# Advancements in the Anti-Diabetes Chemotherapeutics Based on Amino Acids, Peptides, and Peptidomimetics

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**Abstract:** Diabetes Mellitus (DM) is a highly prevalent chronic disease. Recent years have witnessed development of many new oral drugs; novel insulin analogs and their delivery systems for the treatment of patients with either type-1 or type-2 DM. The impetus for developing new antidiabetic drugs comes from the unmet need of pharmacological tools that allow diabetic patients to achieve recommended glucose control targets by precise, safe and effective ways. The number of people afflicted with DM worldwide has increased considerably in recent years and is projected to increase dramatically over the next decades. In the recent times, design and synthesis of bioactive peptides and peptidomimetics has undergone a paradigm shift. Non-proteinogenic amino acids, peptides and peptidomimetics are emerging as novel drug candidates for the treatment of various diseases and/or disorders. This review mainly discusses the advancements in the usage of unnatural amino acids, peptides and peptidomimetics as potential therapeutic agents for the treatment of DM.

**Keywords**: Diabetes, non-proteinogenic amino acids, peptides, peptidomimetics.

## 1. INTRODUCTION

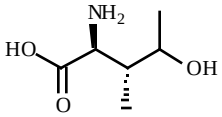
Diabetes Mellitus (DM) is classified as a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, and increased risk of complications from vascular disease [1]. Most patients can be classified clinically as having either type 1 DM or type 2 DM. The Third National Health and Nutrition Examination Survey, conducted in the United States of America between 1988 and 1994, estimated that approximately 16 million people above the age of 20 years suffer from diagnosed and/or undiagnosed DM [1]. Out of this approximately 90–95% suffer due to type 2 DM. The prevalence of DM rose from 4.9% in 1990 to 6.9% of population in 1999, primarily because of an increase in the prevalence of obesity. It is estimated that currently125 million people have diabetes, and by the year 2010 this number is expected to approach 220 million [1]. Type 2 DM is contributor to considerable morbidity in the form of metabolic complications, vision disorders, neuropathy, kidney disease, peripheral vascular disease, ulcerations, amputations, heart disease, stroke, digestive diseases, infection, oral complications, and depression. The associated mortality rate has been estimated at 5.5% of total patient annually, and the disease is known to reduce life expectancy between 5-10 years [2].

Amino acids and peptides are key regulators in various cellular and intercellular physiological responses, and therefore possess enormous potential for the treatment of various pathologic conditions. Despite this potential, amino acids and peptides have seen limited use as clinically viable drugs chiefly due to their high cost and poor drug-like properties. However, with advancement in economical large-scale manufacturing and innovative delivery systems, design and synthesis of these biomolecules has undergone a paradigm shift. Incorporation of novel unnatural amino acids into bioactive peptides at suitable residues has proved to be a powerful tool for understanding ligand-receptor interactions, increasing activity of parent peptides, and peptidomimetic design [3]. This review article encompasses the recent advancement in the area of amino acids, peptides and peptidomimetics in the treatment of diabetes.

## 2. AMINO ACIDS AS ANTIDIABETIC THERAPEUTICS

### 2.1. L-4-Hydroxyisoleucine

L-4-Hydroxyisoleucine (4-OH-Ile) (**1**) Fig. (**1**), a non-proteinogenic amino acid extracted and purified from fenugreek seeds displayed in vitro and in vivo insulinotropic activity of great interest because its stimulating effect is related to the augmentation of glucose concentration in the medium [4,5].




**1**

**Fig. (1).**

Such a glucose dependency is not shared by sulfonylureas; the insulinotropics currently used for the treatment of type 2 DM, and thus hypoglycemia remains a common undesirable side effect of sulfonylurea treatment. Therefore, 4-OH-Ile, found only in plants, because of insulinotropic action is considered as a novel secretagogue with potential application for type 2 DM treatment.

### 2.2. *N*-Acylphenylalanines

In a screening study of numerous compounds for antidiabetic activity, *N*-benzoyl-DL-phenylalanine (**2**) was

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found to possess hypoglycemic activity [6]. The comparison of activities of the enantiomers of compound **2** led to the observation that *N*-benzoyl-D-phenylalanine has activity three times superior to that of **2**; whereas, *N*-benzoyl-L-phenylalanine was found to be inactive. Thus, a series of analogs were synthesized and their structure-activity relationship study resulted in development of *N*-(4-ethylbenzoyl)-D-phenylalanine (**3**) with activity fifty times superior to that of **2**. Further investigations on the structure of the acyl moiety led to potent *N*-(cyclohexylcarbonyl)-D-phenylalanine (**4**) Fig. (**2**) [7]. Potent bio-efficacy of **4** suggested that the planar structure of the acyl group in *N*-benzoyl-D-phenylalanine is not always necessary for activity, and various analogs of **4** with modified cyclohexylcarbonyl group were synthesized and evaluated for blood glucose lowering activity. This resulted in the discovery of a highly active compound, *N*-[(*trans*-4-isopropylcyclohexyl)-carbonyl]-D-phenylalanine (**5**, nateglinide), which showed 20% blood glucose decrease at the oral dose of 1.6 mg/kg in normal fasted mice [7].

The novel oral hypoglycemic agent, nateglinide (**5**) Fig. (**2**) is a non-sulfonylurea insulin secretagogue, and its pharmacokinetic features include rapid absorption and elimination [6,8]. The drug has a dipeptide like structure, and its interaction with peptide transporters PEPT1 and PETP2 that mediate the absorption of various peptide-like drugs was investigated. Nateglinide (**5**) produced potent inhibitory effect on [$^{14}$C]glycylsarcosine uptake by the human colon adenocarcinoma cell line Caco-2 and rat PEPT-transfectants. Kinetic analysis revealed that these inhibitory effects were noncompetitive. Furthermore, $Na^+$-coupled alanine or threonine uptake by Caco-2 cells was not inhibited by nateglinide suggesting that the inhibitory effect of nateglinide on peptide transporters was not due to nonspecific interaction. In conclusion, nateglinide inhibit the transport activity of PEPT1 and PEPT2 though is not transported through these transporters. Thus, nateglinide belong to orally effective novel class of unnatural amino acids, and is a promising antidiabetic agent.

### 2.3. Dipeptidyl-Peptidase IV (DPP IV) Inhibitors

The enzyme and binding protein, dipeptidyl-peptidase IV (DPP IV, EC 3.4.14.5; CD26) has been investigated from two points of view; as a protease, and as binding/co-stimulatory protein. Important DPP IV substrates include neuropeptide Y, endomorphin, peptide YY, growth hormone-releasing hormone, glucagon-like peptides-1 (GLP-1), glucagon-like peptides-2 (GLP-2), gastric inhibitory polypeptide, and paracrine chemokines like RANTES (regulated on activation normal T cell expressed and secreted). Based upon these observations, the potential clinical uses of selective DPP IV inhibitors or DPP IV-resistant analogs, especially for the GLP-1 is determined to enhance insulin secretion and improve glucose tolerance in diabetic animals. Inhibition of circulating DPP IV is predicted to elevate plasma levels of biologically active GLP-1. Orally administered DPP IV inhibitors, Ile-thiazolidide (**6**) and NVP-DPP728 (**7**) Fig. (**2**), have been recently shown to significantly increase the circulation half-life of GLP-1, and amplify insulin secretion in response to oral glucose load, leading to improved glucose homeostasis in insulin-resistant and glucose-intolerant Zucker fatty rats [9].

## 3. PEPTIDES AS ANTIDIABETIC THERAPEUTICS

### 3.1. Orally Active Peptides from Momordica Charantia

Aqueous extract of *M. charantia* (MC) is known to produce hypoglycemic activity in both animals and humans [10-12]. At the same time, juice extracted from *M. charantia* has exhibited blood glucose lowering activity both in normal and streptozotocin (STZ) induced diabetic states in vitro and in vivo [13]. The aqueous extract powder was tested for nephrotoxicity, hepatotoxicity and biochemical parameters such as SGOT, SGPT and lipid profile. The extract did not show any sign of nephrotoxicity and hepatotoxicity as judged by histological and biochemical parameters. Thus, aqueous extract powder of *M. charantia*, an edible vegetable appears to be a safe alternative for

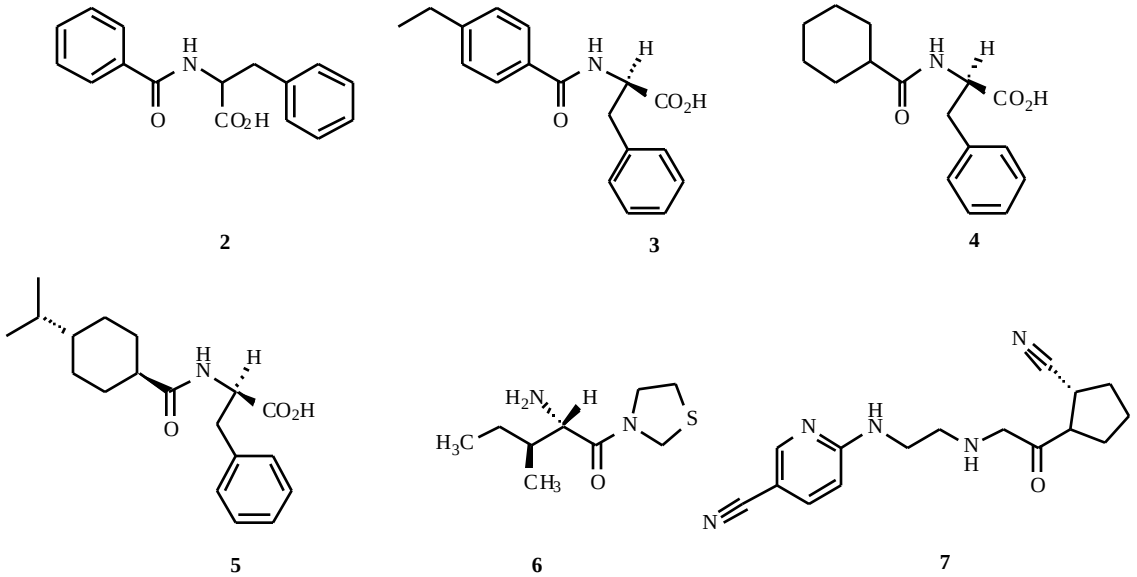



**Fig. (2).**

reducing blood glucose [14]. Taking these observations into account, detailed bioassay guided evaluation of the fruit and the water-soluble fraction of *M. charantia* (MC) was carried out. These studies resulted in the discovery of three orally active hypoglycemic peptides [MC-1 (KTNMKHMAGAAAAGAVVG); MC-2 (KTNMKHMAG-AA); and MC-3 (KTNMKHM)]. It has been suggested that therapeutically effective dose of these peptides will be in the range of about 0.01-1 mg/kg of host weight [14]. The results of various sets of experiments demonstrate that the sequence (KTNMKHM) found in all three peptides is important for reducing blood glucose level. Thus, heptapeptide sequence (KTNMKHM) provides an attractive and convenient approach for further exploration to develop novel peptide and/or peptidomimetic based therapeutics for the treatment of DM.

### 3.2. Glucagon-Like Peptide-1 (GLP-1) Analogs

Glucagon-like peptide-1 (GLP-1) is a 31 amino acid C-terminally amidated gastrointestinal hormone released from the enteroglucagon cells (L-cells) in the small intestine [15-18]. GLP-1 is highly susceptible to degradation by the enzyme dipeptidyl-peptidase IV (DPP IV) resulting in the formation of a truncated peptide, GLP-1(9-36)$NH_2$ [19-22]. The use of GLP-1 has been proposed as a novel therapeutic option for type 2 diabetics with residual β-cell failure because it also stimulates insulin secretion and inhibits glucagon secretion in the patients [23,24]. In vivo studies with minipigs showed that desamino-GLP-1 indeed had a longer biological action and higher potency compared to native GLP-1 [25]. The pigs treated with desamino-GLP-1 when subjected to intravenous glucose tolerance test were found to produce extended action up to 90 min in stimulating insulin secretion and consecutively lowering glucose levels than native GLP-1 [25]. The strategy of using GLP-1 analogs for diabetes therapy has the advantage over pharmacological DPP IV inhibition due to its high specificity that does not lead to ubiquitous inhibition of the degradation of various regulatory peptides. However, the major disadvantage of the usage of GLP-1 is its very short half-life. Therefore, in order to achieve long-term efficacious GLP-1 analogs, synthetic modification on the native compound were carried out to find peptides, which can be injected once or twice daily for possible pharmacological use in type 2 DM. These include NN2211, LY315902, CJC-1131 and exenatide (AC2993) (Table 1) [26].

Studies conducted to evaluate the effect of NN2211 (most effective long acting GLP-1 derivative) on β-cells in patients with type 2 DM indicated that a single subcutaneous dose restored β-cell sensitivity to physiological hyperglycemia thereby supporting the potential that NN2211 has in the treatment for type 2 DM [27,28], and this peptide is currently in Phase II clinical trials [29,30]. On the other

**Table 1. Sequences of Synthetic GLP-1 Analogs**

| **GLP-1 (7-37)** | **HAEGTFTSDVSSYLEGQAA-Lys26-EFIAWLV-Lys34-GRG** |
|---|---|
| NN2211 | HAEGTFTSDVSSYLEGQAA-Lys26*-EFIAWLV-Lys34-GRG |
| CJC-1131 | HAEGTFTSDVSSYLEGQAA-Lys26*-EFIAWLV-Lys34-GR-LyS37* |
| LY315902 | Des- HAEGTFTSDVSSYLEGQAA-Arg26-EFIAWLV-Lys34*-GRG |
| AC2993 | HGEGTFTSDLSKQMEEEAV-Arg20-LFIEWLKNGGSSGAPPPS |
| $Thr^8$-GLP-1 | HTEGTFTSDVSSYLEGQAAKEFIAWLVKGR |
| $Gly^8$-GLP-1 | HGEGTFTSDVSSYLEGQAAKEFIAWLVKGR |
| $Ser^8$-GLP-1 | HSEGTFTSDVSSYLEGQAAKEFIAWLVKGR |
| $Aib^8$-GLP-1 | H-Aib-EGTFTSDVSSYLEGQAAKEFIAWLVKGR |
| $Aha^8$-GLP-1 | H(Aha)EGTFTSDVSSYLEGQAAKEFIAWLVKGR |
| $(Aha^9)_4$-GLP-1 | $HA(Aha)_4$EGTFTSDVSSYLEGQAAKEFIAWLVKGR |
| $(Aha^9)_8$-GLP-1 | $HA(Aha)_8$EGTFTSDVSSYLEGQAAKEFIAWLVKGR |
| GLP-1 D3 | *HAEGTFTSDVSSYLEGQAAKEFIA*WLVKGR |
| GLP-1 D5 | *HAEGTFTSDVSSYLEGQAAKEFIA*WLVKGR |
| GLP-1 D8 | *HAEGTFTS*DVSS*YLEGQAAKEFIA*WLVKGR |
| GLP-1 D12 | *HAEGTFTSDVSSYLEGQAAKEFIA*WLVKGR |
| GLP-1 D21 | *HAEGTFTSDVSSYLEGQAAKEFIA*WLVKGR |
| GLP-1 A11 D | *HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR* |

Lys26*= Lys-{*N*-ε-[γ-Glu(*N*-α-hexadecanoyl)}
Lys37*= Lys-{2-[2-(2-maleimidopropionamido)ethoxy]ethoxy}acetamide
Lys34*= Lys-(octanoyl)
Italics denotes D-amino acids

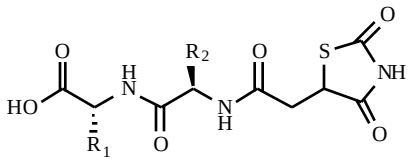
hand, other synthetic GLP-1 analogs, LY315902 and CJC-1131 that reproduced many of the biological actions of NN2211 including prolonged duration of action are in various stages of development.

To further enhance the metabolic stability of the native GLP-1, four analogs where $Ala^8$ of GLP-1 is substituted with threonine ($Thr^8$-GLP-1), glycine ($Gly^8$-GLP-1), serine ($Ser^8$-GLP-1), and α-aminoisobutyric acid ($Aib^8$-GLP-1) were synthesized and screened for proteolytic stability test [31]. All were found to be more resistant to DPP IV in porcine plasma in vitro and in anaesthetized pigs in vivo. All modified peptides bound to the receptor, but only the $Aib^8$ and $Gly^8$ analogs had affinities similar to that of GLP-1. Furthermore, all analogs were active in the isolated pancreas, with the potency order reflecting affinities ($Aib^8$>$Gly^8$>$Ser^8$>$Thr^8$).

In another study, GLP-1 analog in which 6-aminohexanoic acid (Aha) is inserted between histidine and alanine at positions 7 and 8 was studied for biological activity (Table 1) [32]. $Aha^8$-GLP-1 analog (10 nM) was found to be equipotent to GLP-1 (10 nM) in stimulating insulin secretion in RIN 1046-38 cells. As with the case of $Gly^8$-GLP-1, the binding affinity of $Aha^8$-GLP-1 for the GLP-1 receptor in intact Chinese hamster ovary (CHO) cells expressing the human GLP-1 receptor (CHO/GLP-1R cells) was reduced. $Aha^8$-GLP-1 was also shown to stimulate intracellular cAMP production 4-fold above basal at concentrations as low as 0.5 nM. However, it exhibited a higher $ED_{50}$ value when compared to GLP-1 and $Gly^8$-GLP-1. $Aha^8$-GLP-1 administered (s.c.) to fasted Zucker (*fa/fa*) rats lowered blood glucose levels that remained significantly lower up to 8 h. On the other hand, $Aha^8$-GLP-1 did not exhibit an N-terminal degradation product upon incubation with DPP IV (37 ºC, 2 h). At the same time, insertion of four and eight Aha moieties between position 8 and 9 of the GLP-1 sequence [$(Aha^9)_4$-GLP-1 and $(Aha^9)_8$-GLP-1 analogs] resulted in the reduction of biological activity that is directly attributed to the reduced affinity for the receptor and not to any antagonistic effect. These results indicates that insertion of Aha after the 7 position in GLP-1 produces an effective, long-acting GLP-1 analog, which may be useful in the treatment of type 2 DM [32]. In further continuation to design stable and potent GLP-1 analogs, D-amino acid-substituted GLP-1 peptides (Table 1) were also examined to assess the importance of putative peptidase-sensitive cleavage sites present in the GLP-1. However, all of these peptides have exhibited poor binding affinity for the GLP-1 receptor, and none stimulated the production of intracellular cAMP in CHO/GLP-1R cells or insulin secretion in RIN 1046-38 cells. To summarize, it can be concluded that N-terminal modification of GLP-1 confer resistance to DPP IV degradation and some analogs are biologically active with prolonged metabolic stability in vivo, which if associated with greater potency and duration of action, may help to realize the potential of GLP-1 analogs in diabetes therapy [31].

### 3.3. Peptide-Conjugated Thiazolidinediones

To examine the functional and structural features that facilitate the binding of small molecules to PPARs (peroxisome proliferator activated receptors) and to find new activators and/or inhibitors, libraries of thiazolidinedione-peptide hybrids (Gly-Gly-TZD, Gly-Phe-TZD, Gly-Leu-TZD, Phe-Gly-TZD, Phe-Phe-TZD, Phe-Leu-TZD, Leu-Gly-TZD, Leu-Phe-TZD, Leu-Leu-TZD) using solution as well as solid phase methods were synthesized Fig. (**3**) [33].



$R_1$=$R_2$=H, $CH_2C_6H_5$ or $CH_2CH(CH_3)_2$

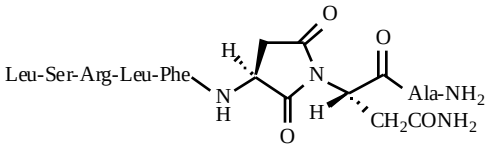
**8**

**Fig. (3).**

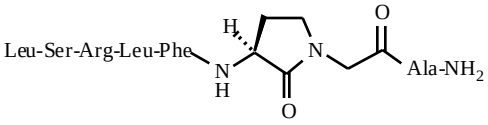
The synthesis of these analogs utilized the known thiazolidinedione antidiabetic agents and structural diversity of the commercial amino acids. Unfortunately, none of the synthesized peptide conjugates exhibited promising antidiabetic activity.

### 3.4. Human Growth Hormone-(6-13) Analogs

The amino-terminal region of human growth hormone (hGH), and specifically the amino acid sequence [Leu-Ser-Arg-Leu-Phe-Asp-Asn-Ala, hGH(6-13)] has been implicated as a functional region for the regulation of energy metabolism



**9**



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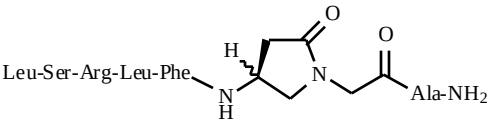

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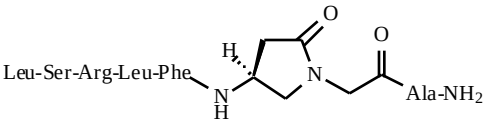


**12**

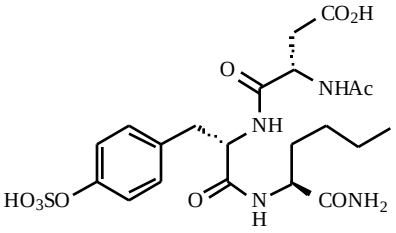
**Fig. (4).**

by exerting an insulin-potentiating action on insulin-sensitive tissues [34,35]. Recent studies have revealed that the cyclization of the aspartate ($Asp^{11}$) residue to form the α-aminosuccinimide ($Asu^{11}$) ring is essential for the biological action of peptides related to the hGH fragment (**9**) Fig. (**4**). However, pharmacological potential of the hGH(6-13) peptides has been restricted by the vulnerability of the α-aminosuccinimide moiety to hydrolytic modification leading to the loss of biological action. It has been successfully found that replacement of α-aminosuccinimide (Asu) ring with compatible and less rapidly metabolized lactam moiety (analog **10**) result in a more stabilized peptide [34]. The hGH(6-13) analog **10** that mimics the stereoelectronic and conformational characteristics of the Asu ring was discovered to be more potent and longer lasting than the aspartimide peptide analog (**9**) [34].

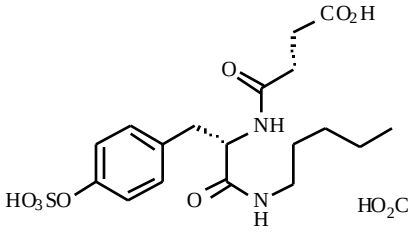
Additional studies resulted in the synthesis of new γ-lactam ring-containing peptides **11**-**12**. Upon evaluation at a dosage of 3 mg/kg with fasted male Wistar rats in the IVITT (intravenous insulin tolerance test) assay, (*S*)-(4-amino-γ-lactam) ring-containing hGH(6-13) analog **12**, in common with the corresponding (*R*,*S*)-(4-amino-γ-lactam) ring-containing hGH(6-13) analog **11** produced a smaller lowering of blood glucose compared to the (*S*)-(3-amino-γ-lactam)-containing hGH(6-13) analog **10** Fig. (**4**). Interestingly, in vivo biological activity of the chiral (*S*)-peptide analog **12** was approximately twice to that of the isomeric counterpart (**11**) in the IVITT assay [35]. These results thus indicate that replacement of Asu moiety with more chemically stable γ-lactam (peptide **10** and **12**) in the hGH(6-13) peptide result in potentiation of the effect of exogenously administered insulin to a significantly greater extent.

### 3.5. Gastric Inhibitory Polypeptide (GIP) Analogs

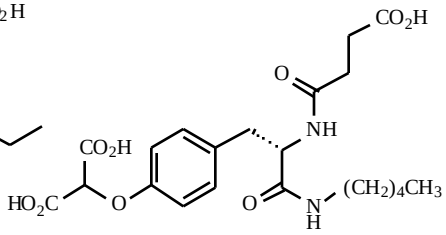
Gastric inhibitory polypeptide (also called as glucose-dependent insulinotropic polypeptide having sequence,



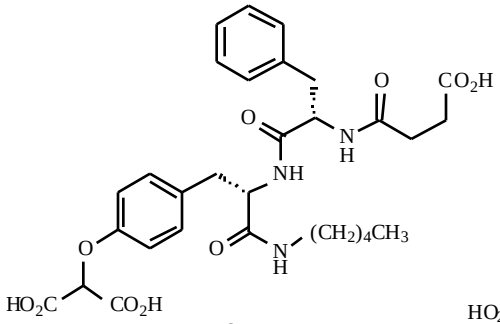
**13.** $K_i$ = 5 μM



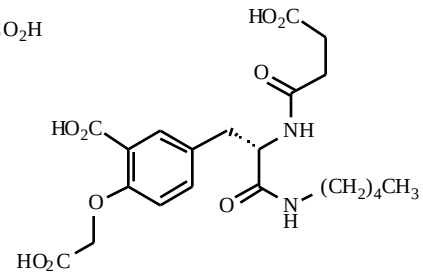
**14.** $K_i$ = 30 μM



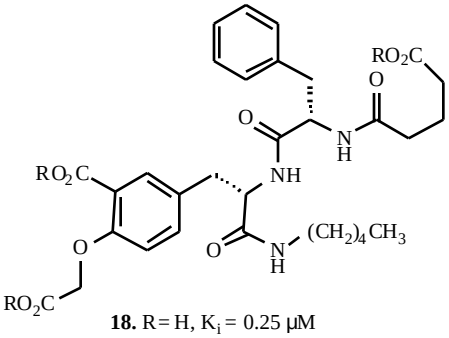
**15.** $K_i$ = 12 μM



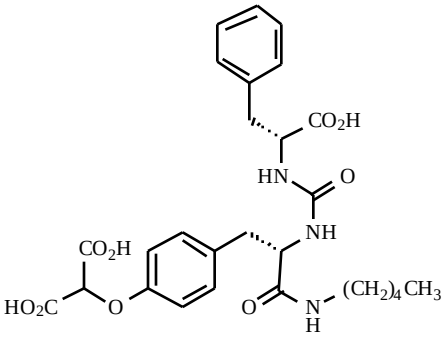
**16.** $K_i$ = 1.2 μM



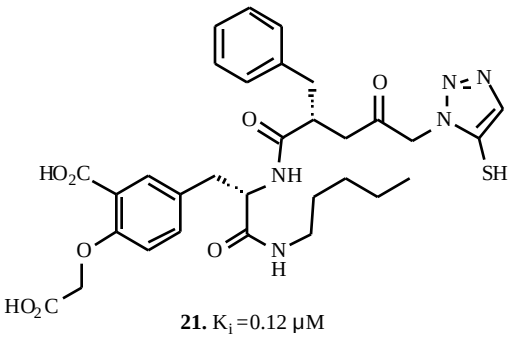
**17.** $K_i$ = 2.8 μM



**18.** R = H, $K_i$ = 0.25 μM
**19.** R = $CH_3$, $K_i$ = 0.40 μM



**20.** $K_i$ = 3.4 μM



**21.** $K_i$ = 0.12 μM

**Fig. (5).**

$^{1}$YAEGTFISDYSIAMDKIHQQDFVNWLLAQKGKKNDW KHNITQ$^{42}$) is an incretin hormone released from the upper small bowel in response to luminal nutrients that amplifies insulin release from pancreatic β-cells. In vitro experiments indicated that [D-Ala$^{2}$]GIP analog is least prone to enzymatic proteolysis, combined with minimal effects on efficacy at the receptor. [D-Ala$^{2}$]GIP was tested in the perfused rat pancreas and bioassay in conscious Wistar and Zucker rats [36]. When injected subcutaneously in normal Wistar, or *fa/fa* Vancouver Diabetic Fatty (VDF) Zucker rats, [D-Ala$^{2}$]GIP significantly reduced glycemic excursions during a concurrent oral glucose tolerance test via stimulation of insulin release. The modified peptide displayed greater in vivo effectiveness compared to GIP, possibly due to resistance to proteolysis. Thus, [D-Ala$^{2}$]GIP with improved plasma stability produce better glucose tolerance when given in supraphysiological doses, and may prove useful in the treatment of diabetes.

### 3.6. Peptide from the Skin Secretion of Rana Pipiens

Few studies have been carried out to exploit amphibian (*Rana pipiens*) granular gland secretions for the discovery of novel peptides, polypeptides and proteins [37]. These studies led to the isolation of a twenty-four amino acid containing peptide (FLPIIAGVAAKVFPKIFCAISKKC) with good insulin-releasing activity. Subsequent database search of the insulin-releasing peptides showed a 100% homology to histamine-releasing pipinin-1 factor (1-609). Thus, study revealed that the skin secretions of *Rana pipiens* frogs contain peptides with insulin-releasing activity, and further exploitation could prove useful to identify antidiabetic agents from natural resources.

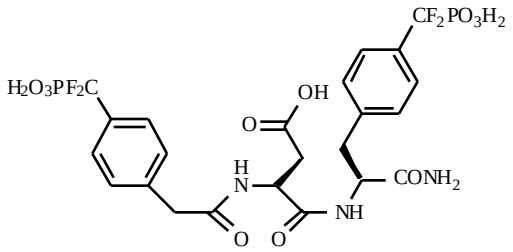
## 4. PEPTIDOMIMETICS AS ANTIDIABETIC THERAPEUTICS

### 4.1. Peptidomimetics as Protein Tyrosine Phosphatase 1B Inhibitors

Protein tyrosine phosphatase 1B (PTP1B) attenuate insulin signaling by catalyzing dephosphorylation of insulin receptors and is an attractive target for potential new drugs for treating the insulin resistance that is central to type 2 DM. PTP1B is an important therapeutic target, and several investigators have described small molecule inhibitors for this target [38-43]. In general, these inhibitors either are not very potent; contain peptide bonds, and other functional groups that make them pharmacokinetically unattractive as lead for therapeutic agents. Furthermore, some of these inhibitors contain functionalities capable of reacting with the catalytically essential Cys$^{215}$ of PTP1B, and thus exhibit irreversible inhibition. Surprisingly, several analogs of cholecystokinin (26-33) [H-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$ (CCK-8)] were found to be potent inhibitors of PTP1B, and a common N-terminal tripeptide, *N*-acetyl-Asp-Tyr($SO_3H$)-Nle-$NH_2$ (**13**) Fig. (**5**), was shown to be essential for inhibition [44-47]. However, tripeptide **13** contains a metabolically unstable sulfotyrosyl group, and because neither a sulfotyrosyl group nor a peptide bond is pharmacokinetically desirable, chemical modification of **13** was done to introduce a stable phosphotyrosine bioisostere, and to minimize peptide-like characteristics of the analogs [48].

This led to the discovery of a novel phosphotyrosine mimic, 2-carboxymethoxybenzoic acid, and incorporation of this bioisostere led to the design of analogs (**14**-**21**) Fig. (**5**) that were >100-fold more potent than the CCK-8. These inhibitors disrupt the binding of PTP1B to activated insulin receptors in vitro and prevent the loss of tyrosine kinase activity that accompanied PTP1B-catalyzed dephosphorylation of insulin receptor. All of these analogs were found to be competitive inhibitor of PTP1B with the activity superior to that of **13**. PTP1B inhibitors (**14**-**21**) when evaluated for the inhibition of LAR, SHP-2, cdc25b, and calcineurin, were either inactive or exhibited <20% inhibition at the concentration 100 μM. These PTP1B peptidomimetic inhibitors with stabilized structure therefore constitute an interesting class of compound with great potential as novel peptidomimetic for the treatment of hypoglycemic disorder.

There is immense curiosity in specific and potent PTP1B inhibitors for biological studies and pharmacological development. However, because of the highly conserved nature of the receptor active site, it is unclear whether selectivity in inhibition can be achieved. More recently, combinatorial approach was designed to target both the active site and a unique peripheral site in PTP1B. Compounds that can simultaneously associate with both sites are expected to exhibit enhanced affinity and specificity. The combinatorial library synthesis and high-throughput analysis produced a small molecule PTP1B inhibitor **22** Fig. (**6**) that is highly potent (Ki = 2.4 nM) and selective [49].




**22**

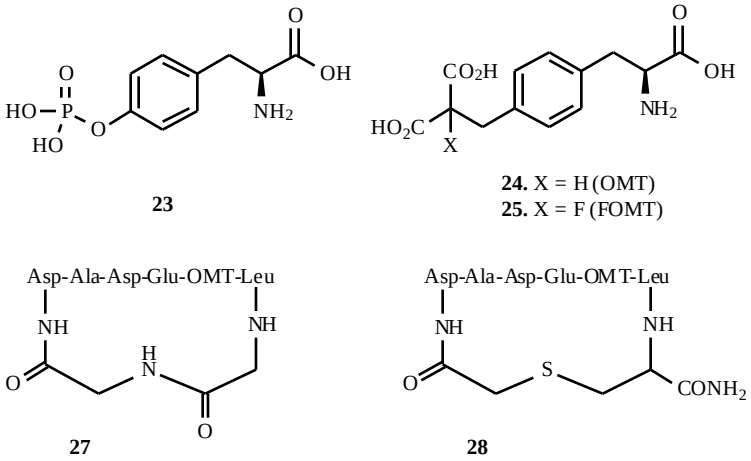
**Fig. (6).**

The results of this study exhibit the discovery of highly potent and highly selective inhibitor for individual members of the large PTPase family of enzymes, and analog **22** is currently undergoing detailed bio-evaluation.

### 4.2. Phosphotyrosyl Mimetic: Use in the Preparation of Tyrosine Phosphatase Inhibitory Peptidomimetics

The phosphotyrosyl (pTyr **23**) pharmacophore Fig. (**7**) is a critical determinant in cellular signal transduction. Its inappropriate expression, either through generation by protein-tyrosine kinases (PTKs) or destruction by protein-tyrosine phosphatase (PTP) contribute to a variety of diseases, including immune disorders, cancers and diabetes. Therefore, agents that modulate PTKs/PTPs, are potentially useful as therapeutics, and considerable synthetic efforts have been expended in the development of compounds, which can mimic the pTyr pharmacophore in physiological systems.

Amongst the most successful examples of non-phosphorus containing pTyr mimetic, are analogs that




**23**

**24.** X = H (OMT)
**25.** X = F (FOMT)

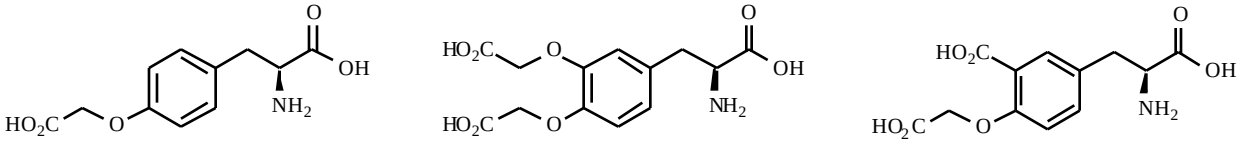
**27** **28**

**Fig. (7).**

utilize the dicarboxylic acid-containing malonate structure as phosphate isostere. These include *O*-malonyltyrosine (OMT, **24**) [50, 51] and fluoro-*O*-malonyltyrosine (FOMT, **25**) Fig. (**7**) [52]. The OMT isostere (**24**) is of particular interest in that it affords new prodrug approaches.

Hexameric peptides of the sequence Ac-Asp-Ala-Asp-Glu-Xxx-Leu-amide (**26**), where Xxx = pTyr (**23**), correspond to incorporation into $EGFR_{988-993}$ peptide [55]. These peptide analogs (**32-34**) Fig. (**9**) were synthesized to derive potent and bioavailable PTP inhibitors where the tyrosyl residue in the sequence has been replaced with non-hydrolysable pTyr mimetic and evaluated in the PTP1 based assay.

The most effective peptide (**34**) Fig. (**9**) of the series where R= 3-carboxy-4-(O-carboxymethyl)-L-tyrosine (**31**)




**29** **30** **31**

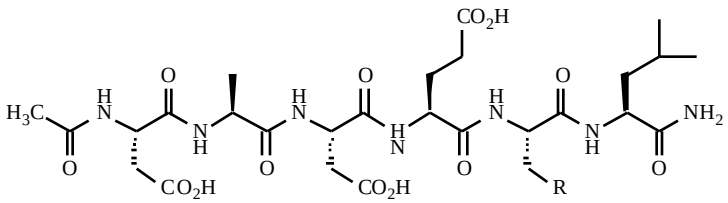
**Fig. (8).**

one of the cytoplasmic autophosphorylation sites of the epidermal growth factor receptor ($EGFR_{988-993}$). This peptide was originally shown to be a high affinity substrate for the rat PTP1 enzyme [53]. Isostere (**24**) was incorporated into $EGFR_{988-993}$ peptide, and subsequently cyclized through the peptide backbone. In a PTP1-based assay, the cyclic octamer (**27**) demonstrated a five-fold increase in potency (Ki= 2.60±0.11 μM); whereas the peptidomimetic analog (**28**) cyclized via a sulfur bridge exhibited very good potency (Ki= 0.73±0.03 μM), and both are amongst the most potent PTP inhibitors yet developed [52,54].

In the further extension, three "carboxylate-based" pTyr mimetic (**29-31**) Fig. (**8**) that represent variants of the original malonate in protected forms are prepared for exhibited a Ki value of 3.6 mM against PTP1, which is equipotent to the parent pTyr containing peptide (**26**).

## 5. CONCLUSIONS

In this review we have concentrated on chemotherapeutic approaches that have been considered in the design and synthesis of amino acids, peptides and peptidomimetics, specifically for the treatment of diabetes mellitus. Amongst the amino acid analogs discussed, 4-hydroxyisoleucine and Ile-thiazolidide have exhibited promise; however, nateglinide constitute the most interesting analog of this class. Nateglinide is an orally effective hypoglycemic agent, and careful development of this promising low molecular weight




**32. R= 29; 33. R= 30; 34. R= 31**

**Fig. (9).**

NCE could lead to potent antidiabetic drugs in future. On the other hand, discovery of orally active peptides from *M. charantia* is highly exciting, and design of additional synthetic analogs along with detailed structure-activity relationship studies is desirable to determine their prospects. Synthetic GLP-1 analogs are highly promising, and first peptide-based antidiabetic agent could emanate from this class of compounds. Similarly, hGH(6-13) analogs belong to a unique structural class of peptides and constitute useful leads for peptide chemists. PTP1B peptidomimetic inhibitors are the focus of much research carried out now-a-days. Similarly, designing pTyr mimetic followed by incorporation into suitable peptides has produced promising NCEs, whose subsequent modification and development to identify drug-molecule is drawing considerable attention of researchers around the world. Although the future of some GLP-1 synthetic analogs, PTP1B inhibitors, hGH analogs, and pTyr mimetic containing peptidomimetics appears bright, several aspects remained to be resolved as none of the drug in clinics yet belong to these classes. These include, designing suitable mode of administration, stability to proteolysis, lack of complete toxicity profiles, and development of appropriate formulations. To conclude, though, as is evident from the cases examined here by way of illustration, interesting and exciting research is being carried out; design of amino acids, peptides, and peptidomimetcis as novel hypoglycemic chemotherapeutic agents still remains a considerable challenge.

## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| SGOT | = | Serum glutamic oxaloacetic transaminase |
| SGPT | = | Serum glutamic pyruvic transaminase |
| RIN 1046 | = | Rat-insulinoma cells |
| Caco-2 cells | = | Calonic adenocarcinoma cells |
| MAP | = | Mitogen-activated protein |
| IVITT | = | Intravenous insulin tolerance test |

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