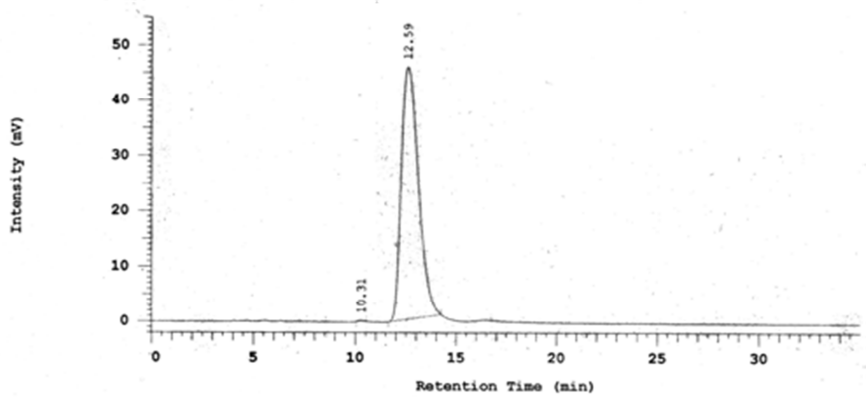


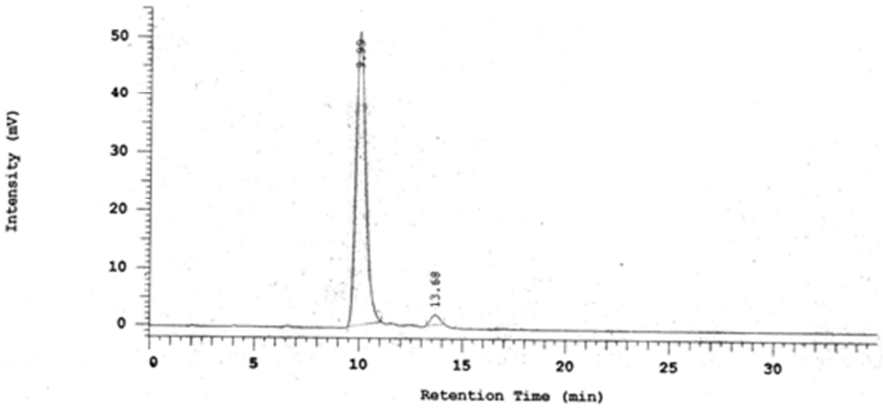
Fig. 9S.[1]H COSY ($CDCl_3$; 400 MHz) spectra of **32a**



(a) HPLC chromatogram of **28*i***



(b) HPLC chromatogram of **31*a***



(c) HPLC chromatogram of **32*a***

Fig. 10S. HPLC chromatograms

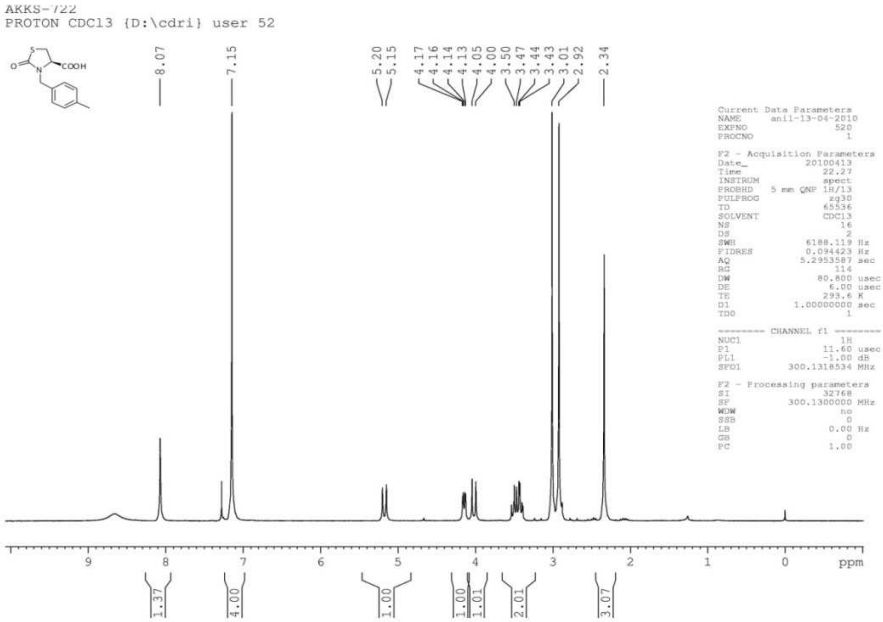


Fig. 11.[1]H spectra of compound **18***a*

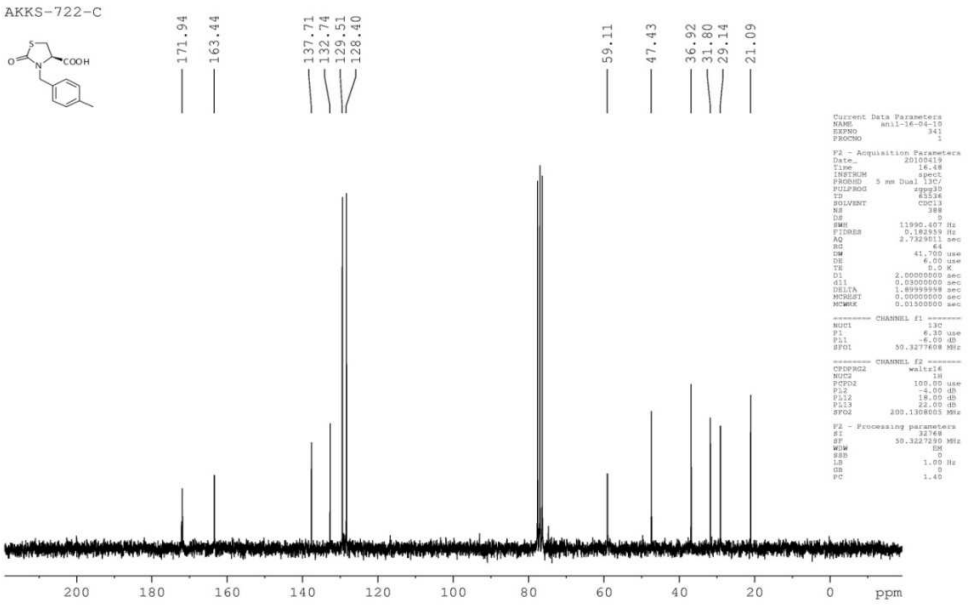


Fig. 12S.[13]C spectra of compound **18a**

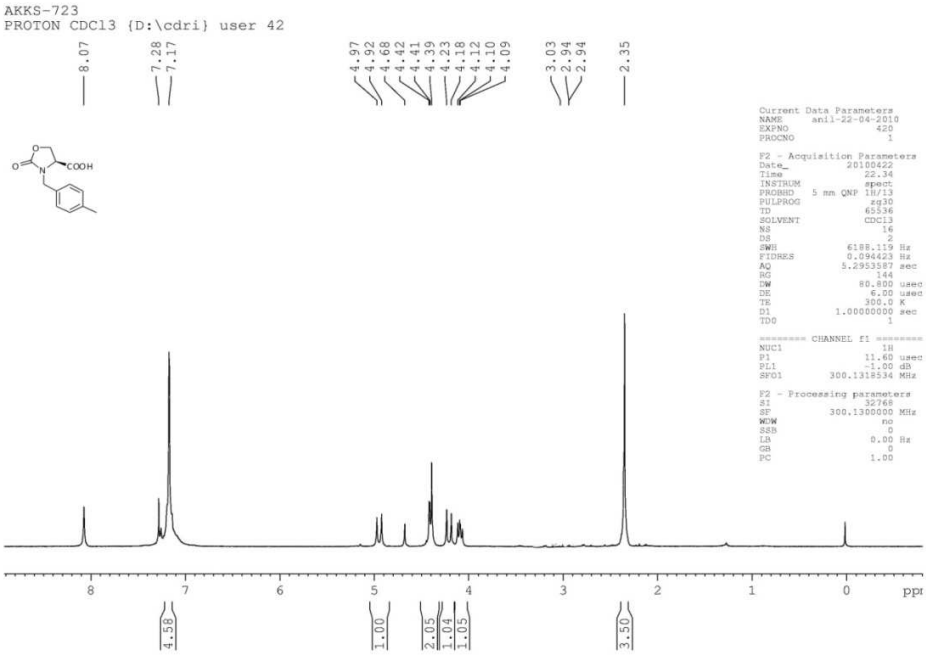


Fig. 13S.[1]H spectra of compound **17a**

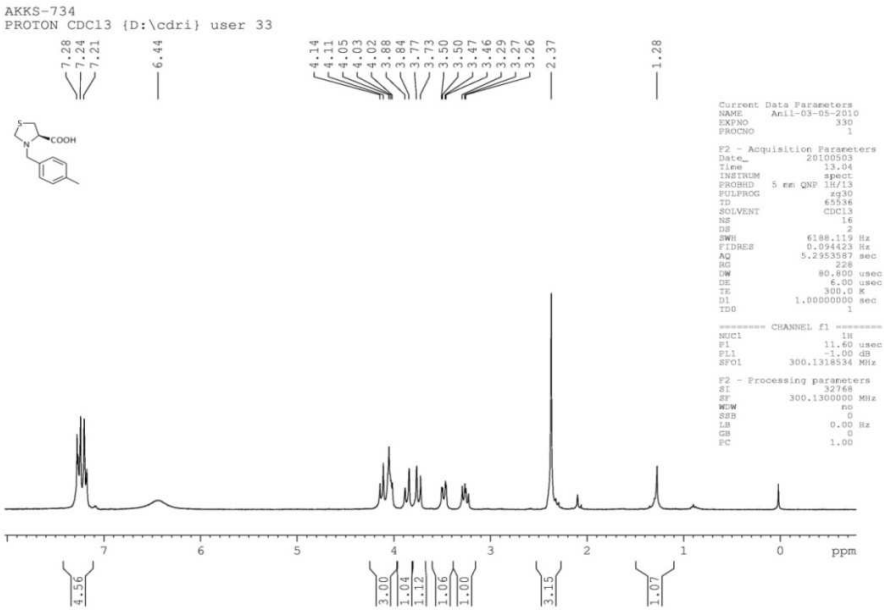


Fig. 14S.**Figure 4**: [13]C spectra of compound **15**

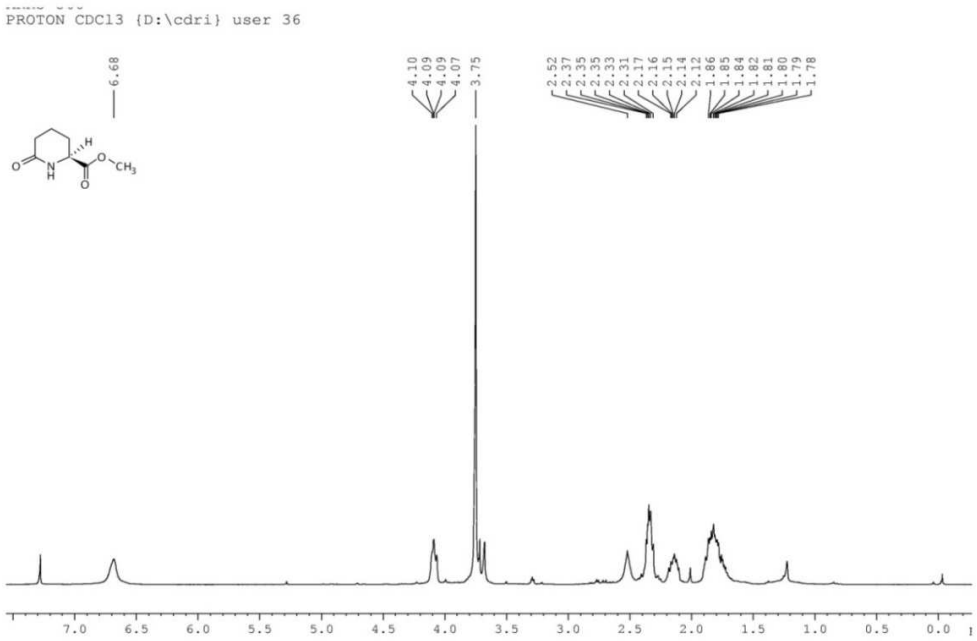


Fig. 15S.[1]H spectra of compound **14**

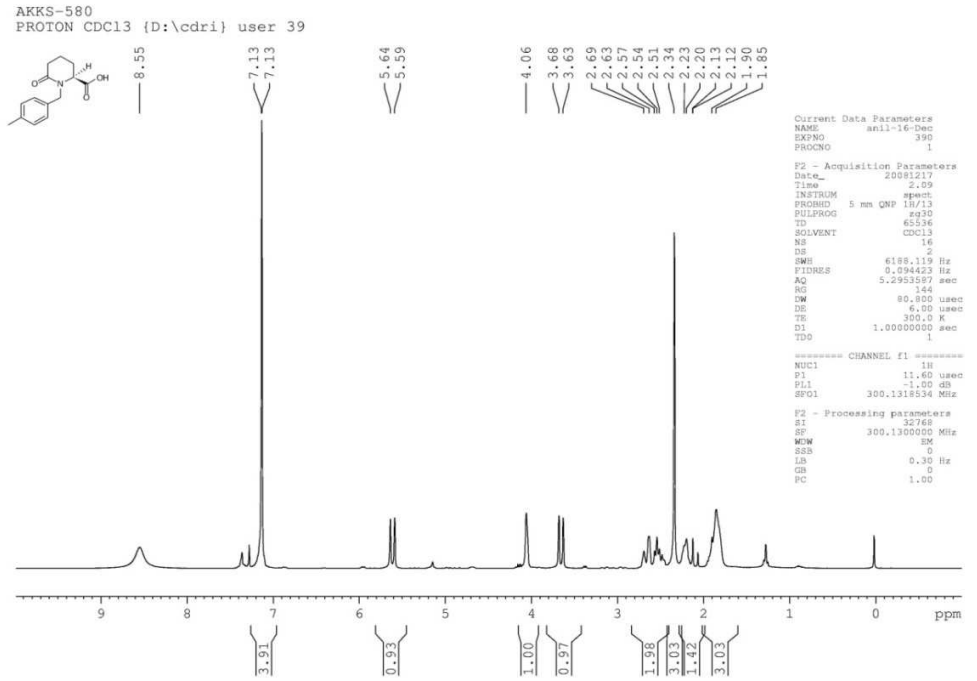


Fig. 16S.[1]H spectra of compound **20**

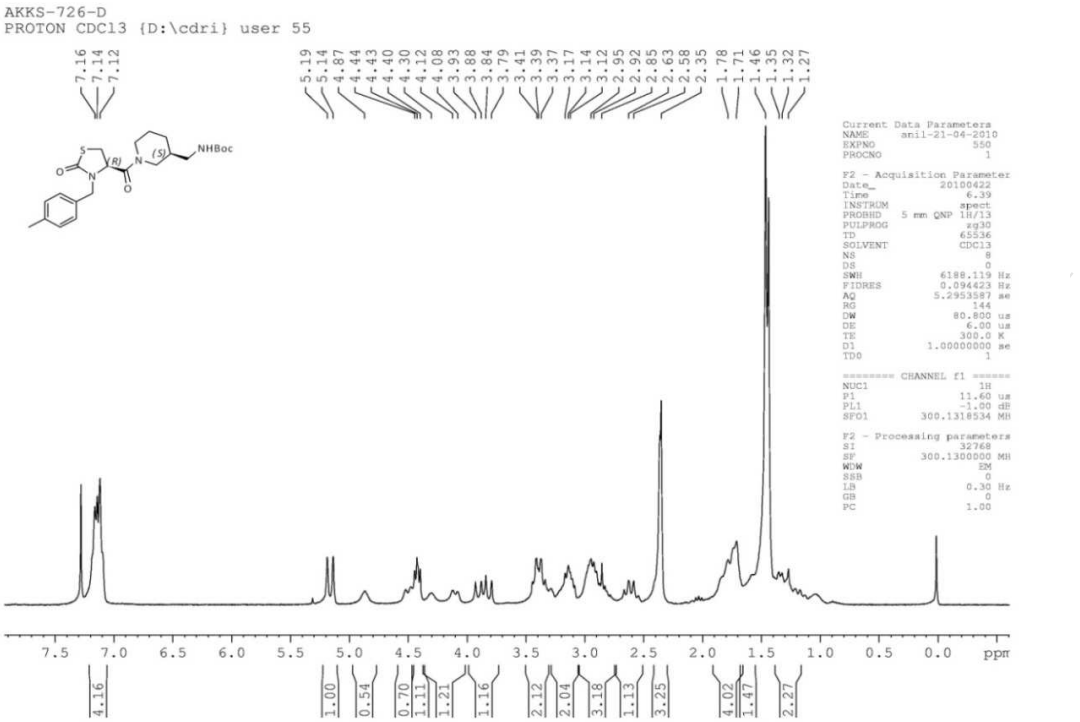


Fig. 17S.[1]H spectra of compound **33b**

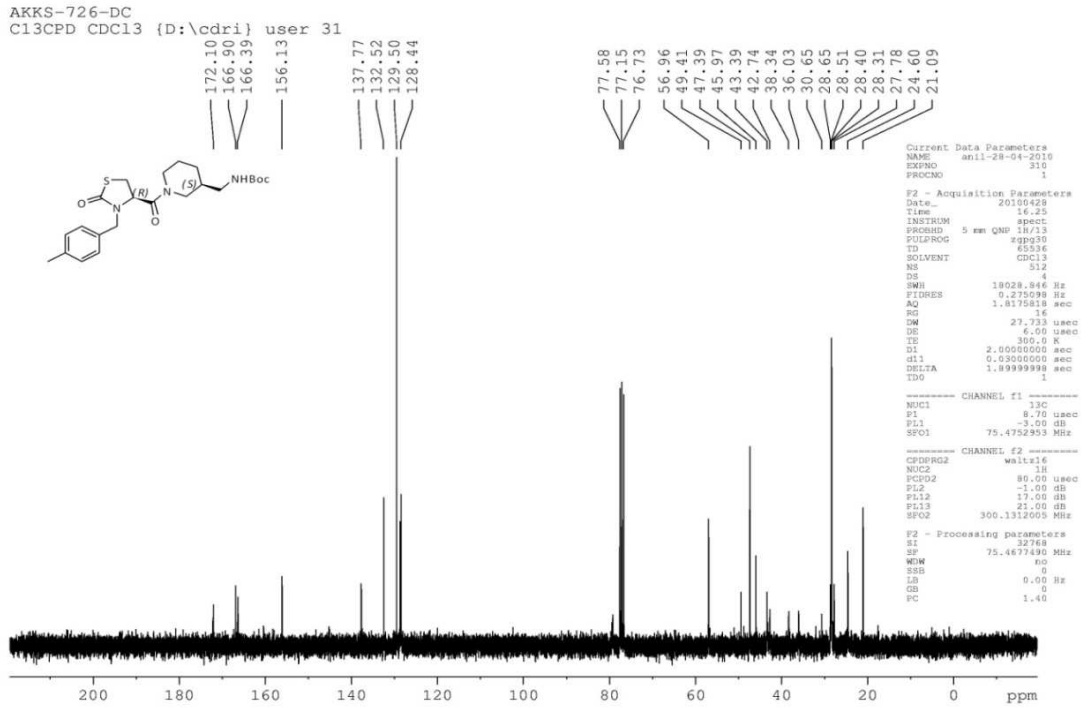


Fig. 18S.[13]C spectra of compound **33b**

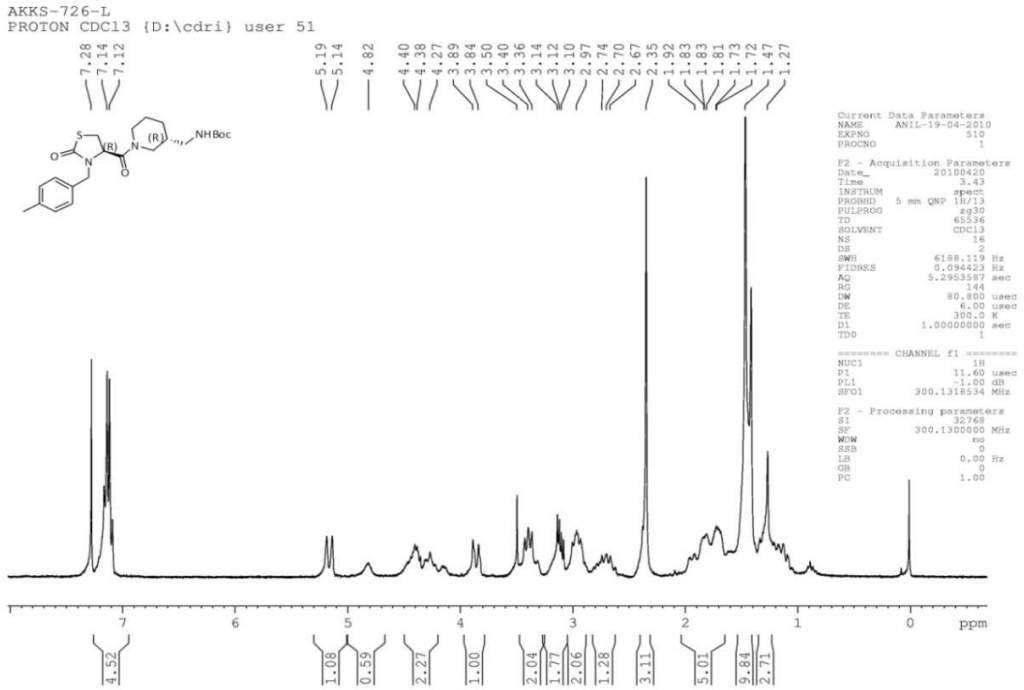


Fig. 19S.[1]H spectra of compound **34a**

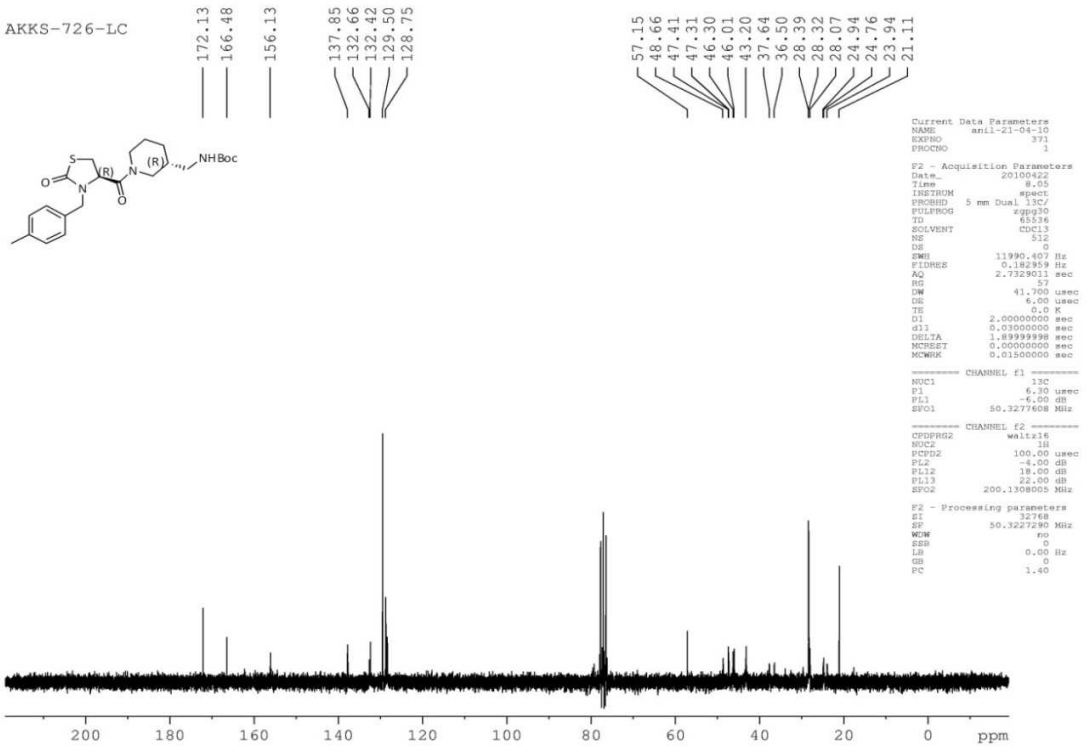


Fig. 20S.[13]C spectra of compound **34a**

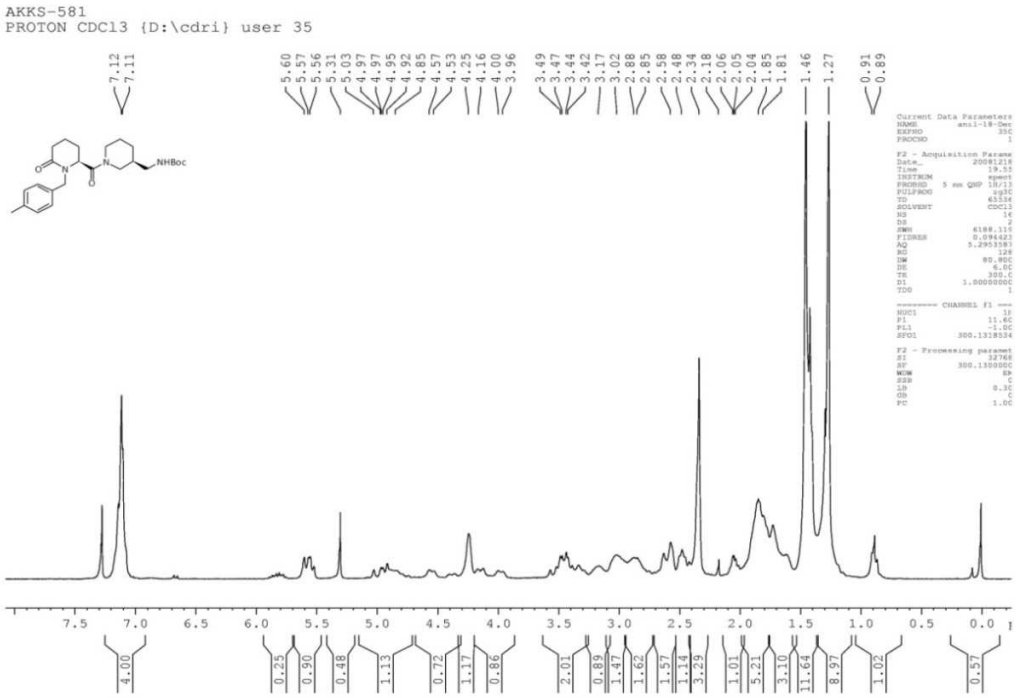


Fig. 21S.[1]H spectra of compound **35a**

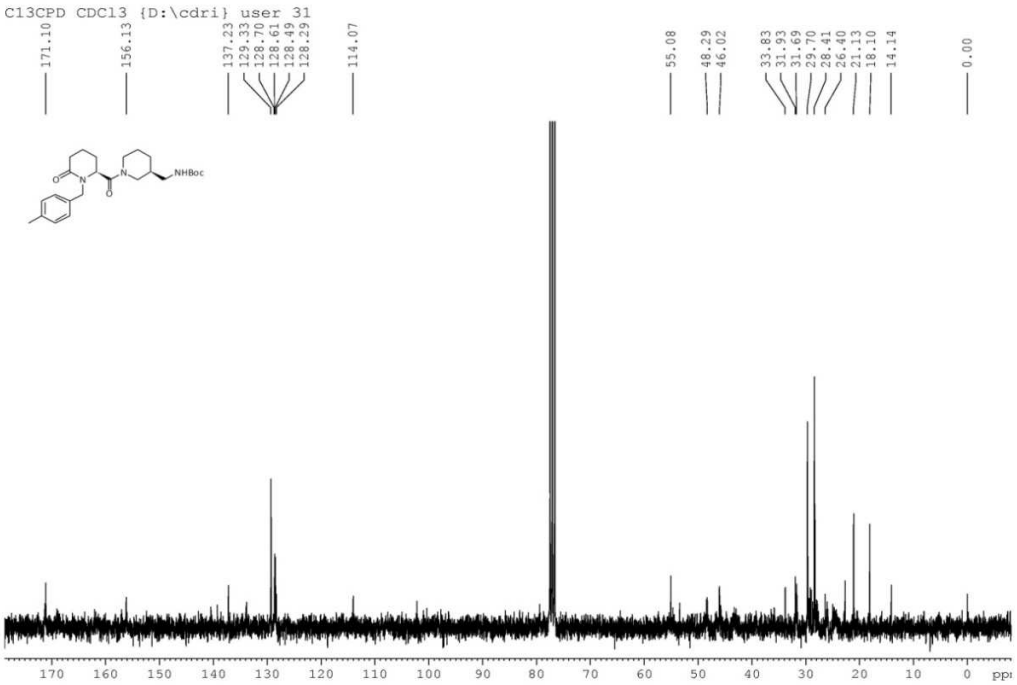


Fig. 22S.[13]C spectra of compound **35***a*