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INSULYSIN INHIBITORS AS NOVEL ANTI-DIABETIC AGENTS: FROM COMPUTATIONAL DESIGN TO PRE-CLINICAL VALIDATION

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ABSTRACT

Background: Diabetes mellitus is a health crisis that has been increasing worldwide in prevalence and has fewer treatment options. The enzyme insulysin (also called insulin-degrading enzyme, IDE) is also important in insulin metabolism and glucose homeostasis. New insulysin inhibitors are coming up as effective therapeutic agents in the management of diabetes.

Objective: This review assesses the potential to use novel insulinase inhibitors as anti-diabetic agents using an integrated approach involving computational and experimental methods in streptozotocin-nicotinamide induced diabetic rat models.

Methods: An extensive literature review was done with the help of such databases as PubMed, Scopus, and Web of Science that included publications dated 2015-2024. Inlay terms were insulin degrading enzyme, insulin-degrading enzyme, in-silico and STZ-NAD diabetic model.

Results: The existing data indicates that insulinase inhibitors show great anti-diabetic effects in a variety of mechanisms such as the increased insulin sensitivity, improved β-cell act, and decreased insulin degradation. In-silico research indicates potential binding affinities and pharmacokinetic characteristics whereas in-vivo research in STZ-NAD models demonstrates better glycemic control and morphology of the pancreas.

Conclusion: Novel insulinase inhibitors offer a perspective in treating diabetes and a combination of in- Silico and in-vivo studies offers a great strength to the clinical efficacy of the compound.

KEYWORDS: Insulysin inhibitors, Insulin-degrading enzyme, Anti-diabetic, In-silico drug design, STZ-NAD diabetic model, Type 2 diabetes

1. INTRODUCTION

Diabetes mellitus (DM) is a persistent metabolic condition that is associated with hyperglycemia caused by impairments in the secretion and/or activity of insulin (1). Diabetes prevalence has become an epidemic in the world with an estimated 537 million adults afflicted with diabetes across the globe as of 2021, which is expected to increase to 783 million by 2045

(2). T2DM is the most prevalent form of diabetes, estimated to contribute to 90-95 percent of all cases of diabetes, and is a major health care burden in most health systems worldwide (3).
Existing interventions at the treatment of diabetes involve life style changes, oral antidiabetic drugs, and insulin therapy. Nonetheless, such treatments do not usually provide long-term glycemic control, and could be linked to such unfavorable effects as hypoglycemia, weight gain, and cardiovascular complications (4). The T2DM progressive nature, which is a reduction in β-cells functionality and rising insulin resistance, requires the emergence of novel treatment methods that can better address the pathophysiology (5).
Insulysin or insulin-degrading enzyme (IDE) is an important zinc metallopeptidase that is highly involved in the dissolution of insulin and other bioactive peptides such as amylin, glucagon, and amyloid-b (6). IDE is also prevalent in insulin-sensitive tissues like liver, muscle, and adipose tissue, in which it controls local insulin concentrations and modulates insulin signalling pathways (7). The specialized and catalytic mechanism of the enzyme and its unique structure are interesting therapeutic targets in diabetes and other metabolic diseases (8).
Recent developments in computational drug discovery have made it possible to find and optimize new insulinase inhibitors with better selectivity and pharmacokinetics (9). Molecular docking, molecular dynamics and pharmacophore modelling are in-silico methods that have been instrumental in the prediction of the drug-target interaction and in lead compounds optimization prior to the costly experimental validation (10). The combination of computational and experimental methodologies is a paradigm shift in drug discovery, which has prospects of converting the speed to develop more efficient anti-diabetic medicines (11).
The streptozotocin-nicotinamide (STZ-NAD) induced diabetic rat model has been used as the gold standard in assessing anti-diabetic agents because it can recreate the pathophysiology of human T2DM (12). This model is a mixture of β-cells destructive effect of streptozotocin plus the protective effects of nicotinamide which produces moderate and stable diabetic state similar to T2DM in humans (13).

2. LITERATURE REVIEW AND METHODOLOGY

2.1 Search Strategy

The systematic literature search has occurred through several electronic databases such as PubMed/MEDLINE, Scopus, Web of science, and Google Scholar covering the articles published in the past five years (since January 2015 and up to December 2024). The search used both the Medical Subject Headings (MeSH) and free-text keywords. The key words used were: insulin-degrading enzyme, insulin-degrading enzyme insulin, insulin-degrading enzyme, insulin-degrading enzyme, insulin-degrading enzyme, in-silico drug design, molecular docking, STZ-NAD model, diabetes rat model.
Search terms were combined using the Boolean operators (AND, OR). The search query was; (insulysin OR insulin-degrading enzyme OR IDE) AND (inhibitor OR antagonist) AND (diabetes OR anti-diabetic OR hypoglycemic) AND (in-silico OR molecular docking OR computational) AND (STZ OR streptozotocin OR animal model).

2.2 Inclusion and Exclusion criteria

Inclusion criteria:

- Original research articles and reviews
- Studies investigating insulysin/IDE inhibitors
- In-silico and in-vivo studies
- Articles published in English
- Peer-reviewed publications
- Studies using STZ-NAD diabetic models

Exclusion criteria:

- Case reports and editorial articles
- Studies not related to diabetes or insulysin
- Non-English publications
- Duplicate publications
- Conference abstracts without full text

2.3 Data Extraction

The extraction of data was done systematically, where the information collected was on the study design, computational procedures, chemical structures of inhibitors, biological activities, mechanism of action, and therapeutic outcomes. The evaluation of quality was performed with the help of proper tools in various studies.

3. INSULYSIN: STRUCTURE, FUNCTION, AND PATHOPHYSIOLOGICAL ROLE

3.1 Molecular Structure and Catalytic Mechanism

Insulysin (IDE) is a 110 kDa zinc metalloprotease that is a member of the M16A subfamily of metalloproteins (14). The enzyme is made up of four homologous domains (IDE-N1, IDE-N2, IDE-C1, and IDE-C2) which shape two bowl-shaped halves with a stretchy hinge region between them (15). The site of catalysis is found at the junction of the N-terminal and C-terminal halves, where the distinct zinc-binding motif, HXXEH, is found (16).

Table 1: Structural Characteristics of Insulysin (IDE)

Parameter	Description	Reference
Molecular Weight	110 kDa	(14)
Number of Amino Acids	1019 residues	(15)
Active Site Motif	HXXEH	(16)
Zinc Coordination	His108, His112, Glu189	(17)
Km for Insulin	0.1-1.0 μM	(18)
Optimal pH	7.0-7.5	(19)
Subcellular Localization	Cytoplasm, Peroxisomes, ER	(20)

The mechanism behind catalysis entails the coordination of a zinc ion by three amino acid residues (His108, His112 and Glu189) and this coordinates a water molecule to nucleophilically attack the peptide bond (17). The enzyme has an atypical inverted zinc coordination in contrast to other metalloproteases, which also helps in its distinct substrate specificity (21).

3.2 PHYSIOLOGICAL FUNCTIONS

IDE has several physiological functions as opposed to insulin degradation. The enzyme participates in metabolism of different bioactive peptides such as amylin, glucagon, atrial natriuretic peptide and amyloid-b (22). This wide substrase specificity implies that IDE is a general peptide hormone signaling and or cellular homeostasis regulator (23).

IDE controls the secretion of insulin in the pancreatic β-cells via autocrine action, by maintaining local insulin levels (24). The enzyme also affects the homeostasis of glucose in the peripheral tissues by changing the sensitivity and uptake of insulin (25). Recent research has also demonstrated other roles of IDE in cellular stress responses, autophagy and mitochondrial functioning (26).

3.3 Pathophysiological Role in Diabetes

The pathogenesis of T2DM and insulin resistance has been suggested to be dysregulation of IDE activity (27). It is possible that elevated IDE expression and activity in diabetic patients can lead to the increased rate of insulin degradation and loss of insulin signaling (28). On the other hand,

population studies have linked genetic variation in the IDE gene with the modified risk of diabetes and insulin sensitivity (29).

4. CURRENT ANTI-DIABETIC THERAPIES AND LIMITATIONS

4.1 Overview of Existing Treatments

Existing pharmacological treatments of T2DM consist of a variety of classes of medications with different mechanisms of action (30). Metformin is the treatment of the first line because it is effective to decrease hepatic glucose synthesis and enhance insulin sensitivity (31). Sulfonylureas, thiazolidinedione, dipeptidyl peptidase-4 (DPP-4) interferent, glucagon-like peptide-1 (GLP-1)-receptor of agonist, sodium-glucose cotransporter-2 (SGLT-2)-inhibitor and insulin preparations are other classes of therapeutics (32).

Table 2: Current Anti-Diabetic Drug Classes and Mechanisms

Drug Class	Mechanism of Action	Primary Benefits	Major Limitations
Metformin	↓ Hepatic glucose production	Weight neutral, CV protective	GI side effects, contraindicated in renal impairment
Sulfonylureas	↑ Insulin secretion	Rapid glucose lowering	Hypoglycemia, weight gain, β-cell exhaustion
TZDs	↑ Insulin sensitivity	Durable glucose control	Weight gain, edema, fracture risk
DPP-4 Inhibitors	↑ Incretin activity	Weight neutral, low hypoglycemia	Modest efficacy, possible pancreatitis
GLP-1 Agonists	↑ Insulin, ↓ glucagon	Weight loss, CV benefits	GI side effects, injection required
SGLT-2 Inhibitors	↓ Renal glucose reabsorption	Weight loss, CV/renal protection	UTI, DKA risk

4.2 Therapeutic Limitations and Unmet Needs

Although several therapeutic solutions exist, there are still serious limitations in the modern management of diabetes (33). Advancing β-cells dysfunction causes failure in treatment with time and most patients have to take combination therapy and eventually become reliant on insulin (34). Hypoglycemia, weight gain and gastrointestinal disturbances are side effects that inhibit treatment adherence and quality of life (35).

The diversity of the pathophysiology of T2DM requires individualized approaches to treatment that is not sufficiently considered by the existing therapies (36). Also, currently used therapies are mainly aimed at managing symptoms instead of addressing disease pathophysiology, which points to the necessity of new therapeutic targets (37).

5. INSULYSIN INHIBITORS: DEVELOPMENT AND CLASSIFICATION

5.1 Historical Development

Insalysin inhibitors Insalysin inhibitors were developed after the discovery of non-specific chelating reagents like 1,10-phenanthroline and bacitracin (38). Initial experiments showed that zinc chelation had the potential to prevent the activity of IDE and extend insulin function in both cellular and animal models (39). Nonetheless, the compounds were non selective and rather toxic which restrained their therapeutic value (40).

In the year 2006, the IDE crystal structure was discovered leading to a breakthrough in the development of inhibitors and development of structure based drug designing methods (41). Further attempts were made to design selective, non-covalent inhibitors, which would regulate the activity of IDE, without causing interference with other zinc-dependent enzymes (42).

5.2 Classification of Insulysin Inhibitors

The existing insulin type 1 inhibitors may be divided into the following categories according to their structure and mode of action (43):

5.2.1 Small Molecule Inhibitors

- **Hydroxamic acid derivatives:** BDM44768, ML345
- **Benzothiazole compounds:** NTE-572
- **Quinoline derivatives:** Various synthetic analogs
- **Natural product derivatives:** Curcumin analogs, flavonoids

5.2.2 Peptide-Based Inhibitors

- **Substrate analogs:** Modified insulin derivatives
- **Allosteric peptides:** Designed to bind regulatory sites

5.2.3 Natural Product Inhibitors

- **Plant-derived compounds:** Quercetin, resveratrol, berberine
- **Marine-derived molecules:** Various bioactive compounds

Table 3: Representative Insulysin Inhibitors and Their Properties

Compound	Chemical Class	IC50 (μM)	Selectivity	Development Stage
BDM44768	Hydroxamic acid	2.4	High	Preclinical
ML345	Hydroxamic acid	0.87	Moderate	Preclinical
NTE-572	Benzothiazole	3.2	High	Research
Quercetin	Flavonoid	15.6	Low	Natural product
Curcumin	Polyphenol	12.3	Low	Clinical trials

5.3 Structure-Activity Relationships

The structure-activity relationship (SAR) studies have revealed essential structural characteristics needed to have potent and selective IDE inhibition (44). The existence of zinc-binding groups e.g. hydroxamic acids or carboxylates is necessary to be active and hydrophobic substituents contribute to selectivity and potency (45). Inhibitor binding is also affected by molecular size and flexibility with the most effective compounds having a moderate molecular weight (300-500 Da) and limited conformational flexibility (46).

6. IN-SILICO APPROACHES IN INSULYSIN INHIBITOR DISCOVERY

6.1 Molecular Docking Studies

Molecular docking has also become an efficient predictor of the binding patterns and affinities of possible IDE inhibitors (47). Crystal structures of IDE in different conformational states at high resolutions have provided meaningful information regarding substrate recognition and inhibitor-binding (48). The docking studies normally use either flexible ligand protocols using rigid or semi-flexible receptor models to allow the receptors to undergo changes in conformation on ligand binding (49).

Recent docking experiments have demonstrated that efficient IDE inhibitors usually occupy the large, enclosed active site cavity and interact with important catalytic residues (50). The zinc ion is an important anchor site of most inhibitors, whereas the presence of hydrophobic interactions between the inhibitor and the amino acids that surround it plays a role in binding affinity and selectivity (51).

6.2 Molecular Dynamics Simulations

Molecular dynamics (MD) simulations can give dynamic information about the interactions of proteins and ligands and support docking predictions (52). These simulations expose how the IDE changes its conformational state in response to the binding of the inhibitor and that the binding is stable over long periods of time (53). The mechanism of allosteric regulation in IDE has also been explained by MD studies, as well as the possible allosteric binding sites that can be used to develop an inhibitor (54).

6.3 Pharmacophore Modeling

Pharmacophore modeling determines the key chemical characteristics needed to inhibit IDE and is used to design new inhibitors (55). Most common pharmacophoric groups are zinc-binding groups, hydrogen bond on the donor/acceptor and hydrophobic sites that complement the enzyme active site (56). These models have been effectively used in virtual screening of library of compounds and lead optimization (57).

6.4 ADMET Prediction

Prediction of the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties in-silico are essential to find drug-like IDE inhibitors (58). There are also computational software like SwissADME, pkCSM and admetSAR, which can quickly determine the pharmacokinetic and safety parameters (59). Important parameters measured are the oral bioavailability, blood-brain barrier penetration, hepatic metabolism, and drug-drug interaction potential (60).

Table 4: ADMET Properties of Selected IDE Inhibitors

Compound	MW (Da)	LogP	HBD	HBA	TPSA (Ų)	BBB	HIA	CYP Inhibition
BDM44768	425.6	3.2	2	6	86.4	Low	High	2D6, 3A4
ML345	389.4	2.8	3	5	94.2	Low	High	2D6
NTE-572	342.8	4.1	1	4	72.1	Moderate	High	3A4

7. STREPTOZOTOCIN-NICOTINAMIDE DIABETIC MODEL

7.1 Model Development and Rationale

The streptozotocin-nicotinamide (STZ-NAD) model of diabetic rats was constructed to address the drawbacks associated with traditional STZ models, especially the high and non-reversible destruction of β-cells, which is not a close replica of human T2DM (61). In several ways, such as enhancing DNA repair and inhibition of poly(ADP-ribose) polymerase (PARP), nicotinamide, a NAD+ precursor, can guard against the effects of STZ on pancreatic β-cells (62).

The STZ-NAD model results in moderate and stable hyperglycemia and maintained secretory ability of insulin, which closely approximates the pathophysiology of human T2DM (63). This model is characterized by such peculiarities as insulin resistance, dysglycemia, dyslipidemia, and the progressive dysfunction of β-cells (64).

7.2 Model Induction Protocol

In the standard protocol, nicotinamide (110-230 mg/kg) is intraperitoneally injected, and 15 minutes after that, STZ (45-65mg/kg) is injected intravenously in citrate buffer (65). Nicotinamide protective effect is time-dependent and it has to be administered in a limited interval prior to STZ treatment (66). The occurrence of diabetes is established 48-72 hours after the induction using the measurement of the level of fasting blood glucose with a level exceeding 250mg/dl confirming the induction of diabetes (67).

Table 5: STZ-NAD Model Induction Protocol

Parameter	Specification	Reference
Animal Species	Wistar/Sprague-Dawley rats	(65)
Age/Weight	8-12 weeks, 200-250g	(66)
Nicotinamide Dose	110-230 mg/kg, i.p.	(67)
STZ Dose	45-65 mg/kg, i.v.	(68)
Time Interval	15 minutes	(69)
Diabetes Confirmation	FBG >250 mg/dL at 72h	(70)
Success Rate	70-90%	(71)

7.3 PATHOPHYSIOLOGICAL CHARACTERISTICS

The STZ-NAD model exhibits several key features that make it suitable for evaluating anti-diabetic compounds (72):

Metabolic Parameters:

- Moderate hyperglycemia (300-450 mg/dL)
- Glucose intolerance
- Insulin resistance
- Dyslipidemia
- Altered body weight

Pancreatic Changes:

- Partial β-cell loss (40-60%)
- Preserved insulin secretory capacity
- Islet morphological changes
- Reduced pancreatic insulin content

Secondary Complications:

- Hepatic steatosis
- Renal dysfunction
- Cardiovascular changes
- Oxidative stress

7.4 Advantages and Limitations

Advantages:

- Closely mimics human T2DM pathophysiology
- Stable hyperglycemia without severe ketosis
- Preserved insulin secretion
- Suitable for chronic studies
- Well-characterized model

Limitations:

- Variable success rates
- Strain differences in susceptibility
- Acute model induction vs. gradual disease progression in humans
- Limited genetic diversity compared to human T2DM

8. IN-VIVO EVALUATION OF INSULYSIN INHIBITORS

8.1 Preclinical Studies Overview

The preclinical studies conducted on the use of insulin-inhibitors on diabetic adult animals have offered important information on their therapeutic effectiveness and safety profiles (73). Research studies have utilized the following experimental designs; (acute and chronic treatment protocols and dose-response studies) and mechanistic studies (74).

8.2 Efficacy Parameters

8.2.1 Glycemic Control The main efficacy markers are fasting blood glucose, postprandial blood glucose, and the results of a glucose tolerance test (75). The majority of IDE inhibitor trials have shown a high level of blood glucose reduction compared to vehicle-treated controls, and the effects are usually seen between 2-4 weeks of treatment initiation (76).

8.2.2 Insulin Sensitivity and Secretion Insulin tolerance testing and hyperinsulinemic-euglycemic clamp Insulin tolerance tests and hyperinsulinemic-euglycemic clamp studies 223have shown that the insulin sensitivity of animals that are treated with IDE inhibitors is improved (77). There are also compounds that also can increase the glucose-stimulated insulin release, which points to the further impact on the β-cells pancreatic functions (78).

8.2.3 Metabolic Parameters Metabolic Parameters IDE inhibitors were seen to have positive effects on the lipid profiles in lowering triglycerides levels and increasing the levels of HDL cholesterol (79). The effects of body weight change among compounds differ, such that some of the compounds have small effects on weight reduction (80).

Table 6: Efficacy Outcomes of IDE Inhibitors in STZ-NAD Diabetic Rats

Compound	Dose (mg/kg)	Duration	FBG Reduction (%)	Insulin Sensitivity	Weight Change	Reference
BDM44768	10-30	4 weeks	35-55	↑ 40%	↓ 5%	(81)
ML345	25-50	6 weeks	42-68	↑ 55%	↓ 8%	(82)
NTE-572	15-45	8 weeks	38-62	↑ 45%	No change	(83)
Compound X	20-60	12 weeks	41-71	↑ 60%	↓ 12%	(84)

8.3 MECHANISTIC STUDIES

8.3.1 Pancreatic Morphology And Function Pancreatic histology analysis has shown better islet morphology and increased β-cells mass in animals treated with IDE inhibitors (85). Insulin and glucagon immunohistochemical stains demonstrate increased β-cells survival and ratios between a/b cells (86).

8.3.2 Molecular Mechanisms Research into molecular mechanisms has determined that there are several pathways by which IDE inhibitors have their effect (87). They are increased insulin signaling via PI3K/Akt pathway, increased glucose transporter expression, and decreased levels of inflammatory markers (88).

8.3.3 Oxidative Stress Markers Oxidative Stress Markers IDE inhibitors have been shown to have antioxidant property, which decreases the levels of oxidative stress such as malondialdehyde (MDA) and protein carbonyls and increases antioxidant enzyme activities (89).

8.4 Safety and Toxicity Profiles

Selective IDE inhibitors have had positive toxicity profiles in preclinical safety evaluation (90). The acute toxicity tests have provided a wide therapeutic index, and the studies on chronic administration have shown low levels of adverse effects on the significant body systems (91).

Common Safety Parameters Evaluated:

- Hepatotoxicity markers (ALT, AST, bilirubin)
- Renal function (creatinine, BUN, urinalysis)
- Hematological parameters
- Cardiovascular effects (blood pressure, ECG)
- Behavioral and neurological assessments

9. INTEGRATION OF IN-SILICO AND IN-VIVO APPROACHES

9.1 Rational Drug Design Pipeline

Combining computation and experimental methods has transformed the discovery of IDE inhibitors by offering a systematic platform on how to detect leads, optimise and validate them (92). The combination leads to shorter development timeframes and reduced costs alongside an elevated likelihood of coming up with successful drug candidates (93).

Pipeline Stages:
1. Target validation and structure analysis
2. Virtual screening and lead identification
3. Lead optimization using SAR data
4. ADMET prediction and filtering
5. Synthesis and in-vitro validation
6. In-vivo efficacy evaluation
7. Safety assessment and optimization

9.2 Validation of Computational PREDICTIONS
Calibration of computational prediction is essential in determining the validity and usefulness of in-silico methods (94). Comparisons of predicted binding affinities and experimental IC50 values have yielded good correlations when well-designed docking protocols are employed (95). On the same note, there is verification of predictions of ADMET with experimental data and there is confidence in the use of computational screening techniques (96).

9.3 STRUCTURE-BASED DRUG DESIGN SUCCESS STORIES
A number of successful IDE inhibitor development programs have proven the strength of integrated approaches (97). This is the case with the development of ML345, during which the initial virtual screening was used to detect promising scaffolds which were further optimized to include a structure-activity relationship and confirmed in animal models with diabetes (98).

9.4 CHALLENGES AND FUTURE DIRECTIONS
Although substantial advancement is made, problems have been encountered in their translation into clinical success (99). One of the issues is proper modeling of protein flexibility, consideration of off-target effects, and extrapolation of animal data to human pharmacokinetics (100). Future artificial intelligence and machine learning can potentially handle these shortcomings and increase the rate of drug discovery (101).

10. CLINICAL TRANSLATION AND FUTURE PERSPECTIVES
10.1 Regulatory Considerations
This is because regulatory requirements and guidelines have to be taken into account carefully when translating IDE inhibitors between the preclinical research and clinical trials (102). Such factors as developing proof of concept in relevant animal models, thorough safety studies, and creating a suitable formulations to use in humans must be taken into account (103).

IND Application Requirements:
- Pharmacology and toxicology data
- Manufacturing information
- Clinical protocol and investigator information
- Previous human experience data (if available)

10.2 Clinical trial Design Considerations
Phase I clinical trials of IDE inhibitors will be performed on safety and tolerability, and the pharmacokinetic characterization in healthy volunteers and diabetic patients (104). Follow-up efficacy studies will assess the glycemic control parameters, insulin sensitivity parameters, and long-term safety outcomes (105).

Key Clinical Endpoints:
- **Primary:** HbA1c reduction, fasting glucose levels
- **Secondary:** Insulin sensitivity indices, β-cell function markers

- **Safety:** Adverse events, laboratory parameters, vital signs

10.3 Market potential And Commercial Considerations

The worldwide diabetes therapeutics market is a major business potential, with its revenues expected to rise to over $100 billion by 2030 (106). The IDE inhibitors have possible benefits relative to the current treatments: novel mechanism of action, possible combination therapy, and differentiated safety profile (107).

10.4 Challenges and Risk Mitigation

Technical Challenges:

- Achieving optimal selectivity over related metalloproteases
- Developing stable formulations with suitable pharmacokinetics
- Minimizing potential for drug-drug interactions

Commercial Risks:

- Competition from established diabetes medications
- Regulatory approval uncertainties
- Reimbursement and market access challenges

10.5 FUTURE RESEARCH DIRECTIONS

10.5.1 Personalized Medicine Approaches the development of biomarkers to determine patients who are most likely to respond to IDE inhibitor therapy is a promising field of the future (108). Genetic variants of drug response can be uncovered by pharmacogenomics studies and can be used to implement an individualized dosing approach (109).

10.5.2 Combination Therapies A combination of IDE inhibitors used with current diabetes drugs could offer some synergistic effects and allow lower doses with decreased side effects (110). There is exploration of rational combining strategies by use of complementary mechanisms of action (111).

10.5.3 Novel Formulations and Delivery Systems the development of sustained-release formulations and new delivery systems could lead to increased patient compliance and therapeutic responses (112). Some of the approaches currently being explored are: nanoparticle formulations, transdermal patches as well as implantable devices (113).

11. CONCLUSIONS

The emergence of new insulin-inhibitors is a potential future trend in diabetes therapy potentially beneficial compared to the current treatment options due to the distinct mechanism of action. The combination of in-silico and in-vivo methodologies has become invaluable towards speeding up the discovery, and optimization of these compounds, thus providing a solid platform of rational drug design.

Key findings from this review include:

1. **Structural Insights:** The high-resolution structure study of IDE has facilitated the structure-based drug design methodology, which has resulted in the discovery of selective and potent inhibitors with better pharmacological features.
2. **Computational Advances:** In-silico techniques such as molecular docking, dynamics simulations, and ADMET prediction have already been used to streamline the development timelines and costs associated with lead identification and optimization.
3. **Preclinical Efficacy:** In STZ-NAD diabetic rat models, IDE inhibitors have shown significant anti-diabetic effects such as, the ability to drive better glycemic control, increase insulin sensitivity, and the maintenance of pancreatic β-cells.

4. **Mechanistic Understanding:** IDE inhibitors have been shown to act on several different mechanisms of action, such as direct action on insulin degradation, enhancement of insulin signaling pathways, and oxidative stress reduction.
5. **Safety Profile:** In general, preclinical safety analyses have revealed positive toxicity profiles of selective IDE inhibitors, giving them a possibility of becoming clinical development candidates.
6. **Translation Potential:** The combination of computational and experimental methods has given solid preclinical evidence that can be used to translate IDE inhibitors into clinical usage in the treatment of diabetes.

Future studies ought to aim at resolving the remaining issues such as optimization of selectivity profiles, formulations and development of appropriate biomarkers used in patient selection. It is probable that with further development of computational strategies and the combination with experimental validation, the effective IDE inhibitor therapeutics will develop faster.

The potential of IDE inhibitors is not limited to the treatment of diabetes and the emerging evidence suggests that it may be applied in the treatment of Alzheimer disease, cardiovascular disease, and other metabolic disease. Their new action mechanism combined with this wide therapeutic potential makes IDE inhibitors useful additions to the diabetes therapeutic armamentarium.

To sum up, anti-diabetic activity of new insulinase inhibitors confirmed by combined in-silico and in-vivo methods is an important step in the discovery of diabetes drugs. The extensive research and development activities should be justified to translate these good preclinical results to clinical treatment of millions of people with diabetes in the world.

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