DISSERTATION II

REPORT

SUBMITTED TO –LOVELY PROFFESSIONAL UNIVERSITY,

FOR THE PARTIAL FULFILLMENT OF THE AWARD

OF

MASTERS OF SCIENCES IN CHEMISTRY

BY

NEERJA THAKUR

(Registration No. 11509979)

UNDER THE GUIDANCE OF

DR. NITIN TANDON

SCHOOL OF PHYSICAL SCIENCES,

DEPARTMENT OF CHEMISTRY

LOVELY PROFESSIONAL UNIVERSITY, INDIA

CONTENT:

CERTIFICATE

This is to certify that the capstone project entitled "Prolineamide derivatives as potential anti-diabetic agents - synthesis and characterization" submitted by NEERJA THAKUR to the Lovely Professional University, Punjab, India is documentation of genuine literature review of coming research work approved under my guidance and is commendable of consideration for the honor of the degree of Masters of Science in Chemistry of the University.

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SUPERVISOR

DR. NITIN TANDON

ASSOCIATE PROFESSOR

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to my supervisor **DR. NITIN TANDON (**Associate professor) at LPU, Punjab from school of physical sciences, department of chemistry for his cooperation and guidance for preparing the pre-dissertation report on DPP-IV inhibitors.

I wish to avail myself of this opportunity to express a sense of gratitude and love to my friends for their support and strength.

NEERJA THAKUR

Reg. no.-11509979

Abbreviations:

Abbreviation	Full form
T2DM	Type 2 diabetes mellitus
DPP IV	Dipeptidyl peptidase 4
DCM	Dichloromethane
MTBE	Methyl tert-butyl ether
DMF	Dimethylformamide
THF	Tetra hydro furan
IR	Infrared Spectrum
^{1}H NMR	Proton Nuclear Magnetic Resonance
DMSO	Dimethylsulphoxide

INTRODUCTION:

Diabetes mellitus disease is caused due to very high level of plasma glucose. Pancreas an organ in our body which is located in between our stomach and spine helps us for digestion and it releases a hormone that is known as insulin in our blood. Insulin hormone helps our blood cells to carry glucose to all our body cells and maintains our body's sugar level. But when glucose in blood is very high it causes disease i.e. diabetes/ prediabetes. Prediabetes is condition before diabetes i.e. when blood glucose is above normal but it is not as higher to cause diabetes. [1] Diabetes consists of two major types:

1. **TYPE I DIABETES:** It is also called as juvenile diabetes. Develops mostly in young people/ in adults. In this body has no longer produce insulin because our immune system has attacked by viruses and bacteria and destroys the cells that make insulin hormone. [1]
2. **TYPE II DIABETES:** These diseases can occur in children. It is generally due to insulin resistance – it is a condition which occurs when our body fat, liver cells and muscles does not use any insulin to provide glucose to body cells for its use for energy. So, body is in need for more insulin. If pancreas fails to make enough insulin, Type-II diabetes occurs. [1]

About 29 million people in United States are affected by diabetes while another 86 million of people are affected by prediabetes. Diabetes leads to diseases like blindness, failure of kidney and nervous damage. It is due to the damage of small vessels and cause micro vascular disease. Arteries become narrower and hardened which cause strokes and heart diseases. The total cost on diabetes in 2012 was calculated to be 245 billion dollars per year in US. It counters about 116 billion dollars in the medical expenditure for diabetes and 69 billion dollars for the premature death and disability due to disease. It is 7^{th} most leading cause of death in US. India has second highest rating of diabetes after China reported by IDF. [3]

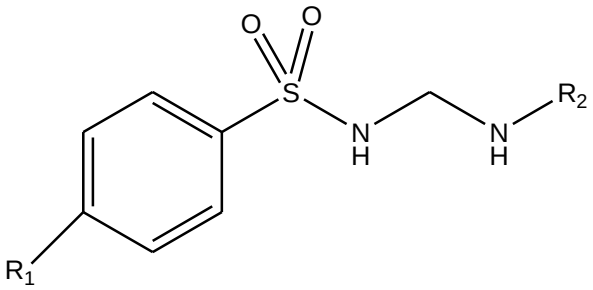
CURRENT THERAPIES FOR DIABETES:

According to the recent survey, the use of oral agents with insulin against diabetes disease in United States is started from 1989 by the National Health Interview Survey (NHIS). We use insulin as treatment for about 43% for patients of age about 18 years or more. Oral agents were

given to about 49% patients and 64% patients were given diet for the treatment of the disease. The ratio of treatment of NIDDM affected patients with insulin has increased with the use of it throughout the year, about 22% for age 0-4 years to 58 for age ≥20 years. Two or more insulin injections were daily taken by 61% of insulin dependent diabetes mellitus (IDDM) patients and 48% of insulin-treatment for NIDDM patients. There are five main classes of oral anti-diabetic agents. These were sulfonylurea (SU), non SU secretagogues (Meglitinides), biguanides, thiazolidinediones (TZDs), alpha glycosidase inhibitors and peptidase-IV. [2]

Oral anti- diabetic agents:

1. Sulfonylurea: Medications in class are: glipizide, glyburide, glimepiride.

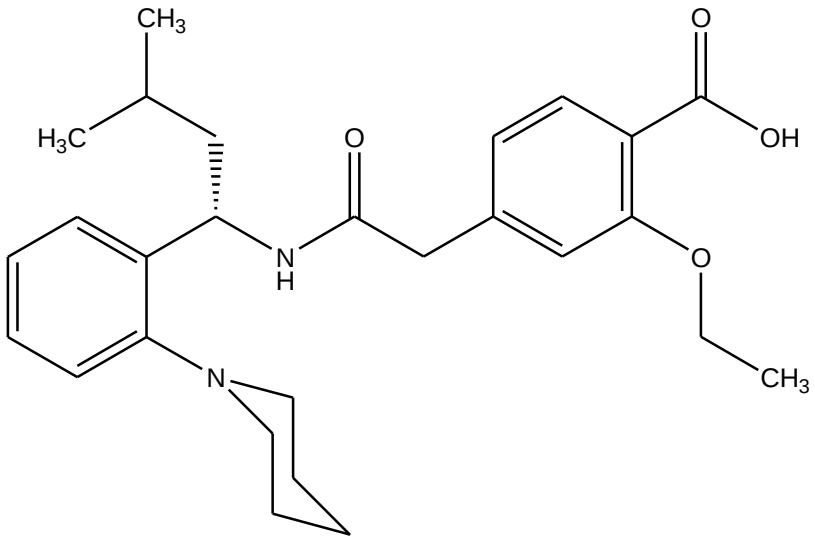


EFFECT: It causes cell membrane depolarization by inhibiting the activity of beta cell KATP channel and it causes rise in insulin secretion. It lead to lowering of hepatic removal of insulin and A1c. The effect of lowering is 1-2%. [4]

ADVANTAGES: It is not much expensive. It is used very commonly used and has extensive experience. [4]

DISADVANTAGES: It causes hypoglycemia, low durability, blunts myocardial ischemic preconditioning, weight gain.[4]

2. NON – SU SECRETAGOGUES: Drug named as meglitinides.

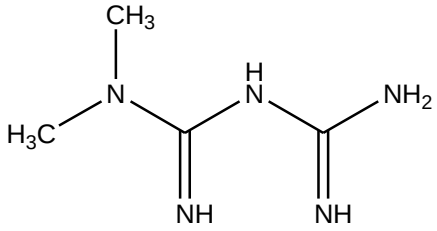


EFFECTS: It increases the amount of insulin produced by pancreas which lowers the blood sugar level. It causes less weight gain and also low blood sugar level as compared to sulfonylurea. It also lowers the heamoglobin A1c by 0.5% to 1.5%. [4]

ADVANTAGES: It work quickly and do not stay in body for long time**.** No weight gain occurs.

DISADVANTAGES: It leads to very low blood sugar level. Joint pain has been often observed. [4]

3. BIGUANIDES (Metformin):

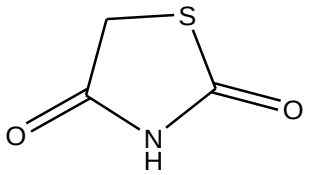


EFFECTS: The activity of AMP- dependent protein kinase rises. AMP- dependent protein activates fatty acid oxidation, uptake of glucose and non-oxidative metabolism and lowers the gluconeogenesis. Net effect is there is decrease in hepatic glucose formation and increase the sensitivity towards insulin; also increases glycogen storage in the muscles and it also lowers the plasma glucose. There is A1c lowering about 1-2%. [4]

ADVANTAGES: It has extensive clinical experience. It lowers the occurrence of hypoglycemia. There is no weight gain and has low cost.

DISADVANTAGES: It causes gastrointestinal side effects and lactic acidosis. It also leads to contraindications (CKD). [4]

4. THIAZOLIDINEDIONES (TZD'S):

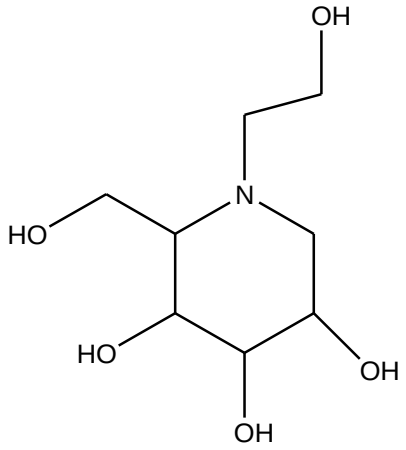


EFFECTS: It does not directly boost the insulin level but it affects the beta cells in the pancreas. Beta cells respond by formation of more insulin to make up the insulin resistance. [4]

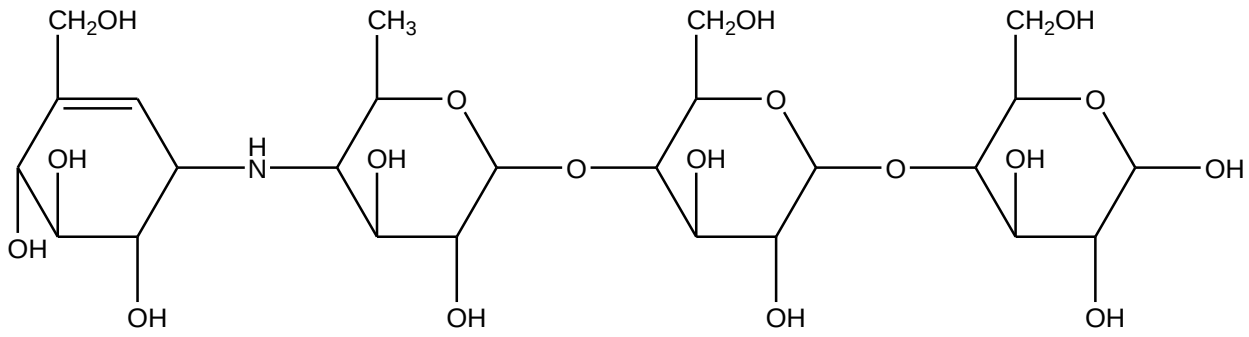
ADVANTAGES: It also lowers the cardiovascular risks of diabetes. These are less potent than sulfonylurea. It lowers the level of free fatty acids in bloodstream.

DISADVANTAGES: It is responsible for weight gain. Rezulin drug withdraw due to fatal liver poisoning. There increased risk of edema due to its use. [4]

5. ALPHA GLUCOSIDASE INHIBITORS: Also called as Acarbose and Miglitol.



Miglitol



Acarbose

EFFECTS: It slow down the rate of digestion of the complex carbohydrates (starches). These do not cause the production of more insulin by pancreas beta cells. It will not lower the blood sugar (hypoglycemia) until and unless it is being used with other medicines. It lowers HA1c by 0.5% to 0.8%. [4]

ADVANTAGES: It does not cause hypoglycemia unless used with other diabetic medicine. It targets on starch rather than pancreas for producing insulin. There is no weight gain.

DISADVANTAGES: It is not suggested if there is problem with digestion. It also leads to diarrhea and belly pain. [4]

6. DPP- IV INHIBITORS:

Types of DPP IV inhibitors: There are two types of DPP-IV inhibitors on the basis of structure. These are:

1. Peptidomimetics: These DPP-IV inhibitors mimic the DPP-IV molecules. Some examples are like vildagliptin, saxagliptins. These inhibitors are less selective towards the DPP-IV compared to DPP-8 or DPP-9

2. Non- peptidomimetics: These DPP-IV inhibitors do not mimic the DPP-IV molecule. Some examples are like sitagliptin, linagliptins

EFFECT: It inhibits the DPP- IV enzyme due to which there is activation of GLP- 1 and GIP. It will raise the concentration of plasma of GLP-1 and GIP by two fold. It will further lead to rise in insulin secretion and inhibits the glucagon secretion and lowers the HA1c by 0.5 to 1%.

ADVANTAGES: There is not any weight gain and hypoglycemia. It is well tolerated drug. It is more potent and selective than other drugs.

DISADVANTAGES: It causes headache, infection of respiratory tract, HA1c efficacy, angioedema, urinary tract infection. It has high cost. [4]

ADVANTAGES OF DPP-IV OVER OTHERS DRUGS: DPP-4 inhibitors are more potent and have more efficacy than other drugs. These are competitive reversible inhibitors. Different types of DPP-IV have variation in their potency when their therapeutic doses are taken. DPP-4 has more selectivity than other drugs. Two types of DPP-IV are usually implicated in preclinical toxicities; these are DPP-8 and DPP-9. To minimize the potential off-target side effects these DPP-IV suppress the T-cell activation and its proliferation. Sitagliptin and alogliptin are highly selective. The DPP-4 inhibitors are eliminated easily from the kidney by active transport. For sitagliptin, about 70% of the dosage is eliminated as the parent molecule and active transport occurs. Clearance of the alogliptin is greater than the normal glomerular filtration and easy elimination occurs. Other drugs cause digestion problem and gastrointestinal diseases. DPP-IV resists the weight gain problem that is present in all other drugs. There is also no chance of hypoglycemia. [4]

ATTEMPTS FOR IMPROVEMENT IN DRUG: Aims of regulating the glycemia to avert osmotic indications of hyperglycemia and avoid the instability of blood glucose over time. Medications according to safety, efficacy, simplicity, cost, risk of hypoglycemia, patient adherence, weight problem. Considerations are made towards efficacy, drug interactions, and side effects. [4]

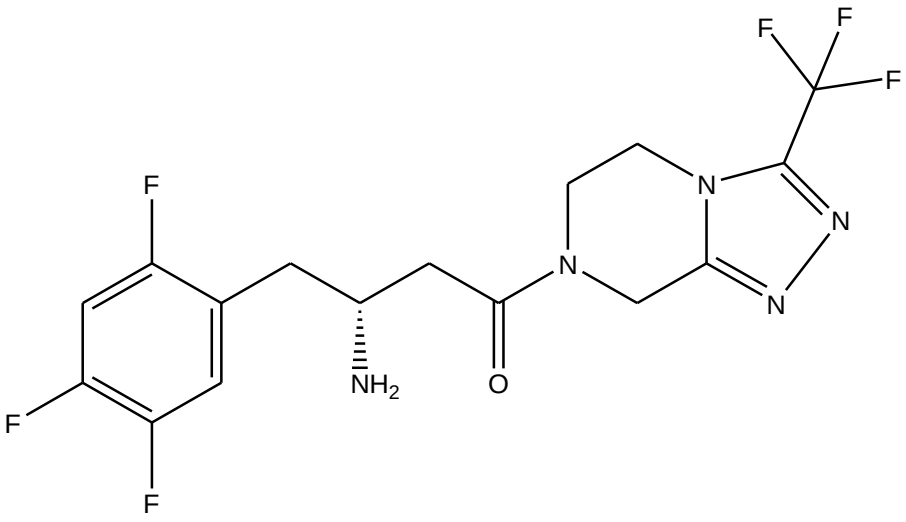
GLIPTINS: ALSO KNOWN AS DPP-IV INHIBITORS.

DPP-IV stands for dipeptidyl peptidase- IV. Eight glyptins are approved for its clinical usages for TYPE-2 diabetes. Till now seventeen glyptins have been discovered. DPP-IV are recently found out and approved by U.S. FDA. DPP-IV inhibitors suppress the activity of DPP-IV enzyme. It is serine protease enzyme which cleaves the incretin hormones, glucagon like peptide-1 (GLP-1) and glucose dependent insulinotropic peptide (GIP).

These hormones are liberated by gut in response to food we take and provide stimulus to secrete insulin and help in its synthesis in glucose – dependent manner. Also incretins restrict glucagon secretion, induce satiety, slow gastric emptying and inhibit hepatic glucose production. By increase in the circulating concentration of incretins, DPP-IV inhibitors improve the glucose control in patient with TYPE-II diabetes. Glyptins development first proved the concept for efficacy in 1998 when NVP-DPP 728 came into existence. Seventeen glyptins have been discovered on the basis of tolerability, potency and their safety and efficacy. Sitagliptin is the first gliptin of the DPP-IV inhibitors for T2DM. [2] The main eight gliptins are:

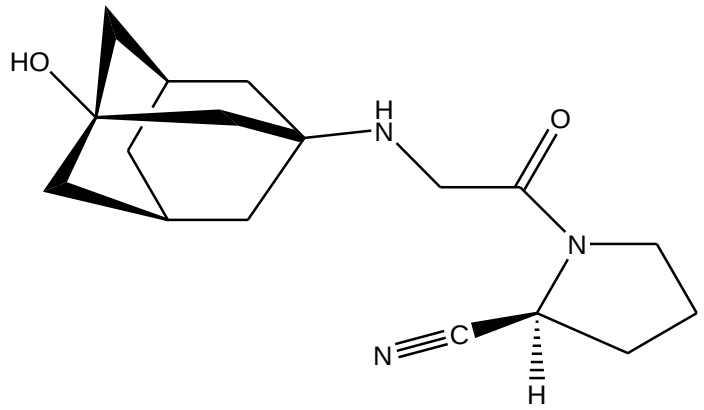
A. PEPTIDOMIMETICS:

1. Sitagliptin: It was discovered by Merck. It is a triazolopiperazine based inhibitor of DPP-IV. It is a potent drug (IC50= 18 nM) and is highly selective over DPP-8 (48000 nM), DPP-9 (>100000 nM).[18] It increases the pancreatic beta cell function. In animal models triazole ring improves the oral bioavailability. It is well tolerated and keeps the body weight neutral. It is used as monotherapy or with combination with Metformin. The binding orientation of the amide carbonyl in sitagliptin is reversed.



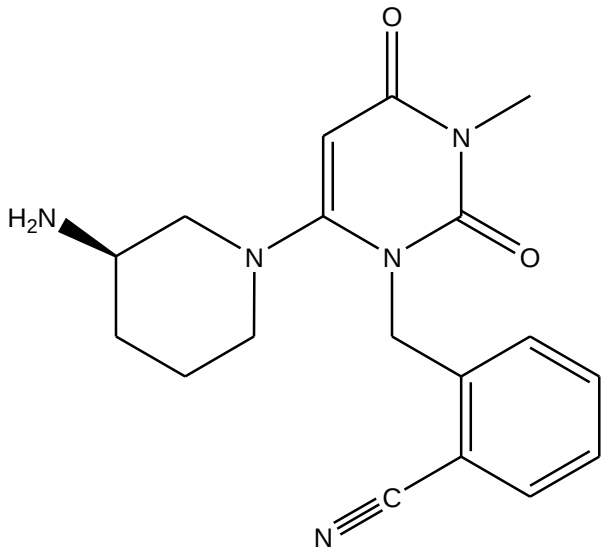
Structure of Sitagliptin

2. Vildagliptins (LAF-237): It is called as the first generation inhibitor and first gliptin of the cyanopyrrolidine class for T2DM. Novartis discovered it. It is selective for DPP-IV against DPP-9 (23 fold) and DPP-8 (>250 fold). [19] The cyanopyrrolidine moiety occupies the S1 pockets whereas the nitrile group covalently forms the imidate adduct with the hydroxyl group of Ser630. Single dose of 100mg of it creates inhibition for about 7 hours (80%). It increases the insulin sensitivity and beta cell function. But it has adverse effects like headache, dizziness, constipation, increase sweating and cough. [2]



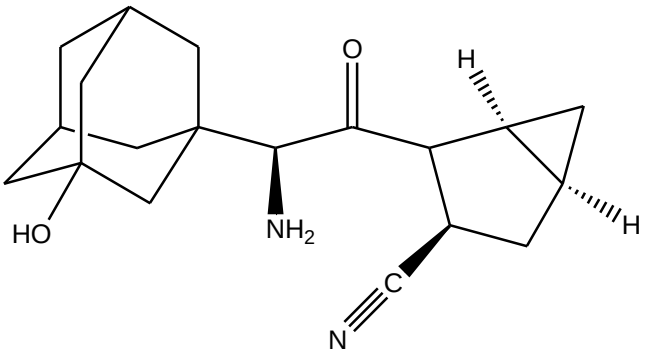
Structure of Vildagliptin

3. Alogliptin: It contains pyrimidinedione scaffold. Ii is the third generation inhibitor of DPP-IV. It is very much potent and has high selectivity over isozymes like DPP-8, DPP-9, DPP-2 and FAP. [32] The cyanobenzyl group presents in the S1 pocket and also makes interaction with the Arg125. It improves the insulin levels and also glucose tolerance. [2]



Structure of Alogliptin

4. Saxagliptin: It is methanopyrrolidine based inhibitor and first generation inhibitor of DPP-IV. It has highest degree of beta branching and cyclopropane moiety in structure. It is extremely potent and also selective for DPP-IV against DPP-9 and DPP-8. Internal cyclization makes it stable. [34] [35] Cyanopyrrolidine moiety occupies the S1 pocket in DPP-IV and forms the covalent bond interaction in between the side chain of Ser630 residue and cyano group. It is used in the combination with other drugs like SU, thiazolidinediones, Metformin. [2]

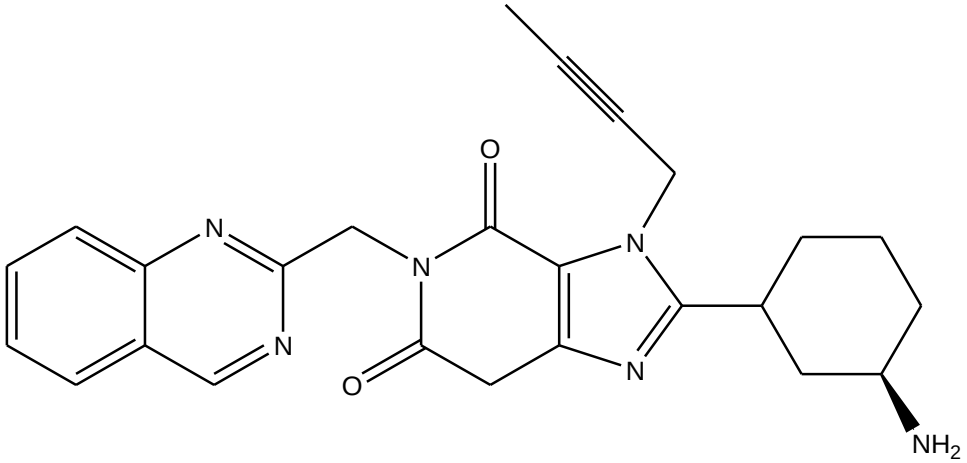


Structure of Saxagliptin

B. NON- PEPTIDOMIMETICS:

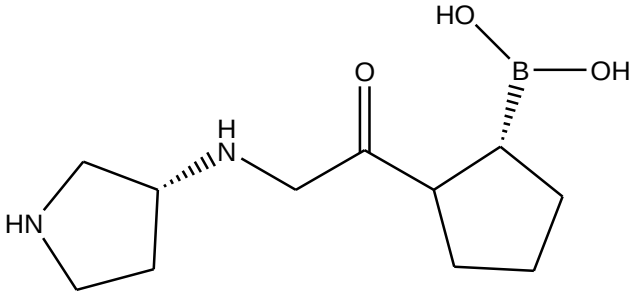
5. Linaglyptins: It is a xanthine based inhibitor and of second generation. It is selective and potent for DPP-IV against DPP-9 (>10000), DPP-8 and homozygous peptidases. Butynyl group

occupied the S1pocket and the S2 pocket is at amino piperidine moiety. Amino group establishes the salt bridge. GLP-1 levels are enhanced by it which is related to beta cell regeneration. The side effects from the drug are limited with no risk of hyperglycemia even at higher dosage. [2]



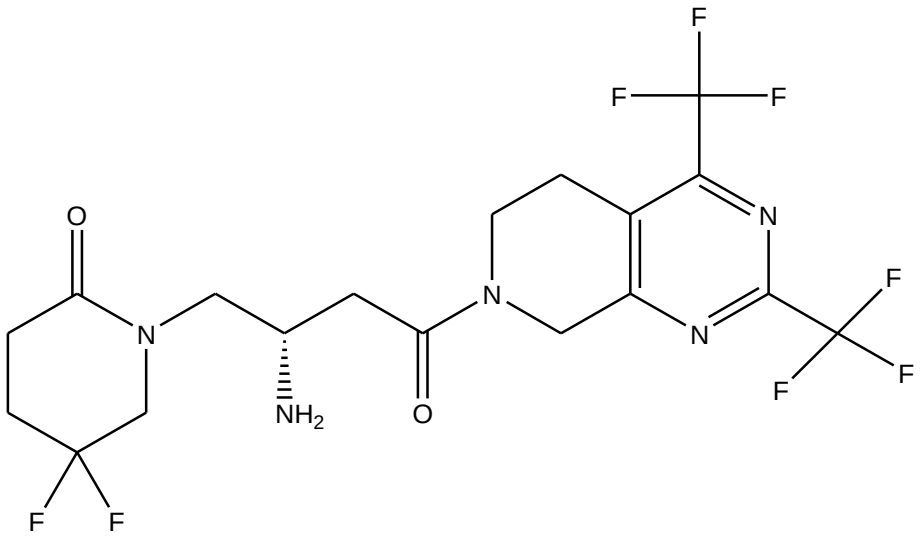
Structure of linagliptins

6. Dutagliptin: The inhibitor consists of boronic acids and is of second generation developed by the Phenomix Corp. It is potent and selective for DPP-IV inhibitors against DPP-9 and DPP-8 (400-fold). It shows around 80% and 50% inhibition of DPP IV in monkeys and dogs, respectively. Precursors for its synthesis are pyrrolidine. [2]



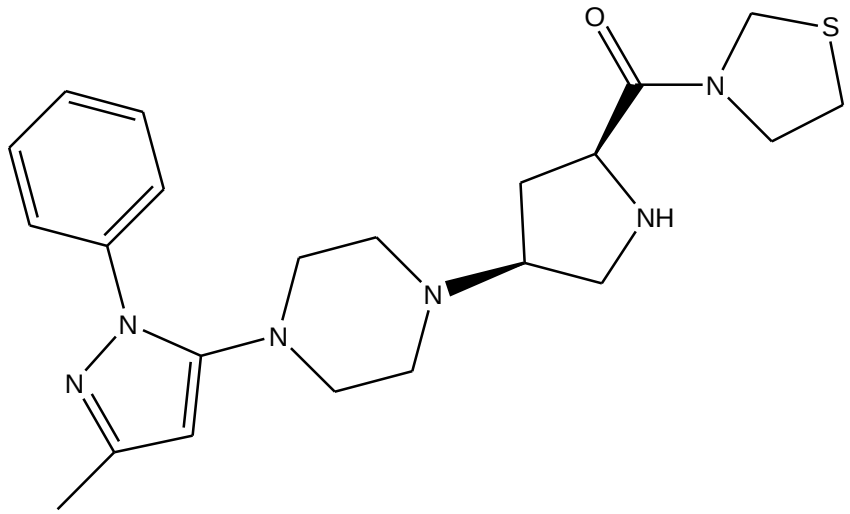
Structure of Dutagliptin

7. Gemigliptin: It is analogue of sitagliptin formed by LG Life sciences Ltd, Korea via derivatizing compounds. It is a potent and long performing inhibitor which has high selectivity (3000-fold) against the isoenzymes. It lowers the blood glucose and elevates the GLP-1 levels. It lowers the HbA1c level (0.77%) with 3.0 mg/kg dosage. [2]



Structure of Gemigliptin

8. Teneligliptin: It is a bicyclic heteroarylpiperazine derivative. It is extremely potent. It is also selective for DPP-IV against DPP-9 (1460 fold) and DPP-8 (703 fold). The thiazolidine moiety present in the S1 pocket which is hydrophobic. The proline moiety contains amino group forms the salt bridge with the diad of Glu205 and Glu206. It inhibits more than 50% of the plasma DPP-IV activity for 24 hours with a single dose of 1.0 mg/ kg. It lowers the increased glucose level in blood. It has been approved for T2DM treatment in Japan. [2]



Structure of Teniligliptin

Mechanism of action Of DPP-IV Inhibitors: -

Peptidomimetics inhibitors are different in mechanism from Non-peptidomimetics inhibitors. They non-covalently form extra-cellular interactions with the residues in the catalytic site of the DPP-4 substrate, and results in the potent, immediate inhibition. On the other hand, Peptidomimetics inhibits the DPP-IV molecule by forming a reversible covalent enzyme–inhibitor complex. This complex formed first bind and then removed from the catalytic site of the DPP-4 molecule very slowly that results into persistent DPP-4 inhibition even when the drug has been inactivated. It means that the even after the free drug has been removed from the blood circulation, the catalytic activity still remain inhibited. It thus explains how saxagliptins and vildagliptin inhibits the DPP-4 activity for longer period in spite of their shorter half-lives. DPP-4 inhibition occur extrcellularly by the specific DPP-4 inhibitors. Due to the extracellular inactivation, the functioning of major intracellularly occurring proteins is preserved, which may account for the lack of immune dysfunction as a result. [5] – [9]

Alpha-series peptidomimetics inhibitors:

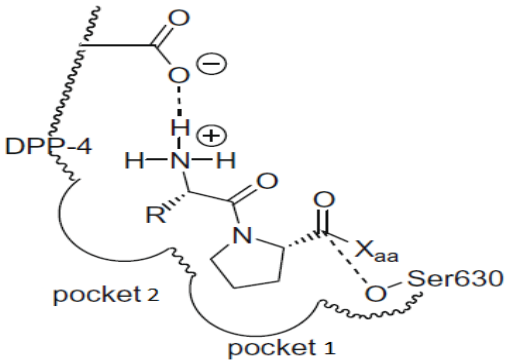


Fig.1 INTERACTION WITH POCKETS OF ENZYMES

Pyrrolidine derivatives have been widely reported as DPP-IV inhibitors due to specificity of DPP-IV molecule for these drugs having an amino-terminal proline at C-2 position. Thus, many DPP-4 inhibitors resemble in cleavage product S1-S2 of dipeptidyl substrate as shown in Fig.1 where at S1 site is proline mimic. In some important inhibitors, electrophile group replace the

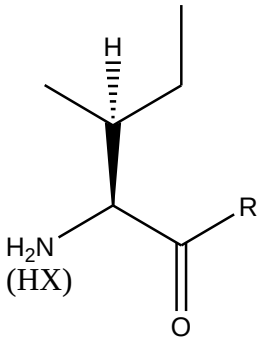
position of the amide moiety in the substrate S1 which results in the formation of adduct with the active site. [29]

Review of literature:

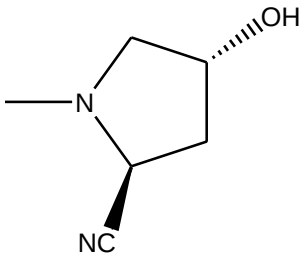
A. Different derivatives:

Hiroshi Fukushima and his coworkers studied the series of 2- cyanopyrrolidine derivatives for its use as DPP-IV inhibitors. [10] Compound 1(b) and 1(e) are 4S derivatives and are more potent than the 4R derivatives which are compound 1(a)and 1(d). In compound 1(k), there is decrease in the inhibitory potency due to substitution of the ketone group at 4th position with IC_{50} value 21 nM. 4(S) fluoride was found to be the most potent analogue as it has least IC_{50} value of 0.6 Nm and it has 480- fold more potency than 4(R) fluoride analogue with IC_{50} value of 290 nM. Compound 8 has also potent inhibitory effect with IC_{50} value of 0.8 nM. It was concluded that among all these compounds 4-flouro-2-cyanopyrrolidine is potent DPP-IV inhibitor which can be used as therapeutic agent in lowering hyperglycemia. [10]

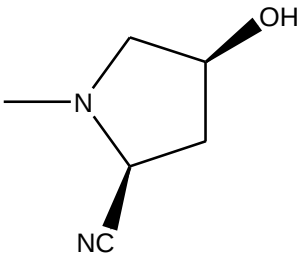
Parent molecule:



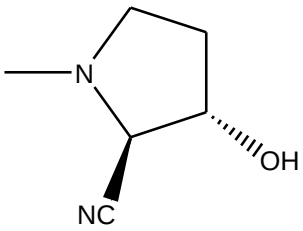
Different R groups are as:



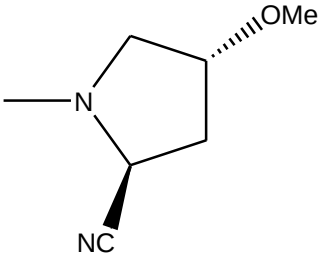
1(a)



1(b)



1(c)



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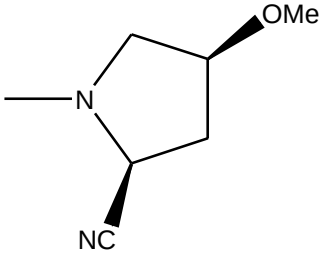

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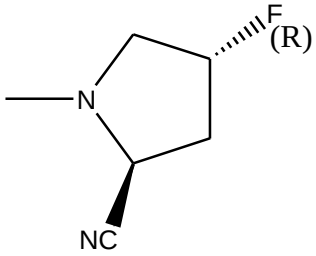

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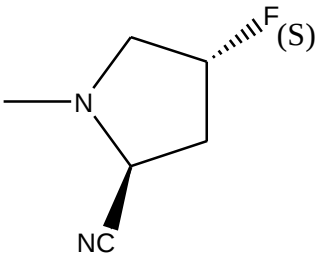

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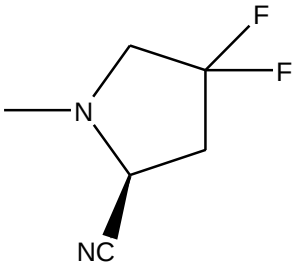

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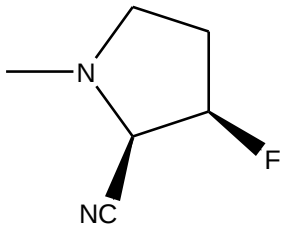
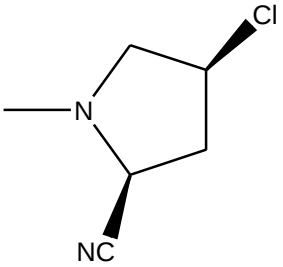


1(i)



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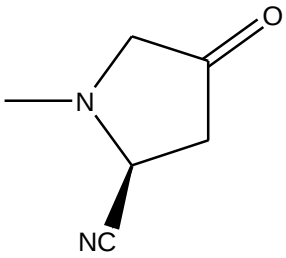
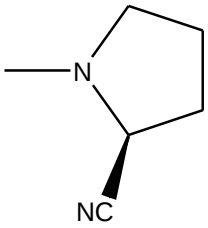
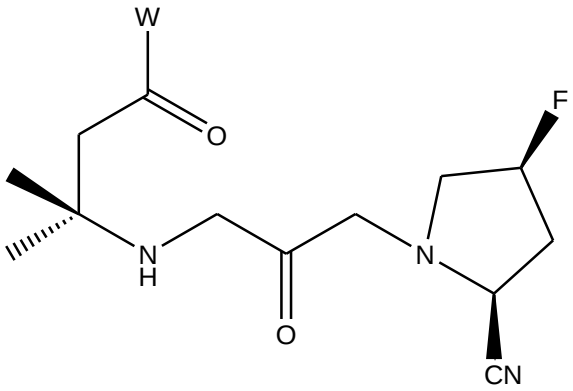


1(k)

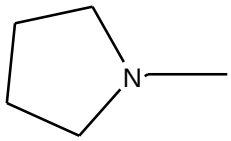


1(l)

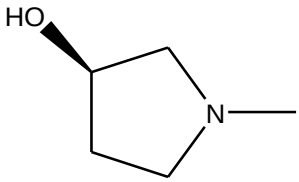


Parent molecule A

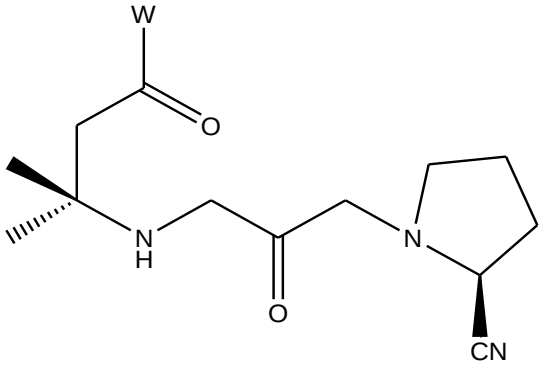
W group can be:



2(a)

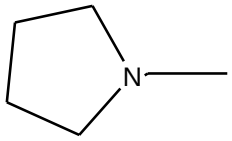


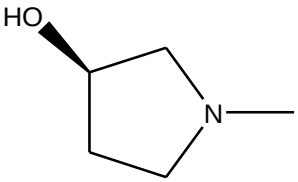
2(b)



Parent molecule B

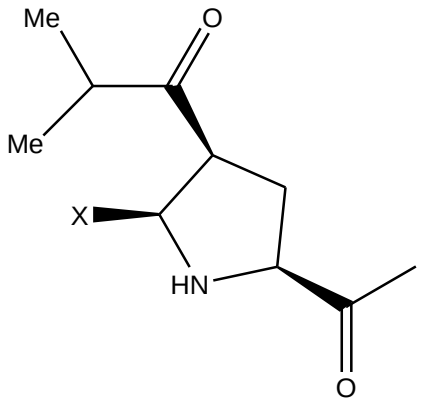
W group can be:





2(c) 2(d)

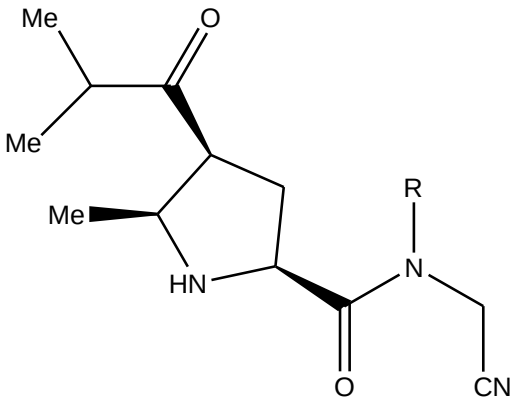
Parent molecule 2 with 2(a) of pyrrolidine compound is a poor DPP-IV inhibitor which has IC_{50} value of 15 nM but have excellent selectivity . Out of these compounds 2 (b) containing group exhibit high potency with the IC_{50} value of < 50 nM against DPP-IV and has excellent selectivity with respect to DPP8 and DPP9 which has IC_{50} value of > 20 nM. Fluoro substitution to the 3-position of cyanopyrrolidine ring gives rise to the 6 fold increase in the DPP-IV inhibitory activity like structure 2 containing 2(c) and 2(d) groups. Unsubstituted structure 1 containing 2(a) group has IC_{50} value of 71nM. Addition of polar hydroxyl group to the third position of the pyrrolidine ring in 1 structure gives rise to the 2.5 fold decrease in the DPP-IV activity with the IC 50 value of 180 nM. [11]



Parent molecule C

X= Me

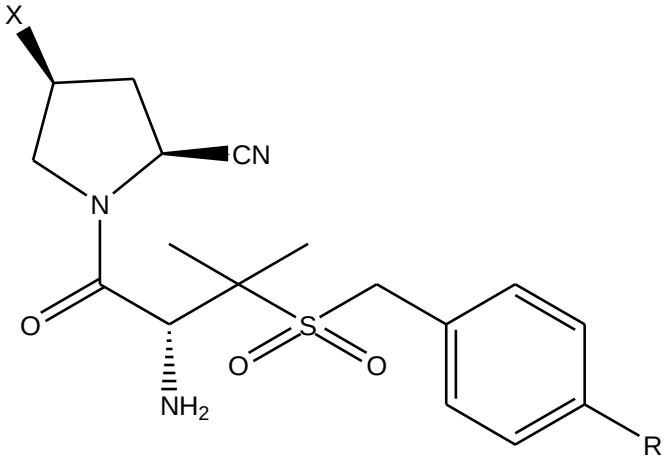
X= Et



R= Me

Parent molecule D R= Et R= H

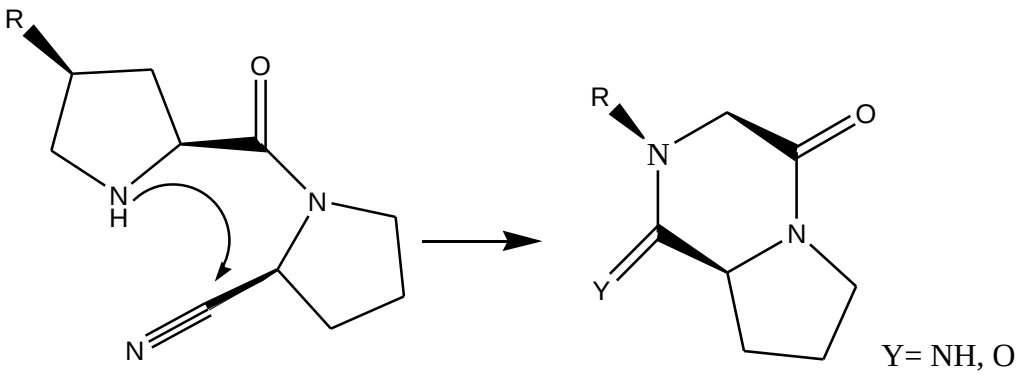
Parent molecule C has IC_{50} value of 10 nM which has X group as Me and 16 nM for the X group as Et. Parent molecule D has IC_{50} value of 13000 nM for R group as H. For R group as Me its value is 25 nM and for R value as Et its value is 24 nM. Parent molecule C is considered as most optimal structure among all including the stereochemistry. Parent molecule D with Me group as R has most potency in vitro activity and its action is of longest duration among all analogs but its action of ex- vivo activity is shorter duration than the structure 1. Structure 2 has greater isozymes selectivity than structure 1. [12]



Parent structure E

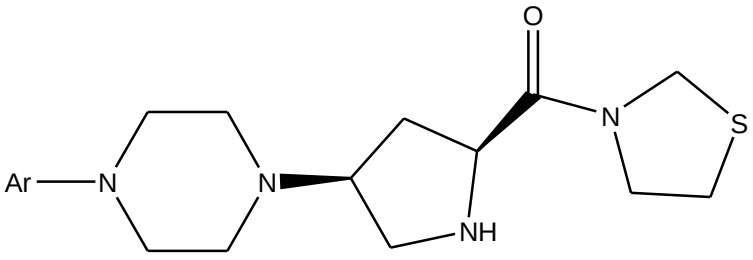
a. X= F b. X= H

Compound containing X=F has longest duration (84% DPP-IV inhibition at 1 mpk in rat at 6 h and >12 h above the IC_{50} at 0.2 mpk in dog). There is difference in the potency and in vivo drug levels of the 2-cyano-4-fluoropyrrolidine derivative with respect to its dis-fluoro counterpart. [15] When fluorine atom incorporated at C4 of the pyrrolidine ring, it imparts the unexpected differences in the compound's pharmacological properties. Compound with F group is more potent than compound with H group. It has better pharmacokinetic properties, and also stable towards the intramolecular cyclization. Both the compounds are very selective against DPP-II. [13]



Intramolecular cyclization of the L-prolyl-(S)-2-cyanopyrrolidine analogues.

The (2S)-2-cyanopyrrolidine class of compounds, such as LAF237 (2)7 and BMS-477118 (3), that includes many potent DPP-IV inhibitors. These compounds are chemically unstable due to the presence of basic amine. On the electrophilic nitrile group of the (2S)-2-cyanopyrrolidine moiety intramolecular attack occurs that results in cyclic products. [14]



Parent molecule F

Ar group is as:

3(a) 5-Cl-2-pyridyl

3(b) 5-CN-2-pyridyl

3(c) 5-NO2-2-pyridyl

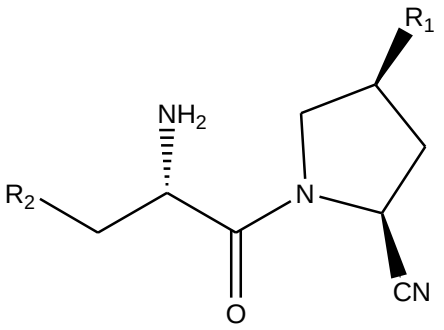
3(d) 5-CF3-2-pyridyl

3(e) 4-CN-2-pyridyl

3(f) 2- pyridyl

From the SAR studies, it was concluded that there is loss of potency due to the absence of an electrophilic trap at the P1 position. Among all the analogues in the report, 5-nitro-2-pyridine analogues 3(c) have high potency with the IC_{50} value of 0.92 nmol/L. It lacks the electrophilic trap at P1 position. Substitution at 4-cyano position on the pyridine ring observed to be most potent on the basis of the IC_{50} value. Compound with analogue 3(a) has IC 50 value = 2.5 nM. Compound with analogue 3(f) has highest IC_{50} value and less potent than all with IC50 value of 2.7nM. Compound 3(b) has IC_{50} value of 1.6 nM. While 3(d) and 3(e) compound show good potency with less IC_{50} value of 1.5 nM and 1.0 nM respectively. [15]

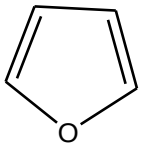
7. Alpha amino pyrrolidine - 2- carbonitrile derivative:



Parent molecule G

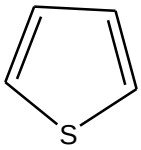
R_1 group is as:

R_2 group is as:

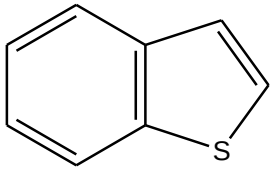


4(a) H

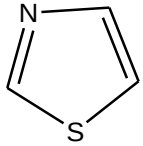
4(b) H



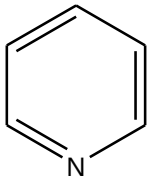
4(c) H



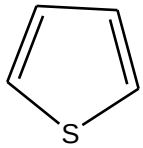
4(d) H



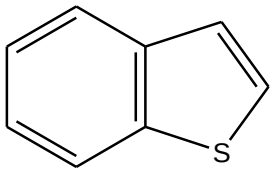
4(e) F



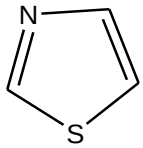
4(f) F



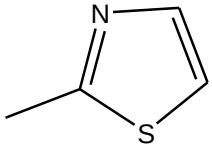
4(g)F



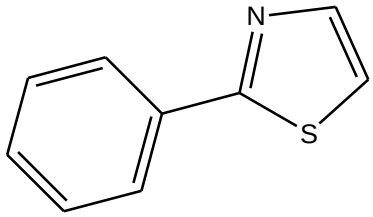
4(h) F



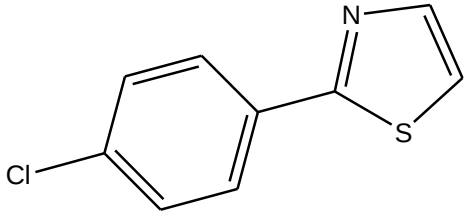
4(i)F



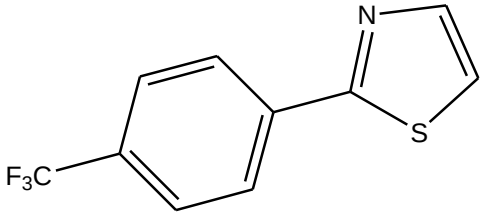
4(j) F



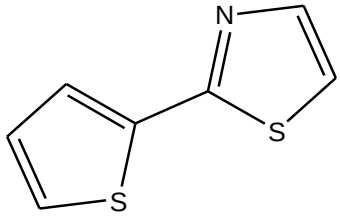
4(k) F



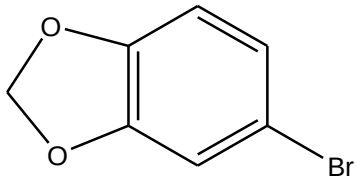
4(l) F



4(m) F

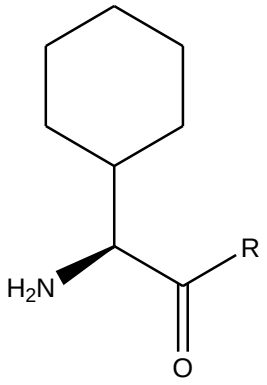


4(n) F



Compound with analogue 4(h) has IC_{50} value of 0.004nM and with analogue 14 has IC_{50} value which is least among all these analogues. Compound with analogue 4(h) is found to have positive results for the diabetic control among all these analogues. It significantly decreases the blood glucose level as compared to other groups. In dose dependent manner, the glucose tolerance capacity is improved by this compound. SAR studies found the hetero atomic moieties substituted to the alpha amino pyrrolidine - 2- carbonitrile derivatives as very selective and

potent DPP-IV inhibitors. Compounds 4(h) and 4(n) have high selectivity and potency as DPP-IV inhibitors and have good pharmacokinetic profiles with good efficacy. [16]



Parent structure H

R group are:

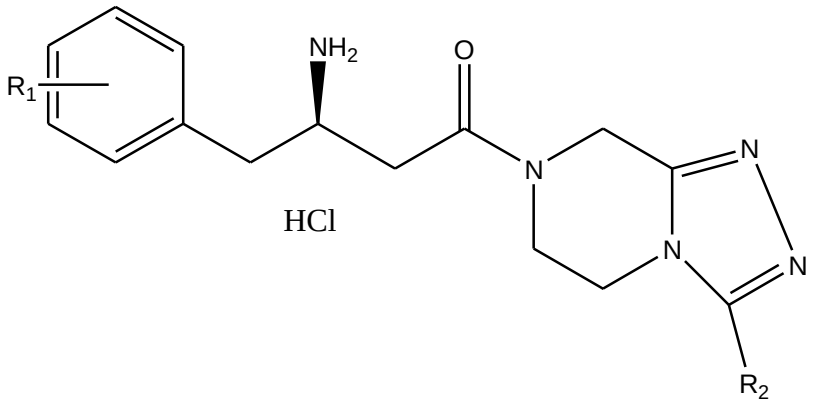
5(a) Pyrrolidine

5(b) (R, R)-3, 4-Difluoropyrrolidine

5(c) (S, S)-3, 4-Difluoropyrrolidine

5(d) cis-3, 4-Difluoropyrrolidine

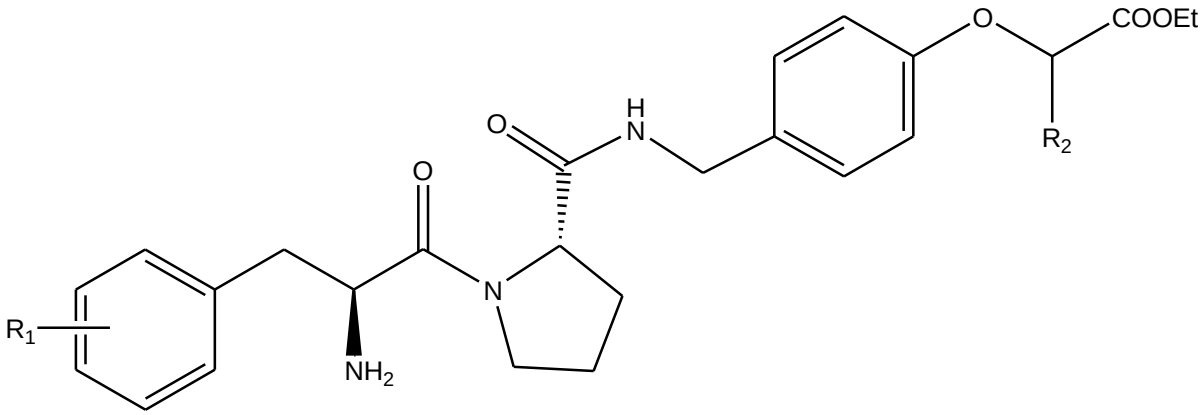
Fluorine has a useful effect on the activity of the pyrrolidide or azetidides. Trans-difluoropyrrolidides has weak activity and its pyrrolidine binding pocket did not tolerate the fluorine atoms which are vicinal at C-3 and C-4 position. [17] In syn stereochemical relationship, vicinal fluorine boosts the activity. [17] Fluorine increase lipophilicity to a small degree and occupy little more space than hydrogen. [17]



Parent molecule I

R 1 can be:	R2 can be:
6(a) 3, 4-di-F	H
6(b) 3, 4-di-F	Et
6(c) 3, 4-di-F	CF_3
6(d) 2, 5-di-F	CF_3
6(e) 2, 4, 5-tri-F	CF_3
6(f) 2, 4, 5-tri-F	H
6(g) 2, 4, 5-tri-F	CF_2CF_3
6(h) 2, 5-di-F	CH_2CF_3

The compound here with the Unsubstituted Triazole analogue 6(a) has 3 fold less activity than the piperazine. It is 7 times more potent than 3, 4-difluorophenyl analogue (IC_{50} value = 139 nM) which lacks the R-benzyl moiety like structure 1.[18] Ethyl analogue 6(b)shows 2-fold increase in potency with respect to the parent compound.

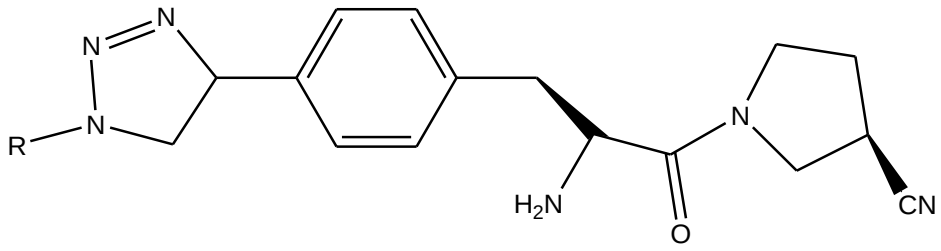


Parent molecule J

R_1 group is as:	R_2 group is as:
7(a) 4-F	H
7(b) 2-F	H
7(c) 3-CN	H
7(d) F	H
7(e) 4-Me	Me
7(f) 3-CN	Me
7(g) 3-F	Me
7(h) H	Et
7(i) 4-Me	Et
7(j) 2- CF_3	Et
7(j) 3-CN	Et
7(k) 3-CF_3	Et
7(l) H	Ph
7(m) 4-CF_3	Ph
7(n) 4-CN	Ph
7(o) 3-CN	Ph
7(p) 3-CF_3	Ph

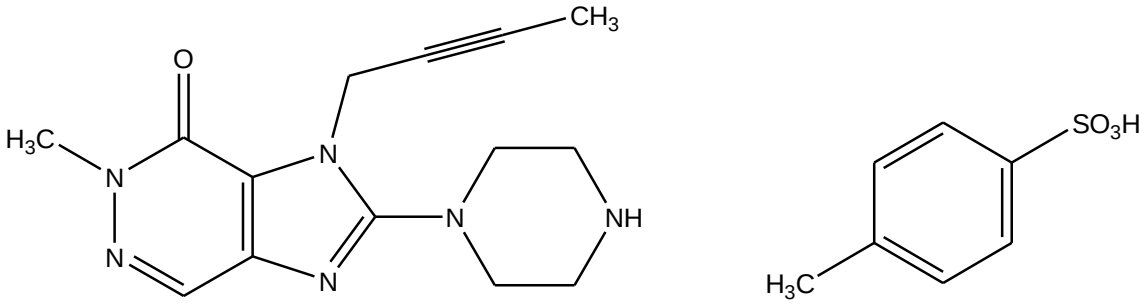
The compounds exhibit the moderate inhibitory activities. The parent compound 1 shows inhibitory activity toward DPP-IV with 98.7 % inhibition at 20 microg/ml. There is not any

improvement in the inhibitory activity due to the introduction of introducing the electron donating and electron withdrawing groups at the para position of the benzene ring of R_1. Compound with R_1 as Me has inhibition activity of 27.7%. Also compound with R_1 as OCH_3, CN, and CF_3 has milder inhibition activity of 48.1%, 29.3%, and 6.0% at 20 micro ⁄ml, respectively. There is decrease in the DPP-IV inhibition due the substitution of electron withdrawing group at ortho and Meta position of the benzene ring. Para substitution of fluorine to the ring has found be essential for DPP-IV inhibition. Fluorine substituted at ortho position has increase in inhibition potency 92.8% inhibition at 20 microg⁄ml. When R1 is taken as fluorine, the inhibition for DPP - IV has increased, like in compounds 7(a) and 7 (b) which have IC50 values of 4.56 and 8.4 microM, respectively. Compound 10, 12 and 15 has better inhibitory activity than other compounds. [19]

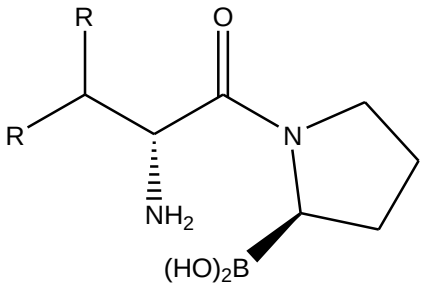


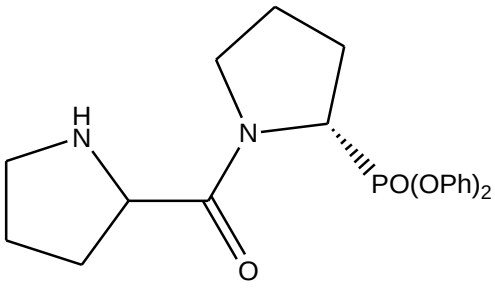
At the N- 1 position of 1, 2, 3-triazole ring substitution had a major effect on activity. When there is substitution of methyl group in the N- 1 position, compound 1 show the inhibitory activity with IC50 value of 0.265 microM. Ethyl-substitution results in IC_{50} value 0.339 microM; n-propyl-substituted, results in IC_{50} value 0.374 microM; n-butyl-substituted results in IC_{50} value 0.331 microM. I-propyl-substituted IC_{50} value 0.527 microM versus n-propyl substituted has IC_{50} value 0.374 microM, i-butyl-substituted has IC_{50} value 0.578 microM versus n-butyl-substituted has IC_{50} value 0.331 microM. Result find that the presence of a small substituent, either cyclic group or a linear alkyl group, at the N-1 position was beneficial to activity. SAR studies on (S)-phenylalanine derivatives lead to the conclusion that cyclopropyl-substituted

compound 11h has most potency in phenylalanine derivatives with an IC_{50} value of 0.247 microM. [20]



E3024 compound has IC_{50} value of 0.13 microM. It shows competition inhibition pattern. It did not affect any inhibition of the human recombinant DPP-8 and DPP-9 even at 100 micromoles/l.[21]





2

1a(R=H)
1b(R=Me)

Structure 1 consists of the boronic acid while structure 2 consists of phosphonate group acts as DPP-4 inhibitors. Both compounds show good inhibitory action against DPP-4. [22]

Research gap and objective of the proposed work:

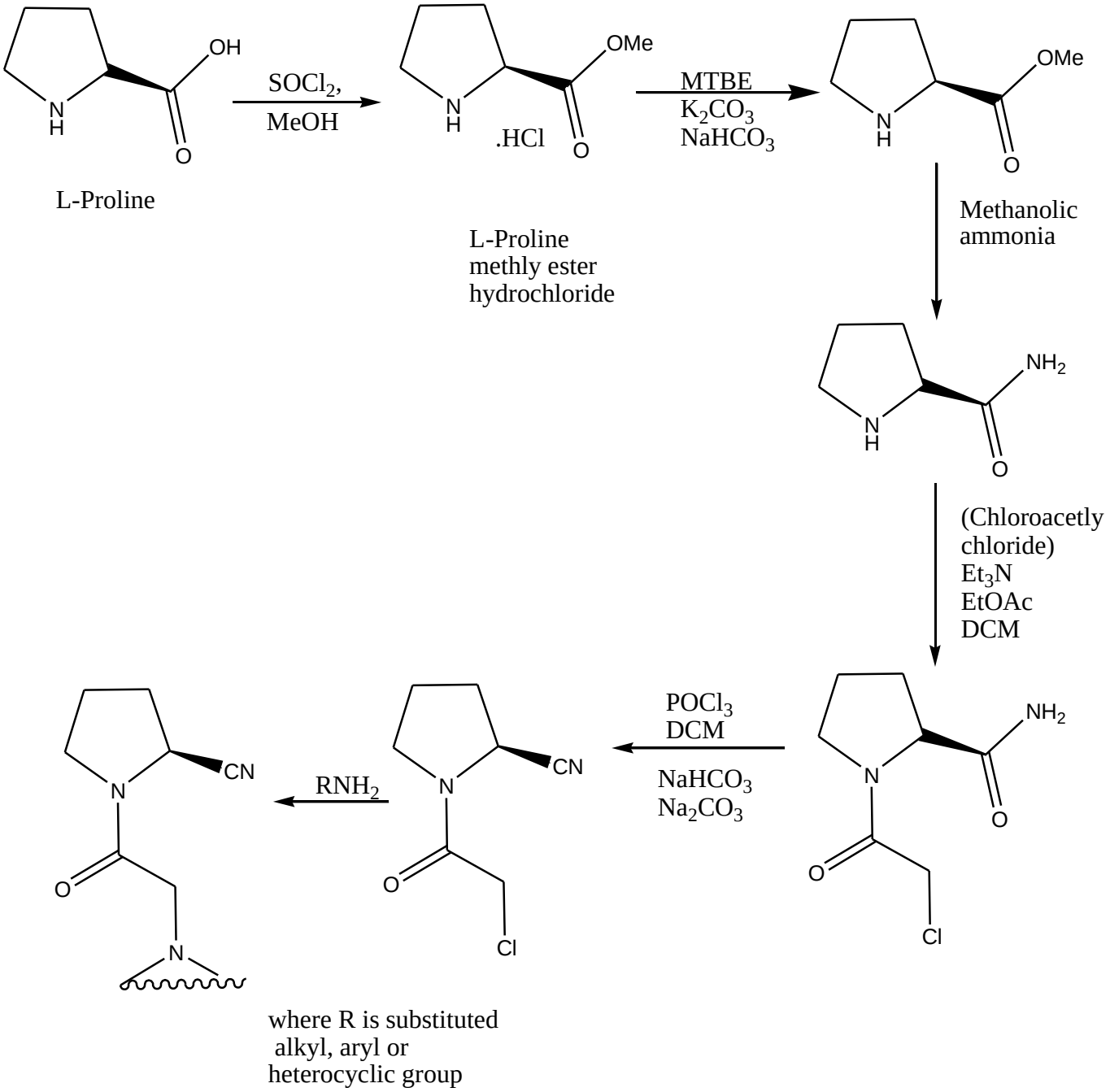
Insulin plays an important role in controlling the glucose level in humans and other vertebrates. In recent few years, there is increased interest in the field of research and development of drug molecules has been observed which cause the secretion of insulin or augmentation of insulin activity. In year 1984, GLP-I have been discovered and since then the research for discovery of novel DPP-IV inhibitor came into interest. The major drawback of GLP-I based therapies is that it has short half-life. By the action of serine protease enzyme GLP-I rapidly degraded to its biologically active form. The bioavailability and half-life of GLP-I increases due to selective inhibition of DPP-IV activity. The increased half life will increase the insulin secretion and also have many advantages in the pharmacology which includes the better health of β-cells. The potential inhibitors of DPP IV were designed and synthesized in wide range most of which are dipeptide mimics. As a result, many peptidomimetics derived compounds have been scientifically approved in market like Sitagliptin and Vildagliptin etc. While many of such peptidomimetic drugs are in the later stages of development. The major disadvantages of these molecules are these are less selective, fairly potent and take lesser time of action. Also, these molecules are difficult to synthesize and very much expensive. Therefore, it is needed to develop more selective and potent drugs which have very long lasting effect. Through the literature survey and SAR studies we have to know that introduction of different substituents at α- position of proline side chain lead to the number of novel molecules which has increased stability and selectivity.

Based upon the this data, we are designing, synthesizing and characterizing the novel peptidomimetics with the different substitution on the proline sites as the key structural feature which is useful in the field of research. The purpose of our present work is as following:

1) Designing and synthesizing novel pyrrolidine derivatives which act as novel DPP IV inhibitors.

2) Structure determination of synthesized derivatives and detection by using different techniques of spectroscopy like IR and NMR spectroscopy.

Action plan:


OH
N
H
O
L-Proline
SOCl2,
MeOH
OMe
N
H
.HCl
O
L-Proline
methly ester
hydrochloride
MTBE
K2CO3
NaHCO3
OMe
N
H
O
Methanolic
ammonia
NH2
N
H
O
(Chloroacetly
chloride)
Et3N
EtOAc
DCM
NH2
N
O
O
Cl
POCl3
DCM
NaHCO3
Na2CO3
CN
N
O
Cl
RNH2
CN
N
O
N
where R is substituted
alkyl, aryl or
heterocyclic group

Material and methods:

Materials:

Sr. No	CHEMICAL	PURCHASED FROM
1.	L-Proline	LOBA
2.	Thionyl chloride	LOBA
3.	Potassium Carbonate	LOBA
4.	Liq. Ammonia	LOBA
5.	Chloroacetyl chloride	LOBA
6.	Tri-ethyl amine	LOBA
7.	Phosphorus oxy chloride	LOBA
8.	Sodium Bicarbonate	LOBA
9.	Sodium hydroxide flakes	LOBA
10.	Methanol	LOBA
11.	tert-Butyl methyl ether	LOBA
12.	Ethyl acetate	LOBA
13.	Dichloromethane	LOBA
14.	Cyclohexane	LOBA
15.	Chloroform	MOLYCHEM
16.	2-mercaptobenzoamine	LOBA
17.	Sodium sulphate (anhy.)	RANKEM
18.	Acetone	LOBA
19.	Sodium hydride	LOBA

Methodology

Instrumentation:

Melting Point:

The Melting Point was determined with a Lab fit electrically heated apparatus (Department of Chemistry, Lovely Professional University).

Infrared Spectroscopy:

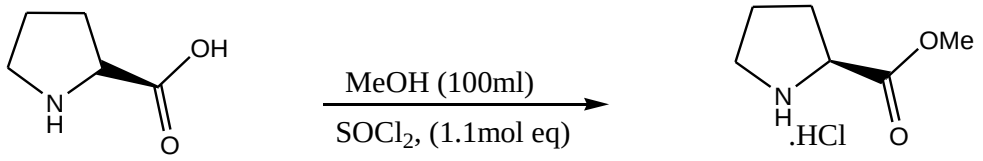
Infrared spectra were obtained using KBr pallets by SHIMADZU FTIR 8400S, Fourier Transform, Infrared spectrophotometer (Department of Chemistry, Lovely Professional University).

^{1}H-NMR:

^{1}H-NMR spectra were recorded on a …… NMR spectrophotometer at …..MHz in DMSO and $CDCl_3$, with TMS as internal reference standard. Samples for 1H-NMR are submitted in Thapar University, Patiala.

Experimental Work

1. Synthesis of L-proline methyl ester .HCl from L-proline:-



L-Proline

$C_5H_9NO_2$
Mol. Wt.: 115.13

L-Proline methyl ester hydrochloride

$C_6H_{12}ClNO_2$
Mol. Wt.: 165.62

1. Charged10 gm of proline in 100 ml of methanol at 25-35°C in100 ml round bottom flask.
2. Cooled reaction mixture to 0-5°C and Charged 7 ml of thionyl chloride (1.1mol eq) drop wise with help of dropping funnel in time interval of 30 min at 0-5°C.

   (**Observation:** The reaction involved here was exothermic.)

3. Allowed reaction mixture to come at room temperature and stirred for 17-18 hours.
4. Distilled off reaction mixture under reduced pressure at 60-65°C to get the viscous liquid.
5. Degas the viscous liquid at 45-50°C for 5 hours under reduced pressure to get the crude product.

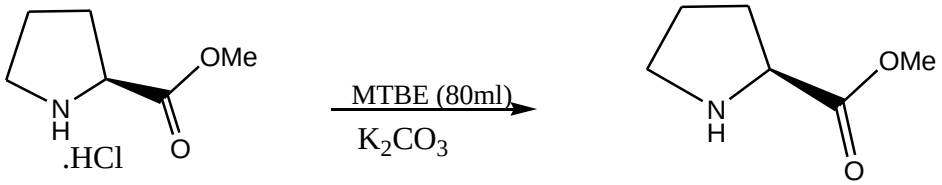
Result:

Theoretical yield = 14.3 gm

Experimental yield = 11.2 gm

% yield = 78.32 %

II. Synthesis of L-Proline methyl ester from L-Proline methyl ester hydrochloride:



L-Proline methyl ester hydrochloride
$C_6H_{12}ClNO_2$
Mol. Wt.: 165.62

L-Prolinemethyl ester
$C_6H_{11}NO_2$
Mol. Wt.: 129.16

1. Cooled 10.6 gm L-Proline methyl ester hydrochloride and 80 ml of MTBE in 250 ml RBF and cooled the reaction mass to 0-5°C.
2. Charged 50 ml of pre-cooled solution of 20% (w/v) K_2CO_3 solution at one time to the above reaction mixture.

(**Preparation of 20% solution of K_2CO_3:** Dissolved 20 gm of K_2CO_3 in distilled water and make the total volume to 100 ml.)

3. Stirred reaction mixture for 15 min and separated organic layer with the help of separating funnel and take it in a 250 ml round bottom flask.
4. Again poured 60 ml of cooled MTBE to aqueous layer and stirred for 15 min at 0-5°C.
5. Separated organic layer with the help of separated funnel.
6. Distilled off the combined organic layer over rotaevaporator under reduced pressure to get the proline methyl ester as liquid.
7. Degas the liquid at 45-50°C for 5 hours under reduced pressure to get the crude product.

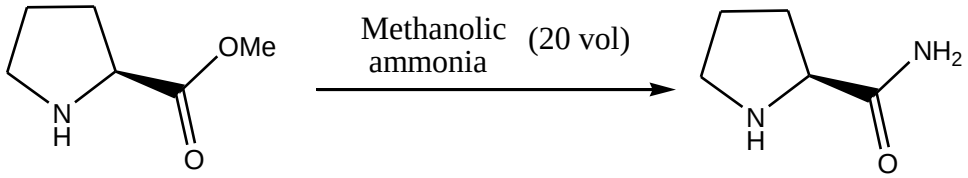
Result:

Theoretical yield= 8.73 gm

Experimental yield= 6.9 gm

% yield= 79.03%

III. Synthesis of Prolinamide from L-Proline methyl ester:



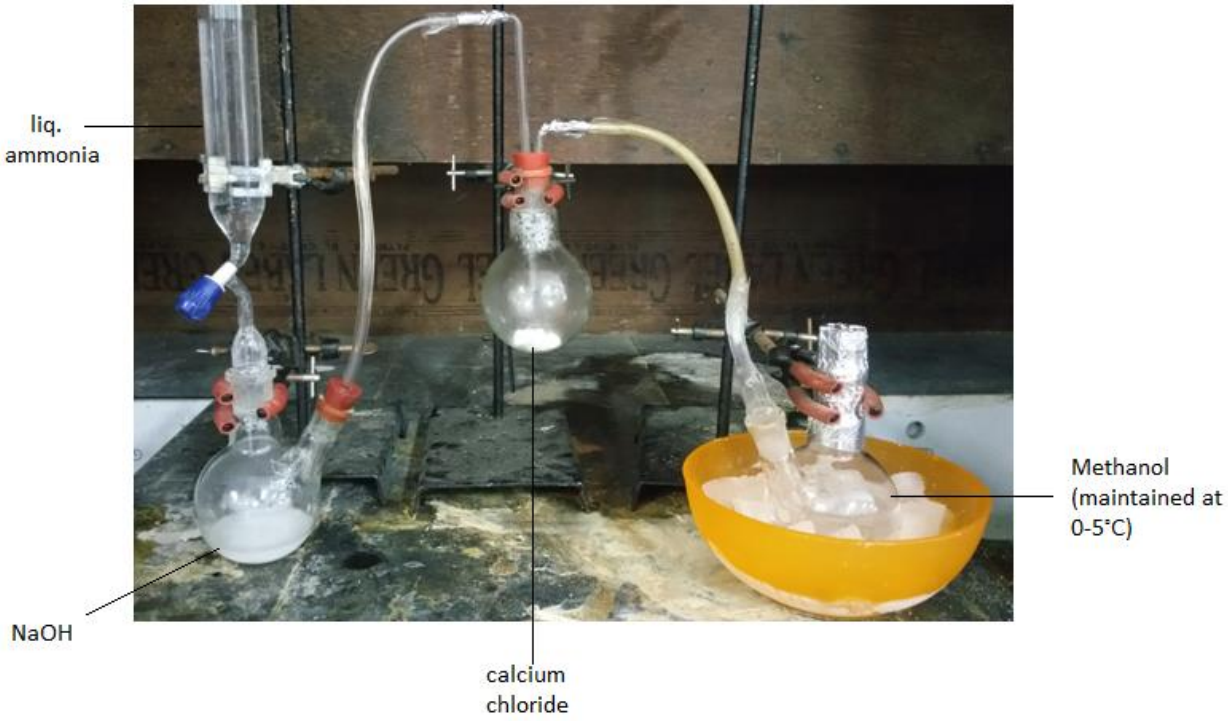
L-Prolinemethylester

$C_6H_{11}NO_2$
Mol. Wt.: 129.16

L-Prolineamide

$C_5H_{10}N_2O$
Mol Wt.114.15

Stage 1: Preparation of methanolic ammonia:



1. Assembled the reaction setup as shown in above picture.

2. Charged 50 gm of sodium hydroxide pellets in 250 ml round bottom flask.

3. Dropwise charged the liq. ammonia to it.

(**Observation:** NH_3 (g) evolved which was purged into the 100 ml methanol maintained at 0-5°C.

(Note: Addition of liq. ammonia needed to be maintained continuously for maintaining positive flow of ammonia gas in the system)

1. Continued NH_3 (g) purging to the methanol and checked weight of the methanol after every two hours.
2. Continued purging till constant weight of the methanolic ammonia solution was achieved.

(**Observation:** Time taken to achieve constant weight was 7-8 hours)

Results:

Initial weight of the methanol: 100 gm

Final weight of the methanolic ammonia solution: 119 gm

%age of ammonia in methanol: 15.96%

Stage-2: Synthesis of L-Prolinamide from L-Proline methyl ester

1. Charged 6.9 gm of L-Proline methyl ester and charged 119 ml methanolic ammonia in it to the round bottom flask and stirred reaction mixture for 6-8 hrs.

2. Distilled off the methanolic ammonia under reduced pressure at 60-65°C.

(Observation: Precipitates were obtained)

3. Charged the precipitates obtained in another round bottom flask along with 60 ml of MTBE and stirred for it for 20 min. Precipitates were filtered and washed with 10 ml of MTBE.
4. Dried the solid at 45-50°C for 5 hours under reduced pressure to get the crude product.
5. Stored dried precipitate of L-prolinamide in anhydrous environment.

Results:

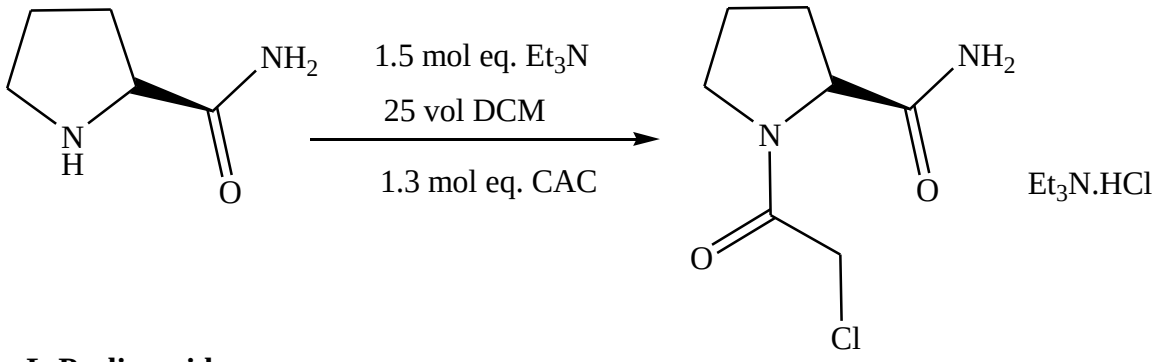
Theoretical Yield= 6.09 gm

Experimental Yield= 4.9 gm

%age yield= 80.45%

Melting Point= 97-103˚C

IV. Synthesis of 2-chloroacetyl pyrrolidine-2-carboxamide from L-Prolinamide:



L-Prolinamide

$C_5H_{10}N_2O$
Mol.Wt.: 114.15

2-chloroacetyl pyrrolidine-2-carboxamide

$C_7H_{11}N_2O_2Cl$
Mol. Wt.: 190.5

1. Charged 10 gm of L-Prolinamide in 250 ml round bottom flask with 100 ml DCM and stirred the reaction mixture.
2. Charged 18.04 ml (1.5 mol eq) of the triethylamine to same round bottom flask and stirred till it become a clear solution.
3. Charged 8.97 ml (1.3 mol eq) of the chloroacetyl chloride in another round bottom flask and added 40 ml of DCM to it.
4. Stirred and maintained the temperature of this reaction mass from 0-5℃ and dropwise charged reaction mass of step-2 to chloroacetyl chloride and DCM solution in the interval of 45-60 min.
5. Stirred this reaction mass for 3 hours.
6. Distilled the reaction mass under reduced pressure and charged 50 ml of ethyl acetate.

7. Heated the reaction mass to 45-50^{o}C and stirred for 30 minutes.
8. Filtered the solid on Buchner funnel under vacuum and washed the solid with additional 10 ml of ethyl acetate.
9. Dried the solid at 45-50^{o}C for 5 hours under reduced pressure to get the crude product.

Result:

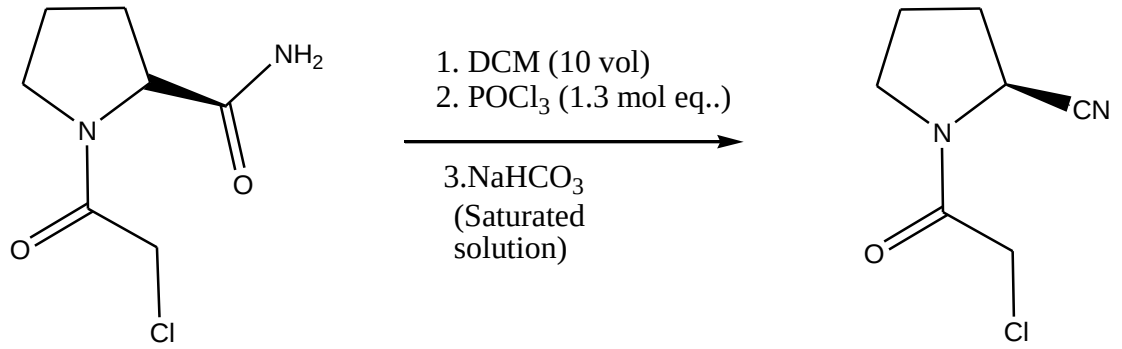
Theoretical Yield= 4.67 gm

Experimental Yield= 2.6 gm

%age Yield= 5.6%

Melting Point= 155- 158℃

V. Synthesis of 2-chloroacetylpyrrolidine-2-carbonitrile from 2-chloroacetyl pyrrolidine-2-carboxamide:



2-chloroacetyl pyrrolidine-2-carboxamide
$C_7H_{11}N_2O_2Cl$
Mol Wt: 190.5

2-chloroacetylpyrrolidine-2-carbonitrile
$C_7H_9N_2ClO$
Mol. Wt. : 172.5

1. Charged 5 gm of 2-chloroacetyl pyrrolidine-2-carboxamide in 50 ml (10 vol) of Dichloromethane in round bottom flask.
2. Charged 5.23 gm (1.3 mol eq.) of $POCl_3$ in the same round bottom flask. Stirred and refluxed reaction mass at temperature 50-60℃ for 6-7 hours.

3. Charged saturated solution of $NaHCO_3$ to same round bottom flask till reaction mass turn basic in nature.
4. Charged 100 ml dichloromethane to obtained solution and stirred for 2mins.
5. Separated organic layer using separating funnel and charged it in a 250ml round bottom flask.
6. Charged 100 ml of DCM in remaining reaction mixture and stirred for 5 mins.
7. Separated organic layer using separated funnel and combined organic layer.
8. Dry it over anhydrous Na_2SO_4 and distilled organic layer under reduced pressure at 45-50^oC.
9. Washed the crude product with hexane and filtered the product.
10. Dried the solid at 45-50^oC for 5 hours under reduced pressure to get the crude product.

Results:

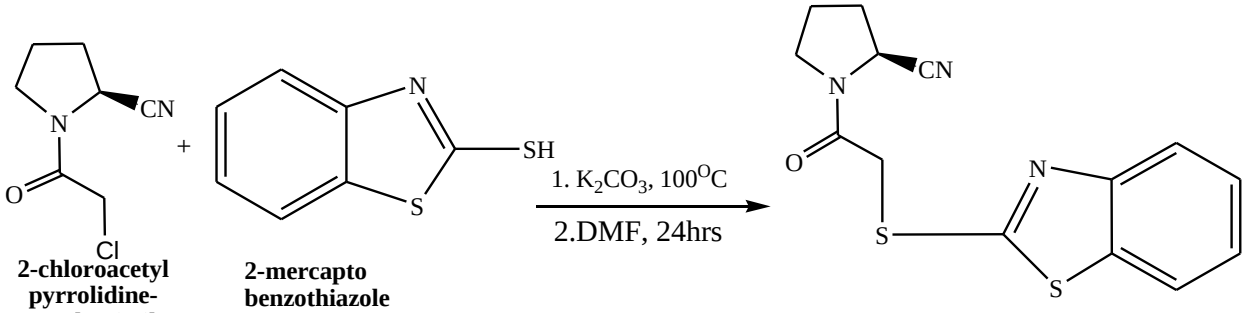
Theoretical Yield= 4.5 gm

Experimental Yield= 3.4 gm

%age Yield= 75.56%

Melting Point= 85-88℃

VI. Synthesis of 2-(mercaptobenzothiazole) acetylpyrrolidine-2-carbonitrile from 2-(chloroacetyl) pyrrolidine-2-carbonitrile:



2-chloroacetyl pyrrolidine-2-carbonitrile
$C_7H_9N_2OCl$
Mol Wt.: 172.5

2-mercapto benzothiazole
$C_7H_5S_2N$
Mol Wt.:167.25

2-mercaptobenzothiazole
$C_{14}H_{13}N_3OCl$

1. Charged 3 gm (1 mol eq.) of 2-(chloroacetyl) pyrrolidine-2- carbonitrile and 3.19 gm (1.1 mol eq.) 2-mercaptobenzothiazole, charged 2.8 gm (1.2 mol eq.) K_2CO_3 and 15 ml DMF in 100 ml round bottom flask.

2. Heated the reaction mixture to $100^{O}C$ and stirred for 24 hours.

3. Cooled the reaction mass to 25-$30^{O}C$ and charged 30 ml of water.

4. Extracted the product with 30 ml of DCM.

5. Separated the DCM layer with the help of separating funnel and again extracted aqueous layer with 30 ml of DCM.

6. Combined DCM layer and washed the DCM layer with 15 ml of water.

7. Distilled off the DCM layer under reduced pressure of 45-$50^{O}C$ to obtain the crude product.

8. The crude product was purified by column chromatography to get desired product at 45 % ethyl acetate and hexane.

Results:

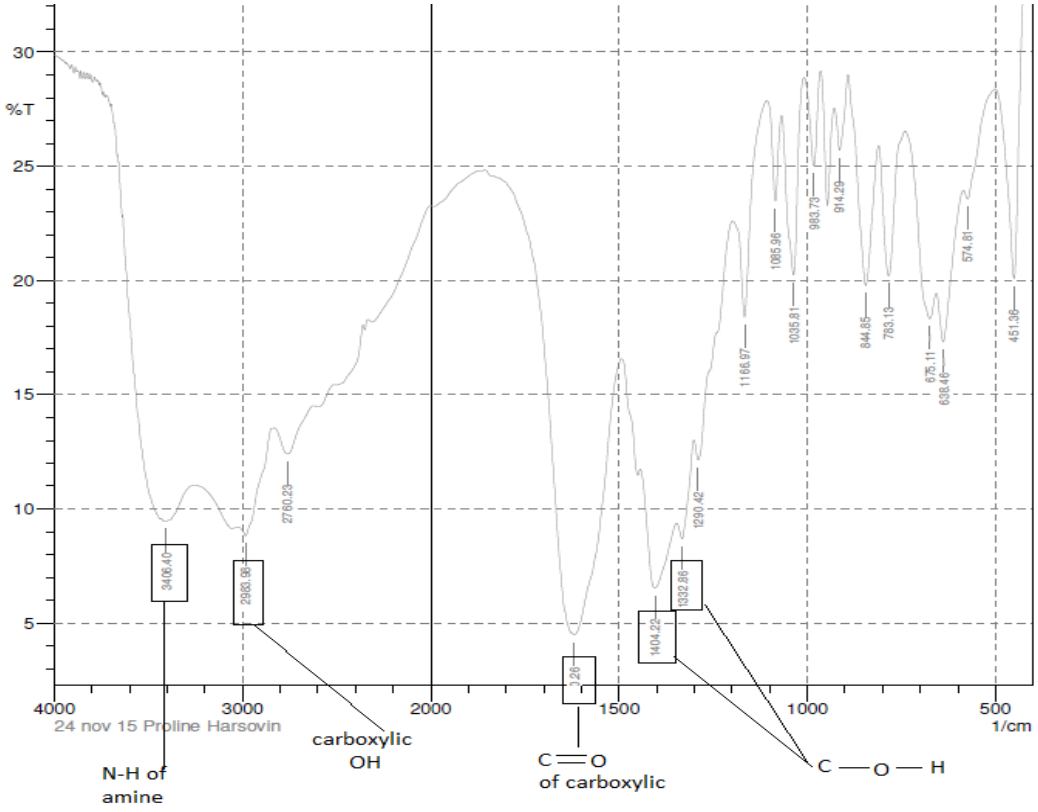
Theoretical yield= 4.76 gm

Experimental yield= 0.99 gm %age yield= 20%

RESULTS AND DISCUSSIONS:

I. Synthesis of L-Proline methyl ester from L-Proline:

1. L-Proline:



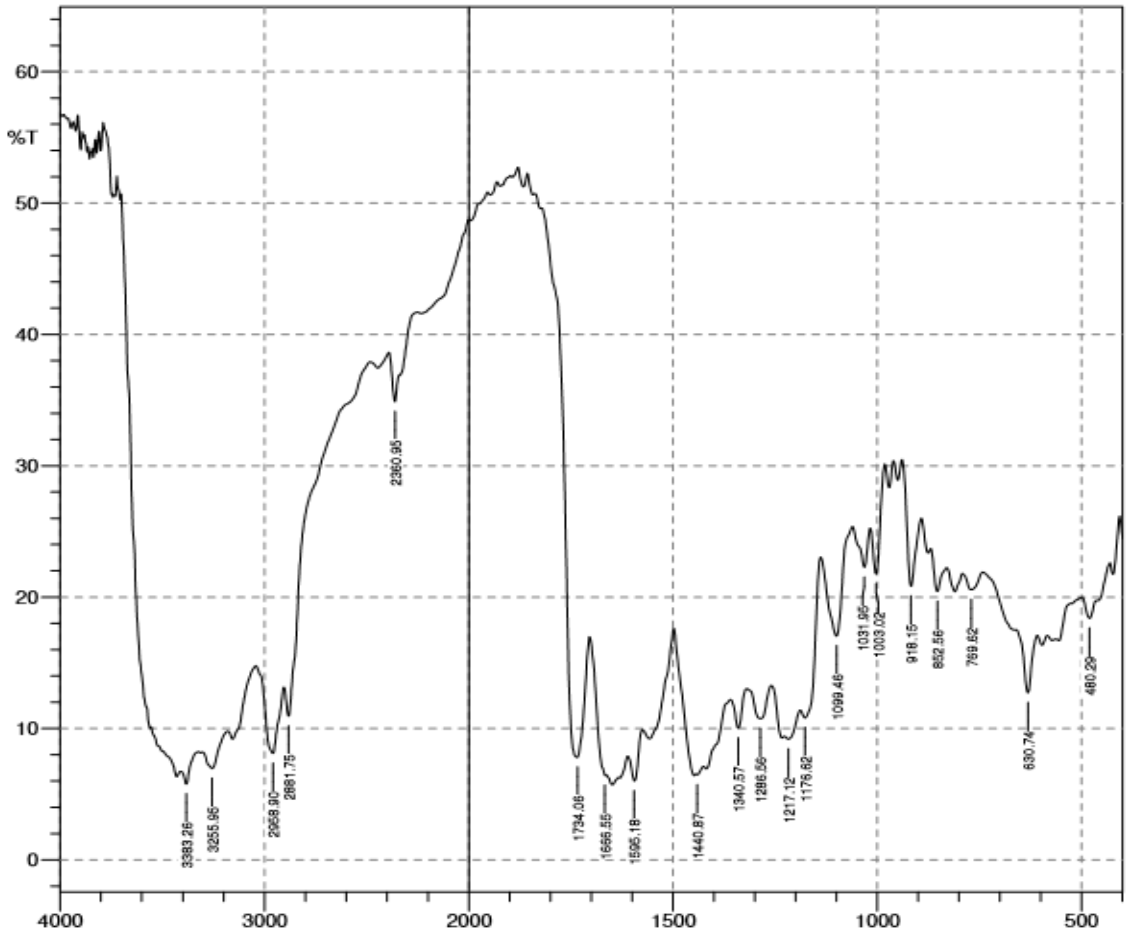
(Fig.1) I.R. Spectra of L-proline

Important Peaks:

1. v(N-H) of amine gave its characteristics absorption peak at 3406 cm^{-1}.
2. v(C-H) of sp^3 C-H gave its characteristic absorption peak at 2983 cm^{-1}.
3. v(O-H) carboxylic OH gave its characteristics peak at 2760cm^{-1}.
4. v(C=O) carboxylic acid C=O gave its characteristics peak at 1698cm^{-1}.

Sample for ^{1}H-NMR spectra submitted.

2. **L-Proline methyl ester:**



(**Fig.2) I.R. Spectra of L-Proline methyl ester**

Important Peaks:

1. v(N-H) stretching of secondary amine gives its characteristics peak at 3383.26cm^{-1}.
2. v(C=O) stretching of ester give its characteristics peak at 1734.06cm^{-1}.
3. v(C-H) of sp^3 C-H gives its characteristics peak at 2958.9cm^{-1}.
4. v(C-O) stretching of ester gave its characteristics peak at 1217.12cm^{-1}.

Sample for ^{1}H -NMR spectra submitted.

Result of TLC method for L-Proline methyl ester:

Sample dissolved in: Methanol

Solvent used: Ethyl acetate and hexane (7:3)

Distance moved by the solvent= 4.2cm

Distance moved by the sample of L-Proline methyl ester= 3.1cm

Distance moved by the reaction mixture= 2.8cm

Distance moved by the L-Proline= 1.5cm

Rf value of L-Proline methyl ester= Distance moved by the L-proline methyl ester/ Distance moved by the solvent

Rf value of L-Proline methyl ester= 3.1/4.2= 0.73

Rf of reaction mixture= 2.8/4.2= 0.6

Rf of the reactant L-Proline= 1.5/4.2= 0.3

Rf value of the product formed is more than the reactant used which means that the reactant L-Proline is being change to the L-Proline methyl ester. Product L-Proline methyl ester rises more in the TLC than the reactant that is L-Proline.

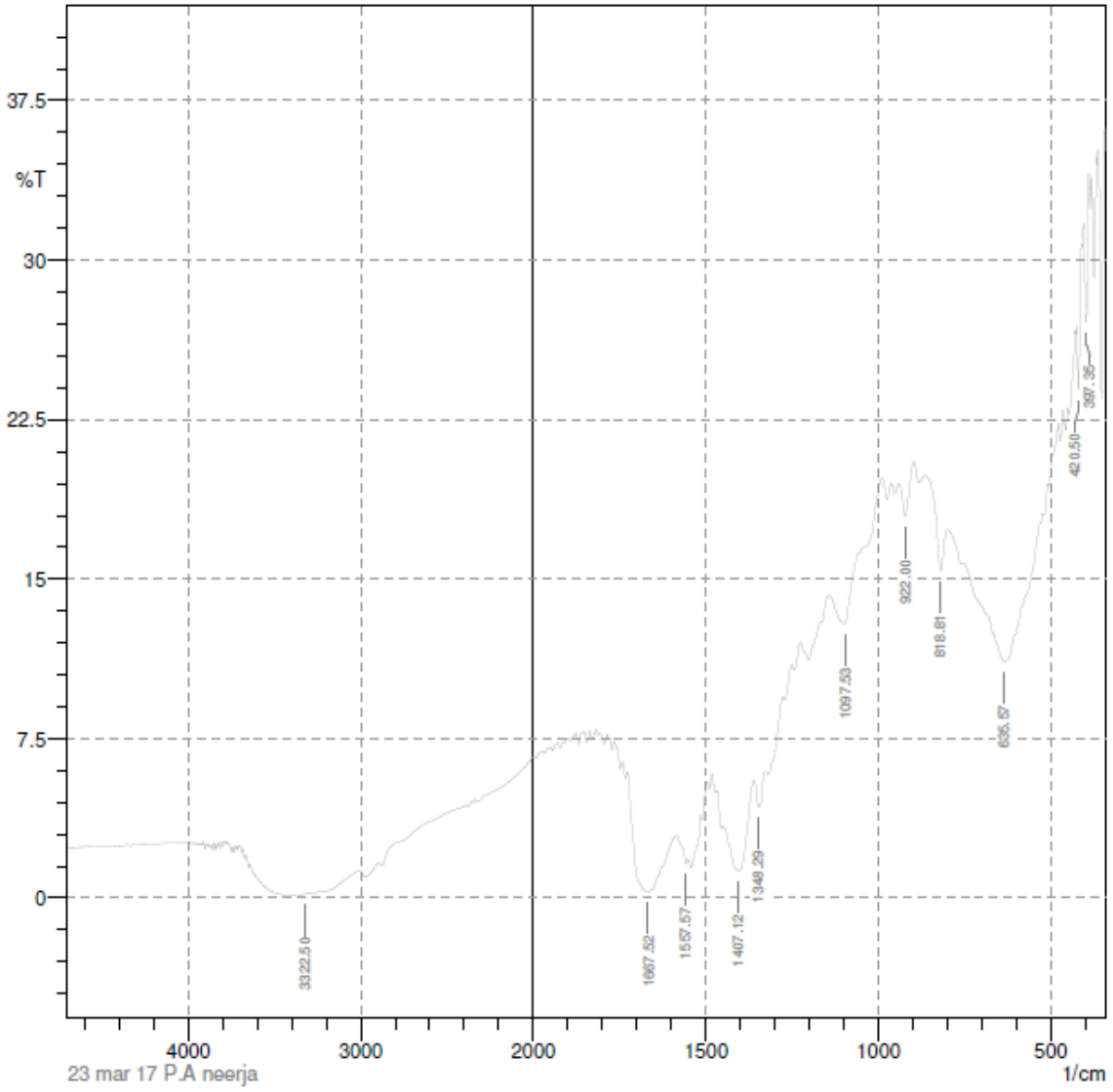
Analysis of IR data of L-Proline methyl ester to predict the synthesis of L-Proline methyl ester from L-Proline:

IR spectra of L-proline give peak at 2760 cm^{-1} which is the corresponding peak of ν (O-H) of carboxylic acid of L-proline. In contrast, there is no such peak observed at this region in IR spectra of L-Proline methyl ester (Fig.2) which supports that carboxylic O-H group has been replaced. Further, ν(C=O) in L-Proline appears at 1698 cm^{-1} due to the presence of O-H adjacent to C=O which withdraw the electron density of C=O while in L-Proline methyl ester, ν(C=O)

appears at higher wavenumber i.e. at 1734.06cm^{-1} which reveals that carboxylic OH in L-Proline has been replaced by OMe.

II. Synthesis of L-Prolineamide from L-Proline methyl ester

3. L-Prolineamide:



(Fig.3) I.R. spectra of L-Prolineamide

Important Peaks:

1. ν(N-H) of amide gives its characteristic peaks at 3322.5cm^{-1}.
2. ν (N-H) symm give its characteristic peaks at 3162cm^{-1}.
3. ν (C-H) of sp3 C-H gives its characteristics peak at 2967cn^{-1}.
4. ν (C=O) of amide gives its characteristics peak at 1667.52cm^{-1}.
5. ν (C-N) amide gives its characteristics peak of 1097.53cm^{-1}.

Sample for ^{1}H-NMR spectra submitted.

Result of TLC method for L-Prolineamide:

Solvent used: Ethyl acetate and hexane (9:1)

Distance moved by L-Proline methyl ester= 4.2cm

Distance moved by L-Prolineamide= 1.3cm

Distance moved by solvent= 5.1cm

Rf value of L-Prolineamide= Distance moved by L-Prolineamide / Distance moved by solvent

Rf value of L-Prolineamide= 1.3/5.1= 0.25

Rf value of L-Proline methyl ester= 4.2/5.1= 0.82

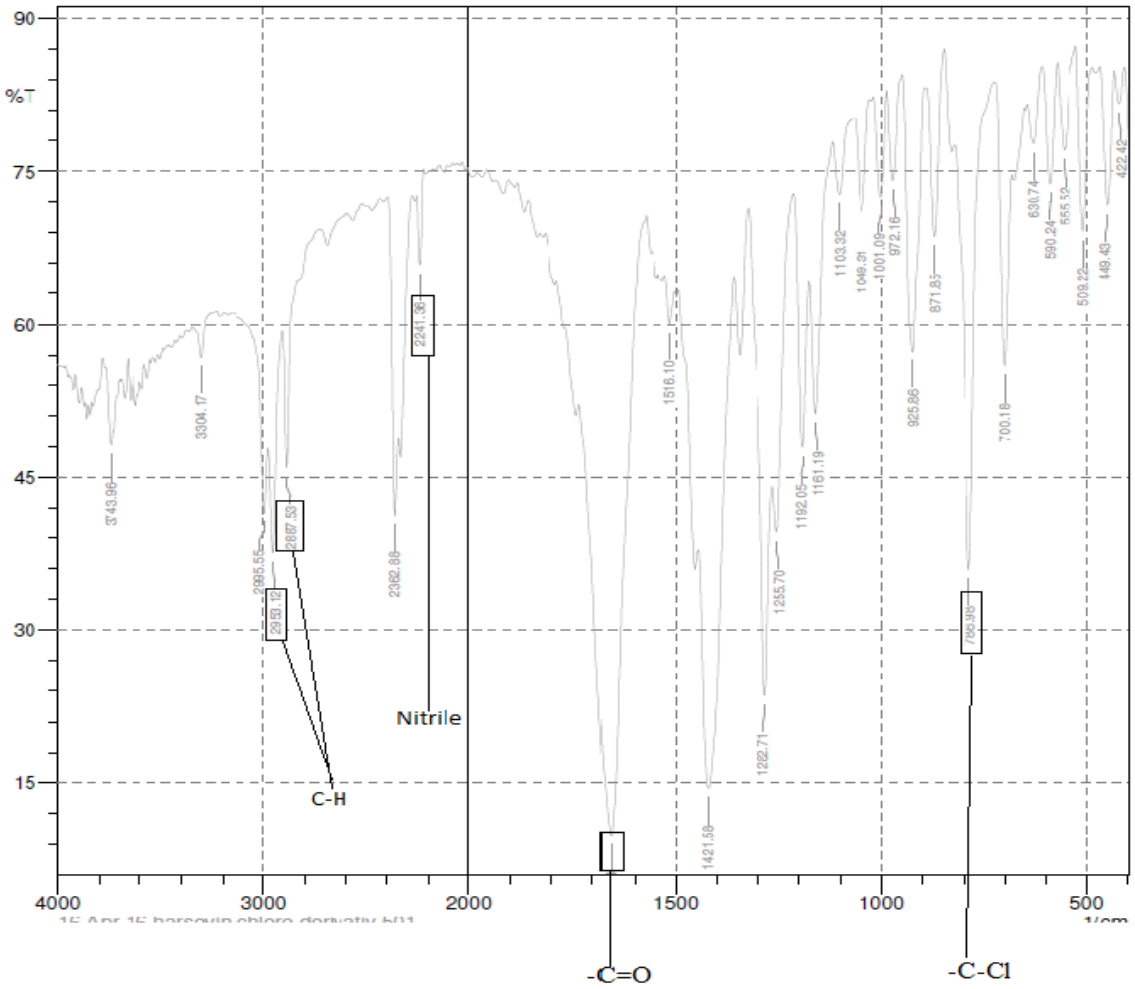
Rf value of L-Prolineamide is lower than Rf value of L-Proline methyl ester. As L-Prolineamide rise less in the solvent system as compared to L-Proline methyl ester which means that the L-Proline methyl ester is being converted into L-Prolineamide.

Analysis of IR data of L-Proline methyl ester and L-Prolinamide in Synthesis of Prolinamide from L-Prolinemethylester*:*

ν(C=O) in spectrum of L- proline methyl ester(Fig.2) appeared within range of 1733-1735 cm^{-1} while ν(C=O) in spectra of L-proilineamide (Fig.3) appears at wavenumber 1667.52cm^{-1}. This decrease in wavenumber of ν(C=O) is due to the shift of the electron density of C=O towards N of amide which support that L-Proline methyl ester has been converted to L-prolinamide.

III. Synthesis of 2-chloroacetylpyrrolidine-2-carbonitrile from L-Prolinamide:

4. 2-chloroacetylpyrrolidine-2-carbonitrile:



(Fig.4) IR spectra of 2-chloroacetylpyrrolidine-2-carbonitrile

Important Peaks:

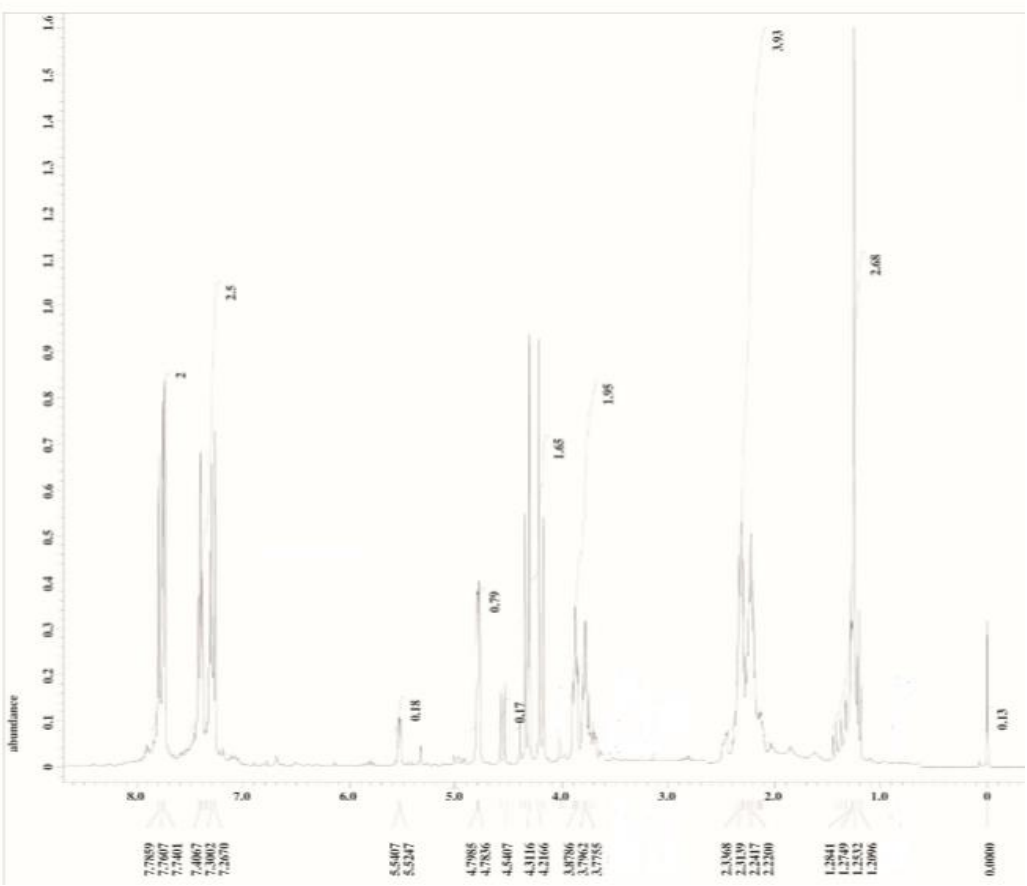
1. ν(C-H) stretching gives its characteristics peak at 2887-2995cm^{-1}.
2. ν($C\equiv N$) gives its characteristics peak at 2241cm^{-1}.
3. ν(CO) gives its characteristics peak at 1658cm^{-1}.
4. ν(C-Cl) gives its characteristics peak at 700cm^{-1}.

Analysis of IR spectra of L-Prolinamide and 2-chloroacetylpyrrolidine-2-carbonitrile to confirm the formation of 2-chloroacetylpyrrolidine-2-carbonitrile:

In IR spectra of L-prolinamide (Fig.3) medium, broad peaks were observed in the region 3322.5 cm^{-1} which corresponds to N-H stretching of amine while in IR spectrum of 2-chloroacetylpyrrolidine-2-carbonitrile (Fig.4) there is no broad or medium band in that region which shows that hydrogen of N-H bond in L-prolinamide has been replaced by acetal chloride group in 2-chloroacetylpyrrolidine-2-carbonitrile. Further, IR spectrum of 2-chloroacetylpyrrolidine-2-carbonitrile contains a sharp but low intensity peak at 2241cm^{-1} corresponding to nitrile group which indicate that the $-CONH_2$ of L-prolinamide has been converted to $C\equiv N$ confirming the formation of 2-chloroacetylpyrrolidine-2-carbonitrile.

IV. Synthesis of 2-(mercaptobenzothiazole) acetylpyrrolidine-2-carbonitrile from 2-(chloroacetyl) pyrrolidine-2-carbonitrile:

5. 2(mercaptobenzothiazole)acetylpyrrolidine-2-carbonitrile:



1-H NMR Peaks: δ 1.20-1.28 (m, 2H, -CH2), 2.22-2.23 (m, 2H, -CH2), 3.77-3.87 (m, 2H, -CH2), 4.21 (s, 2H, -CH2), 4.78-4.79 (m, 1H, -CH2), 7.26-7.40 (m, 2H, Aromatic), 7.74-7.78(m, 2H, Aromatic).

CONCLUSION:

Literature survey has revealed that gliptins have been emerged as potent therapeutic agents for diabetes till date but still it has many side effects (like hypoglycemia and weight loss) associated with present gliptins which are either marketed as drug product or they are in the advance stage of clinical trials. Thus, desired drug bioavailability, efficacy along with the longer duration of action still remain the need of the hour which has led to various structure modifications in gliptin family.

In the present work, an attempt has been made to synthesize the novel pyrrolidine based derivatives which may overcome the present drawbacks associated with the current therapies. The synthesized compounds have been subjected to the various analytical techniques like IR, ^{1}H-NMR, melting point etc. which support their formation. In the mere coming time, these compounds may be explored for their biological activities or docking studies to check their potential applications as DPP IV inhibitors.

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