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(71) Applicant and
(72) Inventor: SANKARANARAYANAN, Alangudi [IN/IN]; B-7, Swastik Park, Premchandnagar Road, Opp Judges' Bunglows, Ahmedabad 380 015, Gujarat (IN).

(74) Agents: AHUJA, Sudhir, D. et al.; D.P. Ahuja & Co., 53, Syed Amir Ali Avenue, Calcutta 700 019, West Bengal (IN).

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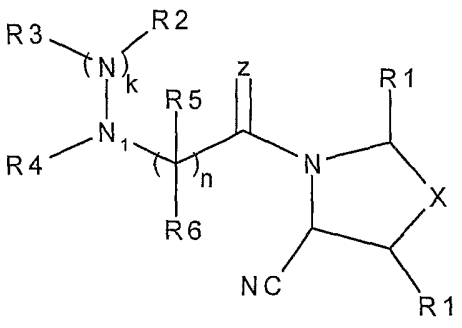


(I)

(57) Abstract: The invention discloses a novel heterocyclic compounds that falls within f the ambit of general formula (I), its stereoisomers, pharmaceutically acceptable salts or solvates wherein X, n,k,z, R1, R2, R3, R4, R5 and R6 are as defined in the specification that are useful in (i) normalizing elevated blood pglucose levels in diabetes, (ii) treating disorders related to glucose intolerance and (iii) for scavenging free radicals of mammals. The invention also discloses pharmaceutically acceptable composition comprising these compounds, method for preparation of the compounds as defined bove and method of treating mammals including human beings by administering an effective amount of said compounds to a subject in need thereof. The invention further discloses use of these compounds in the manufacture of a medicament useful for treatment of different disease conditions as stated above.

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# AZOLIDINECARBONITRILES AND THEIR USE AS DPP-IV INHIBITORS

## FIELD OF THE INVENTION

The present invention relates to novel heterocyclic compounds useful for normalizing elevated blood glucose levels in diabetics and in treating disorders related to glucose intolerance.

These compounds inhibit the enzyme DPP-IV, that degrade the peptide GLP-1, providing for enhanced levels of active GLP-1, a peptide which normalizes elevated blood glucose levels.

These compounds are useful to control blood glucose level in diabetic patients and thereby delay the onset of vascular complications in diabetic patients and also transition to type II diabetes in impaired glucose tolerant patients.

These compounds are also useful in treating disorders related to glucose intolerance like Cushing's syndrome, hyperthyroidism, obesity, hyperglucagonemia, diseases like ulcers, HIV infection, disorders related to increased gastric emptying, acid secretion and hunger, autoimmune disorders like multiple sclerosis, rheumatoid arthritis and Grave's disease (Sedo and Kraml, 1994).

These compounds also exhibit free radical scavenging activity which is useful in treatment of various disease condition caused by accumulation of free radicals in the body cells.

## DESCRIPTION OF THE RELATED ART

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Diabetes mellitus is a clinically and genetically heterogenous group of disorders characterized by abnormally high levels of glucose in the blood. The hyperglycemia is due to deficiency of insulin secretion or to resistance of body cells to the action of insulin, or to a combination of these. Chronic hyperglycemia is a cause of heavy burden of morbidity and premature mortality from diabetic complications. These long-term complications can be delayed by improving glycemic control. None of the currently used medications is capable of reversing an ongoing failure of ß-cell function and reduction in post prandial glucose peak represents an important target for therapeutic strategies.

Although pancreatic insulin secretion is predominantly controlled by blood glucose levels, incretins like the peptide GLP-1 derived from enteroinsular axis have an effect on insulin secretion and therefore on the blood glucose level. It is released from the gut in response to ingested nutrients, which acts on the pancreas to potentiate glucose-induced insulin secretion. GLP-1 has beneficial effects in diabetic patients in normalizing elevated blood glucose levels (Holst J and Deacon C, 1998). GLP-1 has

multifaceted actions, which include stimulation of insulin gene expression, trophic effects on ß-cells, inhibition of glucagon secretion, promotion of satiety, and slowing of gastric emptying. Because of glucose dependency of the peptide and glucagonostatic actions, the glucose lowering effect is self-limiting, and the hormone, therefore does not cause hypoglycemia regardless of the dose.

The pathogenesis of type-2 diabetes ordinarily involves the development of insulin resistance associated with compensatory hyperinsulinaemia followed by progressive beta-cell impairment that results in decreasing insulin secretion and hyperglycemia. Hyperglycemia itself causes additional inhibition of insulin secretion and more insulin resistance (glucose toxicity), which further accentuates the hyperglycemia.(Augustyns K. et al. The unique properties of Dipeptidyl-peptidase IV (DPP-IV/ CD 26) and the therapeutic potential of DPP – IV inhibitors. Current Medical Chemistry 1999; 6:311-327)

Most therapies used at present ultimately fail to control blood sugar level after 3-5 years. This is due to the progressive β-cell failure in the course of the disease and insulin is finally required in most type-2 diabetic patients.

Impaired glucose tolerance and impaired fasting glucose is present in a large population. These abnormalities progress to a large extent to overt diabetes. No therapy has been approved for the prevention or delay of type-2 diabetes in these patients.

Dipeptidyl Peptidase IV (DPP-IV) inhibitors addresses to a large extent the inadequacies of the presently available therapies. It targets not only the β-cell dysfunction but also insulin resistance and increased hepatic glucose output by liver. Thus, it has a more holistic approach towards the treatment of type-2 diabetes. Furthermore, by stabilizing / reversing the progressive β-cell dysfunction, it would prevent the progression of the disease and for the same reason, it has the potential to prevent or delay the occurrence of overt diabetes in subjects with impaired fasting glucose and impaired

glucose tolerance. (Pathogenesis of type-2 Diabetes; Harold E Lebovitz, Drug Benefit Trends 12 (supp A):8-16, 2000).

The presently used antihyperglycemic drugs target either insulin resistance or β-cell dysfunction. Hence, there is a need to address both these pathologies together.

The homeodomain transcription factor, PDX-1 is essential for the early development of the pancreas and the maintenance of the ß-cell phenotype. PDX-1 is known to regulate insulin, GLUT2 and islet amyloid precursor. Under conditions of sustained hyperglycemia, such as in the diabetic state, there is a downregulation of PDX-1 expression and a decrease in insulin secretion (Doyle and Egan, 2001). GLP-1 induces the differentiation of PDX-1 positive pancreatic epithelial cells into insulin-secreting cells. GLP-1 stimulates the expression of transcription factor PDX-1 while stimulating ß-cell neogenesis and may thereby be an effective treatment for diabetes. GLP-1 and a long acting GLP-1 analogue exendin-4, stimulates both ß-cell replication and neogenesis, resulting in increased ß-cell mass and improved glucose tolerance in partial pancreatectomy rat model of type 2 diabetes (Gang et al, 1999).

GLP-$1_{7-36}$ is one of the substrate for the circulating exopeptidase dipeptidyl peptidase IV (EC 3.4.14.5), a post proline cleaving enzyme with a specificity for removing Xaa-Pro or Xaa-Ala dipeptides from the N-terminus of polypeptides and proteins. DPP-IV is widely distributed in tissues like kidney, intestine and placenta, hepatocytes, epithelial cells of pancreatic duct, central nervous system, peripheral nervous system, endothelial cells of blood vessels (Rolf, 1999), and found as soluble enzyme in blood plasma. About 50% of the GLP-$1_{7-36}$ amide released from the L cells is inactivated in the capillary bed surrounding these cells by DPP-IV. Furthermore, single pass through the liver inactivates a large fraction of the remaining active GLP –1 (>40%) (Bork and Xue, 2000). Thus these two processes together with inactivation in the circulatory system and in other organs can be expected to inactivate or remove most of the GLP-1 released from the duodenum and intestine before the peptide can reach the pancreas in the active form. Hydrolysis of GLP-$1_{7-36}$ by DPP-IV yields the truncated

oligopeptide GLP-$1_{9-36}$ and the dipeptide His-Ala. This N- terminally truncated form is not insulinotropic and acts as an antagonist at GLP-1 receptor. GLP-1 is rapidly degraded in the circulation, which results in clearance that exceeds cardiac output and an apparent half-life of 1-1.5 min. The truncated metabolite is eliminated more slowly, with half-lives of 4-5 min for GLP-$1_{9-36}$. It has been speculated that DPP-IV-mediated hydrolysis is the primary mechanism of inactivation of this hormone in vivo (Tina et al, 2001).

Because of rapid degradation, the effects of single injections of GLP-1 are short lasting and for a full demonstration of its anti-diabetogenic effects, continuous intravenous infusion is required. Therefore, it is proposed that the inhibition of DPP-IV, that elevate the levels of active GLP-1 and reduce the level of antagonistic metabolite, may be useful to treat impaired glucose tolerance and perhaps transition to type 2 diabetes.

(Siegel et al (1999) reported that analogues of GLP-1 resistant to degradation by DPP-IV might help to realize the potential of GLP-1 in diabetes therapy.

DPP-IV inhibitor, Isoleucine thiazolidide (P-32/98), completely inhibited the formation of GLP-$1_{9-36}$, an antagonist at GLP-1 receptor, when it was incubated with 30 mM/L GLP-$1_{7-36}$ and serum for 21 hours. Inhibition of circulating DPP-IV enhanced insulin secretion and improved glucose tolerance in response to oral glucose challenge in lean and obese fatty (fa/fa) rats. (Raymond et al, 1998). Also it has improved glucose tolerance in zucker fatty rats (Robert et al, 1999)

It is reported that a DPP-IV inhibitor NVP-DPP-728 i.e. 1-[-2-{(5-cyanopyridin-2-yl) amino}ethylamino]acetyl-2-cyano-(S)-pyrrolidine inhibits DPP-IV activity and improves insulin secretion and glucose tolerance, through augmentation of the effects of endogenous GLP-1. The improvement in prandial glucose homeostasis during DPP-IV inhibition by this molecule suggests that inhibition of this enzyme is a promising target for treating type 2 diabetes (Balkan et al, 2000) Also this molecule showed potentiation of insulinotropic effects of GLP-1 in anaesthetized pig (Carolyn et al, 1998).

These data support a therapeutic approach of drug manipulation of plasma incretin activity by lowering glucose levels in NIDDM and other disorders involving glucose intolerance.

Dipeptidyl Peptidase IV (DPP – IV) is a proline specific protease and is involved in breaking peptide bonds before or after a proline residue. It plays an important role in the regulation of the life-time of biological active peptides like growth hormone releasing factor (GRF), Glucagon-like peptide – I (GLP-I), Gastric Inhibitory Polypeptide (GIP), Glucagon-like peptide – II (GLP-II), β-Casomorphin, morphiceptin, Human Neuropeptide Y, Human Peptide YY (Augustyns K. et al. 1999) DPP – IV is present on the surface of a subset of T-cells (lymphocytes) and has been recognized as CD 26 antigen.

Dipeptidyl peptidase-IV (DPP-IV) is a serine protease, which cleaves N-terminal dipeptides from a peptide chain containing, preferably, a proline residue in the penultimate position. DPP-IV is responsible for inactivating glucagon-like peptide-1 (GLP-1). More particularly, DPP-IV cleaves the amino-terminal His-Ala dipeptide of GLP-1, generating a GLP-1 receptor antagonist, and thereby shortens the physiological response to GLP-1. Since the half-life for DPP-IV cleavage is much shorter than the half-life for removal of GLP-1 from circulation, a significant increase in GLP-1 bioactivity (5- to 10-fold) is anticipated from DPP-IV inhibition. Since GLP-1 is a major stimulator of pancreatic insulin secretion and has direct beneficial effects on glucose disposal, DPP-IV inhibition appears to represent an attractive approach for treating non-insulin-dependent diabetes mellitus (NIDDM). GLP-1 has multifaceted actions, which include stimulation of insulin gene expression, trophic effects on β-cells, inhibition of glucagon secretion, promotion of satiety, and slowing of gastric emptying, all of which contribute to normalizing elevated blood glucose levels (Holst and Deacon, 1998). Because of glucose dependency of the peptide and glucagonostatic actions, the glucose lowering effect is self-limiting, and the hormone, therefore does not cause hypoglycemia regardless of the dose.

The exact biological functions of DPP – IV / CD 26 are still under investigation, but considerable evidence exists for the therapeutic potential of DPP-IV inhibitors.

Although a number of DPP-IV inhibitors have been described, all have limitations relating to potency, stability or toxicity. Accordingly, a great need exists for novel DPP-IV inhibitors, which do not suffer from the above-mentioned limitations.

**Type – II Diabetes Mellitus: -**

DPP – IV is involved in the degradation of GIP and GLP-I. GIP and GLP-I are considered to be most important insulin-releasing hormones (incretins) comprising the enteroinsular axis. The term enteroinsular axis refers to the signaling pathways between the gut and pancreatic islets that amplify the insulin response to absorbed nutrients. Inhibition of circulating DPP – IV with orally administered lie-thiazolidine [DPP-IV inhibitor] enhanced insulin secretion and improved glucose tolerance in response to an oral glucose challenge in lean and obese Zucker rats. The enhanced incretin response was greater in obese than in lean animals, with a more profound improvement in glucose tolerance (Pederson R. A, 1998). This was attributed to disruption of DPP – IV inactivation of GIP and GLP-I, resulting in amplification of enteroinsular axis.

DPP-IV inhibitors would have very little effect on subjects with normal blood glucose levels regardless of dose because its actions are glucose dependent (Qualmann C et al. 1995).

**Hyperthyroidism and glucose intolerance**

In patients with preexisting type I or type II diabetes mellitus, the presence of hyperthyroidism renders blood glucose management more difficult. Influences of thyroid hormone on insulin secretion and cellular metabolism have been implicated on the basis of *in vitro* and animal studies. In rats, thyroxine and triiodothyronine treatment inhibits the delayed phase of glucose-mediated insulin secretion- triiodothyronine being fivefold more potent than thyroxine.

In hyperthyroid states, gluconeogenic precursor (lactate and glycerol) are present in increased concentration in plasma. In rats, increased activity of mitochondrial glycerol, phosphate oxidase increases the capacity for gluconeogenesis from glycerol. It has also been shown in rats and pigs that hyperthyroidism leads to an increase in futile cycling of glucose, which could contribute to hyperglycemia. Increased activity of several enzymes that could be implicated in the increase in gluconeogenesis have been seen in response to thyroid hormone, including glucokinase, pyruvate carboxylase, phospho-enolpyruvate carboxykinase, and glucose-6-phosphatase. Studies in hyperthyroid patients report impairment in insulin suppression of hepatic glucose production. A recent study has also shown the inability to increase the insulin response appropriately to hyperglycemia and increased proinsulin levels, both fasting and in response to a meal (Michael Berelowitz and Lone A Kourides, 2000). Glucose intolerance as a result of hyperthyroidism can be better managed by enhancing the levels of GLP-1 a glucose dependent insulinotrophic agent.

**Obesity and glucose intolerance**

Obesity has been related to insulin resistance and hyperinsulinemia. Visceral obesity is associated with specific changes in skeletal muscle morphology that correlate with insulin resistance and hyperinsulinemia, namely a reduction in capillary density and an increase in the proportion of 'white' or 'glycolytic' fibers which are less insulin sensitive than red (glycolytic) fibers. TNF-alpha is secreted by adipose tissue and its circulating levels parallel total body fat mass. Circulating non-esterified fatty acid (NEFA) levels are raised in obese subjects, especially those with visceral obesity. In the liver, NEFA are oxidised to acetyl CoA, which stimulates pyruvate carboxylase and therefore gluconeogenic production of glucose from pyruvate; hepatic glucose production therefore increases. High NEFA level may also inhibit glucose utilization by skeletal muscle. Increased acetyl CoA levels inhibit pyruvate dehydrogenase, thus decreasing glucose oxidation. The combination of increased hepatic glucose output and reduced peripheral uptake effectively antagonises and would ultimately lead to hyperglycemia

(Ronald T Jung, 1997). Glucose intolerance as a result of the above conditions can be managed better by the elevation of GLP-1 levels (as a result of DPP-IV inhibition).

**Cushing's syndrome and glucose level**

Cushing's syndrome represents a distinctive constellation of clinical features associated with prolonged overproduction of impaired glucose tolerance, overt diabetes (in approximately 20%), loss of libido and impotence. Some of these abnormalities such as obesity, deranged glucose metabolism are directly attributable to increased glucocorticoids. These glucocorticoids stimulate gluconeogenesis in diabetes. Also, they increase amino acid uptake by the liver and kidney and increase the activity of enzymes required for gluconeogenesis and may lead to hyperglycemia (Ronald A DeLellius, 1989)

Glucose metabolism under the above conditions can be managed better by treatment with DPP-IV inhibitors.

**Role of DPP- IV in HIV infection**

**Prevention and treatment of HIV infection**

The role of CD26 in HIV infection is also not completely clear yet, but seems to be important. Some DPP-IV inhibitors are reported to inhibit HIV infection such as pyrrolidine-2-nitriles and an irreversible cyclopeptide inhibitor (Nguyen C et al. 1998).

DPP-IV has been originally described to be a marker of activated T lymphocytes and lately DPP-IV/CD26 molecular identity has been proven CD26/DPP-IV serves as an essential cofactor for HIV entry into $CD^{4+}$ cells and that its enzyme activity is an important condition for this function (Sedo A and Kraml J, 1994). Hence inhibition of DPP-IV could prove useful in the management of HIV infection.

**Immunosuppressant**

It has been shown that DPP-IV / CD26 plays an important role in the immune system by a number of possible mechanisms. The exact mechanism remains to be elucidated, but a few examples are reported where DPP-IV inhibitors are useful immunosuppressants *in vivo*. A dipeptide diphenyl phosphonate ester was able to abrogate acute rejection and prolong allograft cardiac survival (Korom S. et al. 1997).

**Role of DPP-IV inhibitors in ulcers, hyperglucagonemia, gastric emptying and hunger**

DPP-IV inhibitors increase the level of GLP-1. GLP-1 has multifaceted actions, which include stimulation of insulin gene expression, inhibition of glucagon secretion, promotion of satiety, inhibition of food intake and slowing of gastric emptying (Holst JJ and Deacon CF, 1998).

GLP-1 also reduces gastric acid secretion (Michael A Nauck, 1999). Increase in gastric acid secretion is one the main reason for duodenal ulcers. By inhibiting gastric acid secretion, GLP-1 and therefore DPP-IV inhibitors may prove useful for the treatment of ulcers or can be used in combination with other antiulcer agents.

**Diarrhoea**

DPP-IV is involved in metabolic processing of morphiceptin. Co-administration of a DPP- IV and the opiate peptide morphiceptin could be used in case of diarrhoea, as the experiment with DPP-IV deficient rats showed (Tiruppathi, C., et al., Am. J. Physiol. 1993).

**Mucosal Regeneration in patient with Intestinal Disease**

DPP-IV hydrolysis of GLP-2 is responsible for its inactivation. GLP-2 has recently been shown to display intestinal growth factor activity in rodents, raising the possibility that GLP-2 may be therapeutically useful for enhancement of mucosal regeneration in patients with intestinal disease (Drucker, D.J. et al. Diabetes 1998; 47:159). The use of [$Gly^2$] GLP-2, resistant to DPP-IV hydrolysis, increases small bowel weight in mice, predominantly due to a significant increase in villous height (Brubaker P.L. et al. Am. J. Physiol. 1997).

**Growth Hormone Deficiency**

Since GRF is also degraded by DPP-IV, the use of a DPP-IV inhibitor together with GRF could be useful to treat children with growth hormone deficiency (Augustyns K. et al. 1999)

**Neurological and Neuropsychological Disorders**

Administration of a suitable DPP-IV inhibitor leads as a causal consequence to a reduced degradation of the neuropeptide Y(NPY) in the brain of mammals. Such treatment will result in a reduction or delay in the decrease of the concentration of functionally active neuronal NPY (1-36). As a consequence of the resulting enhanced stability of the endogenous NPY (1-36), NPY activity is prolonged thereby resulting among other things in functionally active NPY YI receptor activity thereby facilitating antidepressive, anxiolytic, analgesic, antihypertension and other neurological effects (WO 02/34243 dated 02 May 2002 by PROBIODRUG AG).

**Cancers and Tumours**

DPP-IV is able to bind proteins of the extracellular matrix as a cell adhesion molecule. This has been interpreted from the observation that the DPP-IV inhibitors interfere *in vitro* with the initial spreading of rat hepatocytes on a matrix consisting of fibronectin and collagen. Thus the DPP-IV inhibitors could also be used for the

prevention/treatment of cancer metastasis and tumour colonization (WO 03/002595 dated 09 Jan 2003 by PROBIODRUG AG).

**Free Radical Scavenging Activity**

It has been reported that compounds exhibiting free radical scavenging activity are useful in treatment of Neurodegenerative disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Motor Neuron Disease, Prion Disease etc, (b) Diabetes and Diabetic Vascular Complications, (c) Intestinal Diseases such as Intestinal Ischemia, Radiation Enteritis, Inflammatory Bowel Disease, Gastric and Colorectal Cancers etc., (d) Liver Diseases such as Alcoholic Liver Disease, Chronic Hepatitis C etc., (e) Cancers such as Lung Cancer, Colorectal Cancer, Cervical Cancer, Breast Cancer, Malignant Melanoma etc, (f) Cardiac Diseases such as Atherosclerosis, Myocardial Infarction, Ischemic Stroke, Endothelial Dysfunction etc., (g) Opthalmic Disorders such as Cataract formation, Macular degeneration etc., (h) HIV Diseases, (i) Respiratory Diseases such as Chronic Obstructive Pulmonary Diseases, Asthma etc., (j) Renal Diseases such as Glomerulonephritis, Acute Renal Failure etc.

Neuro-degenerative disorders such as Alzheimer's disease (A.D.), Parkinson's disease (P. D.), Huntington's disease (H.D.), Motor neuron disease (M.N.D), Prion disease.

As people age, their antioxidant levels diminish and these low levels are directly linked to the many diseases associated with aging such as Alzheimer's and Parkinson's disease. One of the leading hypotheses is that oxidative stress induced by ROS damages essential components of the neurons, resulting ultimately in the neuronal death. Oxidative stress is involved in various divergent events leading to neuronal damage, including an increase in membrane rigidity, DNA strand break, and impairment in glucose uptake. Several potential sources of oxidative stress in different neurodegenerative disorders have been well identified (Munch G, et al. 1998).

In A.D. mitochondrial dysfunction, amyloid beta mediated processes; transition metal accumulation and genetic factors are responsible for the redox imbalance (Smith MA, et al. 2000).

Point mutations in Superoxide Dismutase enzymes are known in the familial form of MND.

Disturbances of neuronal energy metabolism have been implicated as a pathogenetic mechanism for H.D. (Browne SE, et al. 1999)

**Diabetes and Diabetic Vascular Complications (DVCs)**

The cause of oxidative stress in diabetes is not yet fully understood but is thought to be due to mitochondrial dysfunction, direct enzyme inhibition by hyperglycemia, auto-oxidation of glucose, and activation of nicotinamide-adenine dinucleotide phosphate (NADPH)-oxidase. Oxidative stress in diabetes is also increased due to weakened defenses due to reduced endogenous antioxidants. The oxidative stress manifests itself as elevated concentrations of lipid peroxidation products, erythrocyte fragility, and decreases in the antioxidant enzyme systems (CAT, GSH Px, SOD). Recent studies also have shown a positive correlation between blood glucose concentration and oxidant-induced lymphocyte DNA damage (E.J. Harper The 24$^{th}$ Annual WALTHAM®/OSU SYMPOSIUM).

ROS are generated during glucose oxidation and formation of advanced glycation end products (AGE). Evidence has accumulated indicating that the generation of ROS plays an important role in the development of DVCs. Many biochemical pathways associated with hyperglycemia such as advanced glycosylation, glucose auto oxidation, and polyol pathway can increase the production of free radicals. Hyperglycemia in diabetic patients leads to excess auto-oxidation of glucose thereby reducing molecular oxygen and yielding oxidizing intermediates such as superoxide ions ($O_2^-$), hydroxyl radicals ($^{\cdot}OH$), and hydrogen peroxide ($H_2O_2$). Free radicals accelerate the formation of

advanced glycosylation end products (AGE), because fragmentation and conformational changes occurring during glycosylation and glucose oxidation have been shown to be dependent upon free radicals. AGEs in turn supply more free radicals; this process is termed as oxidative glycosylation or glycoxidation. These free radicals impair vascular relaxation by inactivating or quenching nitric oxide (NO) and also adversely affect the endothelial function. Evidence also suggests that Maillard reaction acts as an amplifier of oxidative damage in aging and diabetes.

*Intestinal diseases*

Oxidative stress is an important cause of tissue injury that occurs in inflammation and ischemia. ***Intestinal ischemia, radiation enteritis, inflammatory bowel disease,*** and promotion of ***gastric and colorectal cancers*** are some of the gastro-intestinal conditions where oxidative stress is implicated in the pathogenesis.

*Liver diseases*

***Alcoholic liver disease***- Ethanol induces an increase in lipid peroxidation either by enhancing ROS or decreasing the level of endogenous antioxidants. Ethanol also induces variety of cytochrome P450 enzymes in microsomes and xanthine oxidases in cytosol. The role of these enzymes in the generation of oxidative stress has been well established in various studies (Ishii H, et al. 1997).

Chronic hepatitis C- Enhanced oxidative stress initiates a fibrogenesis cascade in the liver of patients with chronic hepatitis C. Evidences are coming up supporting an oxidative stress pathway leading to active fibrogenesis in chronic hepatitis C. This fibrogenesis cascade characteristic of severe chronic hepatitis C (e.g., oxidative stress, induction of c-myb, activation of stellate cells, and collagen gene expression) is stimulated by ROS.

*Cancers*

Oxidative damage to DNA is a result of interaction of DNA with ROS, in particular the hydroxyl radical. The hydroxyl radicals produce multiple modifications in

DNA. Oxidative attack by OH radical on the deoxyribose moiety leads to the release of free bases from DNA, generating strand breaks with various sugar modifications and simple abasic (AP) sites.

ROS also interact with and modify cellular protein, lipid, and DNA, which results in altered target cell function. The accumulation of oxidative damage has been implicated in both acute and chronic cell injury including possible participation in the formation of cancer. Acute oxidative injury may produce selective cell death and a compensatory increase in cell proliferation. This stimulus may result in the formation of newly initiated preneoplastic cells and/or enhance the selective clonal expansion of latent initiated preneoplastic cells. Similarly, sublethal acute oxidative injury may produce unrepaired DNA damage and result in the formation of new mutations and, potentially, new initiated cells. ROS, therefore, can have multiple effects in the initiation stage of carcinogenesis by mediating carcinogen activation, causing DNA damage, and interfering with the repair of the DNA damage.

Benefits of various antioxidants in preventing or treating following cancers have been extensively studied.

1) Lung cancer
2) Colorectal cancer
3) Cervical cancer
4) Breast cancer
5) Malignant melanoma

***Oxidative stress in cardiac diseases***

Lifelong high levels of antioxidant nutrients are supposed to protect against the development of heart disease. High doses of antioxidants in the month following an acute heart attack have been shown to significantly reduce the number of deaths, as well as the extent of cardiac damage in non-fatal cases.

It is currently thought that increase in oxidative stress is involved in the pathophysiology of endothelial dysfunction that accompanies a number of cardiovascular risk factors including hypercholesterolemia, hypertension and cigarette smoking. It also plays a pivotal role in the evolution of clinical conditions such as atherosclerosis and heart failure. Oxidative stress can activate redox-sensitive kinase cascades and transcription factors such as $NF_KB$ and AP-1, with resulting increases in the expression of factors associated with an inflammatory response and cellular proliferation. There are three enzyme systems producing reactive oxygen species in the vascular wall: NADH/NADPH oxidase, xanthine oxidoreductase, and endothelial nitric oxide synthase (Zalba G.et al, 2000, Rosenfeld ME, 1998).

Atherogenesis is regarded as the outcome of interactions among multiple stimuli. Endothelial dysfunction plays a key role in the development of **atherosclerosis**. Elevated homocysteine concentrations are associated with rapid onset endothelial dysfunction, which is another mechanism by which increased oxidative stress contributes to atherosclerosis. Oxidation of low-density lipoprotein plays an important role at several steps in atherogenesis. Oxidative stress also activates $NF_KB$, which induces expression of genes controlling cytokine expression and leukocyte adhesion to vascular wall. (Maxwell, et al. 1997).

Animal studies have provided evidence by suggesting that free radicals may promote thrombosis, directly damage vascular cells and other tissues, and interfere with vasomotor regulation with the clinical sequelae of **myocardial infarction and ischemic stroke.**

In tissues where oxygen supply becomes used up following ischemia, as in myocardial ischemia, the enzyme xanthine oxidase is changed to a form that has potential to reduce oxygen to superoxides. On readmission of oxygen e.g. by reperfusion there is a burst of free radical generation. ROS are formed at an accelerated rate in post-ischemic myocardium. Thus biochemical damage due to free radicals contributes to the ischemic injury.

Oxidative stress also seems to be one of the mechanisms that may produce membrane defects and result in intracellular calcium overload, and cardiac contractile dysfunction in the stunned myocardium.

*Macular degeneration and cataract*

Oxidative damage to lens of the eye with increase in age has a major contribution in cataract formation. Macular degeneration is also being recognized as a consequence of oxidative damage.

*HIV disease*

Perturbation of anti-oxidant defense system has been observed in various tissues in HIV patients. Oxidative stress may contribute to several aspects of HIV disease pathogenesis such as viral replication, inflammatory response, and decreased immune cell proliferation, loss of immune function, apoptosis, chronic weight loss. Antioxidants may offer a promising treatment to HIV patients.

*Chronic obstructive pulmonary diseases (COPD)*

Alteration in the alveolar and lung metabolism of glutathione is widely recognized as a central feature of many inflammatory lung diseases including COPD. These changes are a result of the alteration in the gene expression of the gamma-glutamyl cystine synthase (Gamma-GCS), the rate-limiting enzyme in glutathione synthesis. Oxidative stress is implicated in the pathogenesis of COPD, since it results in inactivation of anti proteinases, airspace epithelial injury, mucus hypersecretion, increased influx of neutrophils into the lungs, transcription factor activation and gene expression of pro-inflammatory mediators (MacNee W, et al. 2001).

*Renal Disease*

ROS have been implicated not only in the genesis of different forms of renal disease, predominantly experimentally induced **glomerulonephritis**, but also in different forms of **acute renal failure**.

*Asthma*

Although the pathogenesis of asthma is not fully defined, a typical feature is an increase in the number of inflammatory cells in the lung. Such cells generate ROS, which are involved in the pathophysiology of asthma, including airway smooth muscle contraction, increased airway reactivity, and increased vascular permeability.

**Effect of antioxidant status on immunologic function**

The immune system is particularly sensitive to oxidative stress, primarily because immune cells rely heavily on cell-to-cell communication to work effectively. Peroxidation of cell membranes compromises membrane integrity and disrupts intracellular signaling.

*Cataract:*

**Oxidative damage to lens of eye with increase in age has been a major contribution in cataract formation.**

Thus, by scavenging the free radicals, the following diseases can be treated or controlled :

1) Neurodegenerative disorders
   (a) Alzheimer's Disease
   (b) Parkinson's Disease
   (c) Huntington's Disease
   (d) Motor Neuron Disease
   (e) Prion Disease

2) Diabetes and Diabetic Vascular Complications

3) Intestinal Diseases
   (a) Intestinal Ischemia

(b) Radiation Enteritis

(c) Inflammatory Bowel Disease

(d) Gastric and Colorectal Cancers

4) Liver Diseases

(a) Alcoholic Liver Disease

(b) Chronic Hepatitis C

5) Cancers

(a) Lung Cancer

(b) Colorectal Cancer

(c) Cervical Cancer

(d) Breast Cancer

(e) Malignant Melanoma

6) Cardiac Diseases

(a) Atherosclerosis

(b) Myocardial Infarction

(c) Ischemic Stroke

(d) Endothelial dysfunction

7) Opthalmic Disorders

(a) Cataract formation

(b) Macular degeneration

8) HIV Disease

9) Respiratory Diseases

(a) Chronic Obstructive Pulmonary Diseases (COPD)

(b) Asthma

10) Renal Diseases

(a) Glomerulonephritis

(b) Acute Renal failure

## OBJECTS OF THE INVENTION

The first object of the present invention is to provide a new class of compounds which normalize elevated blood glucose levels in diabetic patients thereby delaying diabetic complications and preventing transition to type II diabetes in impaired glucose tolerant patients.

These compounds exhibit in vitro DPP-IV inhibitory activity. DPP-IV inhibitors enhance the level of active GLP-1, which would be advantageous in treating hyperglycemia. Added advantage is that there is no risk of hypoglycemia, since GLP-1 increases glucose mediated insulin secretion. Due to non-peptide nature of the compounds, they can be conveniently administered orally. The increase in GLP-1 level in the active form provides for multifaceted action in respect of increase in insulin level, decrease in glucagon level, neogenesis of pancreatic ß-cell, stimulation of insulin gene expression, and promotion of satiety, all of which contribute to beneficial effects in a diabetic patient.

Another object of the invention is to provide a method of treatment of a diabetic patient with glucose intolerance by administration of the compounds of the invention or pharmaceutically acceptable salts thereof either singly or in combination with drugs for anti-diabetic or other therapies for Cushing's syndrome, hyperthyroidism, HIV infection, obesity, ulcers, disorders related to hyperglucagonemia, gastric emptying and hunger in required dosage in admixture with pharmaceutically acceptable diluents, solvents, excepients, carriers or other media as may be appropriate for the purpose.

A further object of the invention is to provide a class of compounds having free radical scavenging activity which are useful for treatment of (a) Neurodegenerative disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Motor Neuron Disease, Prion Disease etc, (b) Diabetes and Diabetic Vascular Complications, (c) Intestinal Diseases such as Intestinal Ischemia, Radiation Enteritis, Inflammatory Bowel Disease, Gastric and Colorectal Cancers etc., (d) Liver Diseases such as Alcoholic Liver Disease, Chronic Hepatitis C etc., (e) Cancers such as Lung Cancer, Colorectal Cancer, Cervical Cancer, Breast Cancer, Malignant Melanoma etc., (f) Cardiac Diseases such as Atherosclerosis, Myocardial Infarction, Ischemic Stroke, Endothelial Dysfunction etc., (g) Opthalmic Disorders such as Cataract formation, Macular degeneration etc., (h) HIV Diseases, (i) Respiratory Diseases such as Chronic Obstructive Pulmonary Diseases, Asthma etc., (j) Renal Diseases such as Glomerulonephritis, Acute Renal Failure etc.

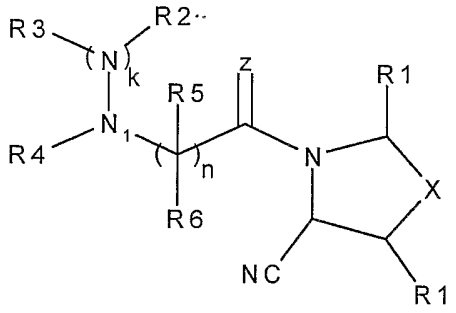
Yet another object of the present invention is to provide a method of preparation of these compounds.

A still further object of the invention is to provide a pharmaceutical composition comprising said compound in association with a pharmaceutical acceptable carrier, diluent or excepients.

Yet another object of the invention is to provide a method of treatment and/or prophylaxis of mammals including human beings for diseases relating to glucose intolerance and/or disease conditions caused by accumulation of free radicals in the body cells.

## SUMMARY OF THE INVENTION

The present invention provides novel compounds represented by general formula (I) and its pharmaceutically acceptable salts, which is to be understood as also including its derivatives, analogs, tautomeric forms, stereoisomers, polymorphs and their pharmaceutically acceptable solvates, which are useful for one or more of (i) normalizing elevated blood glucose levels in diabetes, (ii) treating disorders related to glucose intolerance and (iii) scavenging free radicals from body cells.




(I)

wherein

X is O, S, SO, $SO_2$, NR7 or CHR1 ;

n is null or 1;

k is null or 1;

Z is O, S, and NR7 ;

R1 at two positions are independently selected from hydrogen or a substituted or unsubstituted group selected from linear or branched ($C_1$-$C_{12}$)alkyl, ($C_2$-$C_{12}$)alkenyl, ($C_3$-$C_7$)cycloalkyl, ($C_5$-$C_7$)cycloalkenyl, bicycloalkyl, bicycloalkenyl, heterocycloalkyl, aryl,

aryloxy, aralkyl, aralkoxy, heteroaryl, heteroaralkyl, heteroaryloxy, heteroaralkoxy, wherein one or more heteroatoms are independently selected from O, N or S;

R2, R3, R4 and R7 are independently selected from hydrogen, perhaloalkyl, -(CO)NR8R9, -(CO)R8,-(CO)OR8, -SO2R8, -SOR8, substituted or unsubstituted groups selected from linear or branched ($C_1$-$C_{12}$)alkyl, ($C_2$-$C_{12}$)alkenyl, ($C_3$-$C_7$)cycloalkyl, ($C_5$-$C_7$)cycloalkenyl, bicycloalkyl, tricycloalkyl amidino bicycloalkenyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, wherein one or more heteroatoms are independently selected from O, N or S;

R5 and R6 are independently selected from by hydrogen or a substituted or unsubstituted group selected from linear or branched ($C_1$-$C_{12}$)alkyl, ($C_2$-$C_{12}$)alkenyl, ($C_3$-$C_7$)cycloalkyl, ($C_5$-$C_7$)cycloalkenyl, bicycloalkyl, bicycloalkenyl, heterocycloalkyl, aryl,aralkyl, heteroaryl,heteroaralkyl, wherein one or more heteroatoms are independently selected from O, N or S;

R8 and R9 are independently selected from hydrogen or a substituted and unsubstituted group selected from linear or branched ($C_1$-$C_{12}$)alkyl, alkoxyaryl, alkoxyalkyl, alkoxycycloalkyl, alkoxyaryl, perhaloalkyl, ($C_2$-$C_{12}$)alkenyl, ($C_3$-$C_7$) cycloalkyl, perhalocycloalkyl, haloheterocycloalkyl, cyanoheterocycloalkyl, perhaloheterocycloalkyl, ($C_5$-$C_7$) cycloalkenyl, bicycloalkyl, bicycloalkenyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, perhaloaryl, perhaloheteroaryl;

wherein in the groups represented by R1, R2, R3, R4, R5, R6, R7, R8 and R9 when substituted, the substitutents are optionally and independently bridged by –(CO)-, -CH2(CO)-(CO)O, -(CO)NH-, -CH2(CO)NH-, -NH-, -NR8-, -O-, -S-, -(SO)-, -(SO2)-, -(SO2)NH-, -NHCH2(CO)NH-, -NH(SO2)-, -O(CO)- or –NH(CO)-; and are selected from hydrogen, halogen, hydroxy, nitro, cyano, amino, oxo, oxime, unsubstituted or substituted by R10 for the groups selected from linear or branched ($C_1$-C8) alkyl, ($C_3$-$C_7$)cycloalkyl, tricycloalkyl, alkylcycloalkyl, alkoxyalkyl, perhaloalkyl, perhalocycloalkyl, aryl, aralkyl, alkylaryl, alkylheteroaryl, aralkoxylalkyl, perhaloaryl, alkylheterocycloalkyl, heterocycloalkyl, perhaloheterocyclyloalkyl, heteroaryl, heteroaralkyl, alkylaryl, perhaloheteroaryl, acyl, acyloxy, acylamino, alkylamino, arylamino, aralkoxy, alkoxyalkyl, alkylthio, thioalkyl, arylthio, thioaryl, carboxylic acid or its derivatives, or sulfonic acid or its derivatives wherein the groups / substituents present on same or adjacent atoms such as carbon or nitrogen, together optionally and independently may form a five or a six or a seven membered ring optionally containing one or more double bonds and optionally containing one or more heteroatoms selected from O, N or S;

and wherein

R10 is independently selected from halogens, hydroxy, nitro, cyano, amino, alkoxy carbonyl alkyl, -SO2NH alkyl, -SO2NH aryl, oxo or oxime, and pharmaceutically usable hydrates and salts thereof;

with the proviso that,

if k is null, then R4 and R6 together form an optionally six or seven membered ring, which optionally contains two to three heteroatoms independently selected from O, S and NR7 with R1 as hydrogen, and $N_1$ is attached to hydrogen.

As used herein, aryl and heteroaryl ring includes up to two conjugated or fused ring systems.

Pharmaceutically acceptable salts forming part of this invention are intended to include not limited to salts of the carboxylic acid moiety such as alkali metal salts like Li, Na and K salts; alkaline earth metal salts like Ca and Mg salts; salts of organic bases such as lysine, arginine, guanidine, diethanolamine, choline, trimethamine and the like; ammonium or substituted ammonium salts and aluminum salts. Salts may be acid addition salts which defines but not limited to sulfates, nitrates, phosphates, perchlorates, borates, hydrohalides, acetates, perhaloacetates, tartrates, maleates, citrates, succinates, palmoates, methanesulfonates, benzoates, salicylates, hydroxynaphthoates, benzensulfonates, ascorbates, glycerophosphates, ketoglutarates and the like.

The invention also provides a process for preparation of the compounds as defined above. The invention further provides pharmaceutical composition comprising compounds of the invention in association with a pharmaceutically acceptable carrier, diluent or excepient.

The invention also provides a method of treatment of mammals including human beings in disease conditions resulting from glucose intolerance and/or accumulation of free radical in the body cells by administering an effective compound of compounds of the invention to the subject in need thereof.

The invention further provides use of the compounds of invention in the manufacture of a medicament useful for treatment of diseases conditions resulting from glucose intolerance and/or accumulation free radical in the body cells.

## BRIEF DESCRIPTION OF ACCOMPANYING DRAWING

Fig. 1 : The results of invivo study for compounds 25 and 27 vis-à-vis the vehicle has also been shown in Fig.1 of the drawing.

## DETAILED DESCRIPTION OF THE INVENTION

The representative compounds of formula (I) as referred above are listed in Table1 below which can be conveniently prepared, by methods as described hereinafter. These compounds may exist both as diastereomeric mixtures or as the diastereomerically pure or enantiomerically pure compounds.

## Table-1 : Representative Compounds

| Comp No. | R1 | R2 | R3 | - R4 – R6 - | R4 | R5 | R6 | k | n | X | Z | Salt |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | H | H | H | - | 1-[{5-chloro pyridin-2-yl carbamoyl}-methyl]-piperidine-4-yl | H | H | 1 | 1 | S | O | 3CF3COOH |
| 2 | H | H | H | - | 1-[{5-bromothiazol-2-ylcarbamoyl}methyl]-piperidine-4-yl | H | H | 1 | 1 | S | O | 3CF3COOH |
| 3 | H | H | H | - | 1-[2-amino-2-oxoethyl] piperidine-4-yl | H | H | 1 | 1 | S | O | 2CF3COOH |
| 4 | H | H | H | - | 1[{4,5-dimethylthiazol-2-ylcarbamoyl}methyl]piperidine-4-yl | H | H | 1 | 1 | S | O | 3CF3COOH |
| 5 | H | H | H | - | 1-[{5-cyano pyridin-2-yl carbamoyl} methyl]-piperidine-4-yl | H | H | 1 | 1 | S | O | 3CF3COOH |

| Comp No. | R1 | R2 | R3 | - R4 – R6 - | R4 | R5 | R6 | k | n | X | Z | Salt |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | H | H | H | - | 1-[{2-chloro pyridin-3-yl carbamoyl} methyl]-piperidine-4-yl | H | H | 1 | 1 | S | O | 3CF3COOH |
| 7 | H | H | H | - | 1-[{2-fluorobenzylcarbamoyl}-methyl]piperidine-4-yl | H | H | 1 | 1 | S | O | 2CF3COOH |
| 8 | H | H | H | - | 1-[2-phenoxyethyl]piperidine-4-yl | H | H | 1 | 1 | S | O | 2CF3COOH |
| 9 | H | H | H | - | 1-[{5-chloro pyridin-2-yl carbamoyl} methyl]-piperidine-4-yl | H | H | 1 | 1 | CH 2 | O | 3CF3COOH |
| 10 | H | H | H | - | 1-[{cyclohexylcarbamoyl} methyl]piperidine-4-yl | H | H | 1 | 1 | S | O | 2CF3COOH |
| 11 | H | H | H | - | 1-[{3-isopropoxy propan-1-ylcarbamoyl}methyl]-piperidine-4-yl | H | H | 1 | 1 | S | O | 2CF3COOH |

| Comp No. | R1 | R2 | R3 | -R4-R6- | R4 | R5 | R6 | k | n | X | Z | Salt |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 | H | H | H | - | 1-[{thiophene-2-yl-ethyl carbamoyl}methyl]piperi dine-4-yl | H | H | 1 | 1 | S | O | 2CF3COOH |
| 13 | H | H | H | - | 1-[{3-chloro-4-fluorophenyl-1-yl carbamoyl}methyl]piperi dine--4-yl | H | H | 1 | 1 | S | O | 2CF3COOH |
| 14 | H | H | H | - | 1-[{4-ethoxycarbonylmethyl thiazol-2-yl-carbamoyl}methyl]piperi dine-4-yl | H | H | 1 | 1 | S | O | 3CF3COOH |
| 15 | H | H | H | - | 1-[((3,4-methylenedioxyphenyl)c arbamoyl)methyl]piperidi n-4-yl | H | H | 1 | 1 | S | O | 2CF3COOH |
| 16 | H | H | H | - | 1-[{(4-aminosulphonylphenyl) carbamoyl}methyl]piperi dine-4-yl | H | H | 1 | 1 | S | O | 2CF3COOH |

| Comp No. | R1 | R2 | R3 | - R4 – R6 - | R4 | R5 | R6 | k | n | X | Z | Salt |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | H | H | H | - | 1-[(3-cyclopropylamino-3-oxo) propyl]piperidin-4-yl | H | H | 1 | 1 | -S- | O | 2CF3COOH |
| 18 | H | H | Methoxycarbonyl- | - | 1-[{5-chloro pyridin-2-yl carbamoyl} methyl] piperidine-4-yl | H | H | 1 | 1 | S | O | 3HCl |
| 19 | H | H | H | - | 1-[{thiazol-2-yl carbamoyl}methyl]piperidine-4-yl | H | H | 1 | 1 | S | O | 3CF3COOH |
| 20 | H | H | H | - | 1-[(2-(2-methoxyethyl)amino-2-oxo)ethyl]-piperidine-4-yl | H | H | 1 | 1 | S | O | 2CF3COOH |
| 21 | H | H | H | - | 1-[{pyridine-2-yl carbamoyl}methyl]piperidine-4-yl | H | H | 1 | 1 | S | O | 3CF3COOH |
| 22 | H | H | H | - | 1-[ 3-pyridineacetyl] piperidine-4-yl | H | H | 1 | 1 | S | O | 2CF3COOH |

| Comp No. | R1 | R2 | R3 | - R4 – R6 - | R4 | R5 | R6 | k | n | X | Z | Salt |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 23 | H | H | H | - | 1-[ {benzthiazol-2-yl carbamoyl}methyl]piperi dine-4-yl | H | H | 1 | 1 | S | O | 3CF3COOH |
| 24 | H | H | H | - | 4-[(5-methylpyrazin-2-ylcarbonyl) amino] cyclohex-1-yl | H | H | 1 | 1 | -S- | O | 3CF3COOH |
| 25 | H | H | H | - | 1-[{5-cyano pyridin-2-yl carbamoyl} methyl]-piperidine-4-yl | H | H | 1 | 1 | S | O | 3HCl |
| 26 | H | H | H | - | 1-[{2-chloro pyridin-3-yl carbamoyl}methyl]-piperidine-4-yl | H | H | 1 | 1 | S | O | 3HCl |
| 27 | H | H | H | - | 1-[{(4-aminosulphonylphenyl) carbamoyl}methyl] piperidine-4-yl | H | H | 1 | 1 | S | O | 2HCl |

| Comp No. | R1 | R2 | R3 | - R4 – R6 - | R4 | R5 | R6 | k | n | X | Z | Salt |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 28 | H | H | H | - | 1-[(4-chlorophenyl carbamoyl)-methyl]-piperidin-4-yl | H | H | 1 | 1 | S | O | 2HCl |
| 29 | H | H | H | - | 1-[{benzthiazol-2-yl carbamoyl}methyl] piperidine-4-yl | H | H | 1 | 1 | S | O | 3HCl |
| 30 | H | H | H | - | 1[{4,5-dimethylthiazol-2-yl carbamoyl} methyl]piperidine-4-yl | H | H | 1 | 1 | S | O | 3HCl |
| 31 | H | H | H | - | 1-[(pyrimidin-2-yl-carbamoyl)-methyl]-piperidin-4-yl | H | H | 1 | 1 | S | O | 2HCl |
| 32 | H | H | tert-butyloxycarbonyl | - | H | H | H | 1 | 1 | O | O | - |

**The representative compounds of the invention listed in Table I can be identified by their following chemical names :-**

a)3-[1-oxo-2-(1-(1-(2-oxo-2-(5-chloropyridin-2-yl)aminoethyl) piperidin-4-yl)) hydrazino]ethyl (s)-(+)-4-cyanothiazolidine tris-trifluoroacetate (Compound no. 1 )

b)3-[1-oxo-2-(1-(1-(2-oxo-2-(5-bromothiazol-2-yl)aminoethyl)piperidine-4-yl))hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 2)

c)3-[1-oxo-2-(1-(1-(2-oxo-2-amino)ethyl)piperidine-4-yl))hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate. (Compound no. 3)

d)3-[1-oxo-2-(1-(1-(2-oxo-2-(4,5-dimethylthiazole-2-yl) aminoethyl) piperidin-4-yl)) hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 4)

e)3-[1-oxo-2-(1-(1-(2-oxo-2(5-cyanopyridin-2-yl)aminoethyl)piperidin-4-yl))hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 5)

f)3-[1-oxo-2-(-1-(-1-(2-oxo-2-(2-chloropyridyl-3-yl)aminoethyl) piperidin-4-yl))hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 6)

g)3-[1-oxo-2-(1-(1-(2-oxo-2-(2-flurobenzyl)amino ethyl)piperidine-4-yl))hydrazino]ethy cyanothiazolidine, bis-trifluoroacetate (Compound no. 7)

h)3-[1-oxo-2-(1-(1-phenoxyethyl)piperidin-4-yl)hydrazino] ethyl-4-cyano-thiazolidine, bis-trifluoroacetate. (Compound no. 8)

i)3-[1-oxo-2-(1-(1-(2-oxo-2-(5-chloropyridin-2-yl)amino ethyl) piperidin-4-yl))hydrazin ethyl-2-cyanopyrrolidine, tris-trifluoroacetate. (Compound no. 9)

j)3-[1-oxo-2-(1-(1-(2-oxo-2-cyclohexyl)aminoethyl)piperidin-4-yl)hydrazino]ethyl-4-cyanothiazoline, bis-trifluoroacetate. (Compound no. 10)

k)3-[(1-oxo-2-(1-(1-(2-oxo-2-(3-isopropoxy propan-1-yl)amino ethyl) piperidine-4-yl))hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate. (Compound no. 11)

l)3-[1-oxo-2-(1-(1-(2-oxo-2-(2-(thiophene-2-yl) ethyl) aminoethyl) piperidine-4-yl))hydrazino]ethyl - 4- cyanothiazolidine, bis-trifluoroacetate. (Compound no. 12)

m)3-[1-oxo-2-(1-(1-(2-oxo-2-(3-chloro-4-fluoro-phenyl)aminoethyl)piperidine-4-yl)) hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate. (Compound no. 13)

n)3-[1-oxo-2-(1-(1-(2-oxo-2-(4-ethoxycarbonyl methyl thiazole-2-yl) amino ethyl) piperidine-4-yl)) hydrazino] ethyl -4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 14)

o)3-[1-oxo-2-(1-(1-(2-oxo-2-(3,4-methelenedioxyphenyl) amino ethyl ) piperidine -4-yl)) hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate. (Compound no. 15)

p)3-[1-oxo-2-(1-(1-(2-oxo-2-(4-aminosulphonylphenyl) aminoethyl)piperidin-4-yl)) hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate. (Compound no. 16)

q)3-[1-oxo-2-(1-(1-(3-oxo-3-cyclopropyl)amino propyl)piperidin-4-yl)) hydrazino]ethyl-4-cyanothiazolidine, bis-trifluoroacetate (Compound no. 17)

r)3-[1-oxo-2-(1-(1-(2-oxo-2-(5-chloropyridin-2-yl) amino ethyl )piperidine-4-yl)-2-methoxycarbonyl) hydrazino]ethyl-4-cyanothiazolidine, trihydrochloride. (Compound no. 18)

s)3-[1-oxo-2-(1-(1-(2-oxo-2-(thiazole-2-yl)-aminoethyl)piperidin-4-yl))hydrazino] ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 19)

t)3-[1-oxo-2-(1-(1-(2-oxo-2-(2-methoxyethyl)aminoethyl)piperidin-4-yl))hydrazino]ethyl-4-cyanothiazolidine, bis -trifluoroacetate. (Compound no. 20)

u)3-[1-oxo-2-(1-(1-(2-oxo-2-(pyridin-2-yl)aminoethyl)piperidine-4-yl))hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 21)

v)3-[1-oxo-2-(1-(1-(3-pyridylacetyl) piperidine-4-yl))hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate. (Compound no. 22)

w)3-[1-oxo-2-(1-(1-(2-oxo-2-(benzothiazole-2-yl)piperidine-4-yl))hydrazino] ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 23)

x)3-[1-oxo-2-(1-(1-(5-methylpyrazine-2-ylcarbonyl)amino-4-cyclohexyl)) hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 24)

y)3-[1-oxo-2-(1-(1-(2-oxo-2-(5-cyano pyridin-2-yl)aminoethyl)piperidin-4-yl))hydrazino]ethyl-4-cyano thiazolidine, trihydrochloride. (Compound no. 25)

z)3-[1-oxo-2-(1-(1-(2-oxo-2-(2-chloro pyridin-3-yl)aminoethyl)piperidin-4-yl))hydrazino]ethyl-4-cyano thiazolidine, trihydrochloride. (Compound no. 26)

aa)3-[1-oxo-2-(1-(1-(2-oxo-2-(4-amino sulphonyl phenyl)aminoethyl) piperidin-4-yl))hydrazino]ethyl-4-cyano thiazolidine, dihydrochloride. (Compound no. 27)

bb)3-[1-oxo-2-(1-(1-(2-oxo-2-(4-chlorophenyl)aminoethyl)piperidin-4-yl)) ydrazino]ethyl-4-cyano thiazolidine, dihydrochloride. (Compound no.28)

cc)3-[1-oxo-2-(1-(1-(2-oxo-2-(benzothiazole-2-yl)aminoethyl)piperidine-4-yl)) hydrazine]ethyl-4-cyanothiazolidine, trihydrochloride. (Compound no. 29)

dd)3-[1-oxo-2-(1-(1-(2-(4,5-dimethylthiazole-2-yl)aminoethyl)piperidin-4-yl)) hydrazino]ethyl-4-cyano thizolidine, trihydrochloride. (Compound no. 30)

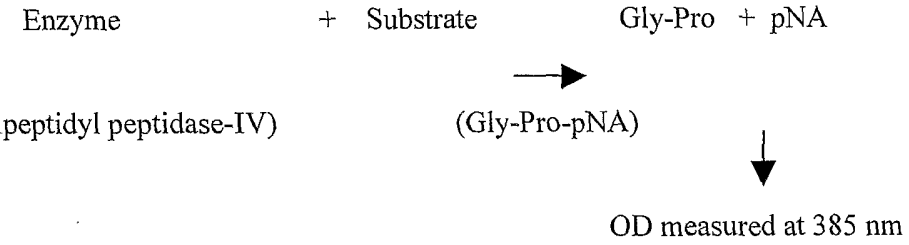
ee)3-[1-oxo-2-(1-(1-(2-cyclopropyl-1-yl)aminoethyl)piperidin-4-yl)) hydrazino]ethyl-4-cyano thiazolidine, dihydrochloride. (Compound no. 31)

ff)3-[-oxo-2- (2-ter-butyloxycarbonyl) hydrazino] ethyl-4-cyanooxazolidine. (Compound no. 32)

**Assay of DPP-IV enzyme inhibitory activity.**

The assay method is a modified method (as described by Welch et al, 1998) based on spectrophotometric determination of the product formed by penultimate proline cleaving activity of the enzyme.

The following equation explains the principle of the assay method:




Gly-Pro-pNA: Glycine-Proline-p-nitroanilide

Assay protocol involves incubation of the enzyme dipeptidyl peptidase IV with the test substance at 30° C for 30 min followed by addition of this reaction mixture to the substrate Gly-Pro-pNA that was equilibrated at 30° C for 2 min. The enzyme cleaves the substrate at penultimate proline and releases p-nitroanilide, the optical density of which is

measured at 385 nm. The formation of p-nitroanilide will be reduced in the presence of inhibitor. Optical density is measured for 2 hours for every 10min using a spectrophotometer and $V_{max}$ is calculated to find the activity of new

chemical entities. The activity of molecule is expressed in terms of % inhibition. Atleast three different concentrations were tried out for each of the test substances. The percentage inhibitions for each of the concentrations were plotted and an $IC_{50}$ of the test compound was worked out. The enzyme inhibitory activity of different test compounds were compared based on the $IC_{50}$ values.

The percentage inhibition %I, is calculated using the formula:

$\% I = [(1-v_i/v_0)]*100$ where $v_i$ and $v_0$ are the $V_{max}$ values with and without the test substance, respectively.

**Reagents and their preparation:**

*Substrate solution*: 0.5 mM in 45 mM phosphate buffer
Substrate used: Gly-Pro-p-nitroanilide (Source: Sigma-Aldrich Co. Germany)
M. Wt of Gly-Pro-p-nitroanilide =328.8
3.288 mg substrate in 1 ml 45 mM phosphate buffer was prepared as stock solution.
0.25 ml of this stock solution was diluted to 5 ml to get 0.5 mM substrate solution (90 µl to be added in each well). The stock solution of the substrate was used within three days of preparation.

*Enzyme solution*: Porcine DPP-IV (Sigma-Aldrich Germany) was used throughout the study. 0.4 mU in 80 µl of Tris. HCl buffer was prepared. Fresh solutions were prepared everyday for the assays.

*Inhibitor solution*:

The compounds of the present invention were dissolved in their respective vehicles.

Various concentrations of inhibitor were used: 0.391μM, 0.781μM, and 3.125 μM.

Solutions of inhibitor were prepared and used on the same day.

Experimental Procedure:

Different concentrations of inhibitors vehicle, substrate and enzyme were prepared as per standard procedures. 280 μl of enzyme solution (0.4-mU/80 μl in Tris HCl buffer) was added to the eppendorf containing 70 μl solution of inhibitor or vehicle and mixed. This reaction mixture was incubated for 30 min at 30 °C. The 96 well plate containing substrate solution was thermally equilibrated in the spectrophotometer for 2 min at 30°C. Later 100 μl of the enzyme-inhibitor pre-incubation solution was added to respective wells in a 96 well plate. Each concentration of the inhibitor was tested in triplicates.

The rate of change in UV absorbance (in presence of various concentrations of the inhibitor) was measured at 385 nm, with respect to wells containing only 0.5 mM substrate in 45 mM phosphate buffer as blank at every 10 min for 2 hours after adding enzyme-inhibitor mixture to wells containing substrate solution.

**Table 2**:

The inhibitory activity of the compounds on DPP-IV enzyme activity

| Compound No. | $IC_{50}$ (μM) Mean ±SD |
|---|---|
| 1 | 4.1±0.4 |
| 2 | 0.226±0.005 |
| 3 | 0.817±0.04 |
| 4 | 0.185±0.01 |
| 5 | 0.090±0.02 |

| Compound No. | $IC_{50}$ (μM) Mean ±SD |
|---|---|
| 6 | 0.119±0.015 |
| 7 | 0.510±0.05 |
| 8 | 0.676±0.07 |
| 9 | 3.7±0.5 |
| 10 | 0.506±0.06 |
| 11 | 0.468±0.005 |
| 12 | 0.415±0.03 |
| 13 | 0.342±0.035 |
| 14 | 0.362±0.060 |
| 15 | 0.240±0.025 |
| 16 | 0.0985±0.012 |
| 17 | 1.77±0.167 |
| 18 | 40.44±6.40 |
| 19 | 0.205 ± 0.011 |
| 20 | 1.027 ± 0.035 |
| 21 | 0.210 ± 0.004 |
| 22 | 0.667 ± 0.062 |
| 23 | 0.104 ± 0.009 |
| 24 | 0.485 ± 0.010 |
| 25 | 0.13±0.03 |
| 26 | 0.144±0.003 |
| 27 | 0.07±0.01 |
| 28 | 0.547±0.045 |
| 29 | 0.167±0.007 |
| 30 | 0.715±0.06 |
| 31 | 217.3±9.07 |
| 32 | 150.24±43.01 |

(Values are mean ± SD of the three.experiments except for compound Nos. 18 & 24)

**Effect of Compound no. 25 and Compound no. 27 on Oral Glucose Tolerance in Neonatal Streptozotocin-induced (n0 STZ) diabetic rats.**

Glucose levels in the body are tightly controlled by insulin. Many factors contribute to insulin release. Administration of glucose by oral route causes an increase in blood glucose levels as it gets absorbed and this increase in the glucose level is brought down by the release of insulin as it increases glucose uptake in skeletal muscles and adipocytes. Glucose stimulated insulin release is impaired in diabetes. By pretreatment with drug that releases or stimulates insulin release before taking food/glucose, glucose levels can be tightly controlled in diabetics too. Oral glucose tolerance test is one of the laboratory markers to test pre-diabetic or diabetic condition and to evaluate insulin secretogogues and/or releasers.

## METHODOLOGY

**Principle of glucose estimation by glucometer (One Touch, Lifescan, USA)**

Each $cm^2$ of the test strip contains the following reactive ingredients in the approximate concentration listed below:

| | |
|---|---|
| Glucose oxidase | 14 IU |
| Peroxidase | 11 IU |
| 3-methyl-2 benzothiazolinonehydrazone hydrochloride | 0.06 mg |
| 3-dimethylaminobenzoic acid | 0.12 mg |

Glucose and oxygen react in the presence of glucose oxidase yielding gluconic acid and hydrogen peroxide. Hydrogen peroxide subsequently oxidizes the dyes in a reaction mediated by Peroxidase producing a blue colored form of the dyes (Marks and Dawson, 1965). The intensity of the blue color is proportional to the glucose concentration in the sample.

**Animals**

Wistar rats were made type 2 diabetic by injecting streptozotocin (STZ) at the dose of 90 mg/kg intraperitoneally on the day of birth (n0STZ) to male pups (Portha et al, 2001). Animals showing overnight fasted blood glucose levels between 7-10 mM at the age of 10 –12 weeks were used for the study. On the day of the experiment, animals were fasted overnight with free access to water.

**Oral Glucose Tolerance Test (OGTT) (Pospisilik et al, 2002)**

External jugular vein of overnight fasted n0STZ rats was cannulated using polyethylene cannula with heparinized (100 IU/ml) saline under ether anesthesia and exteriorized at the back of the neck. After the animal was recovered from the anesthesia, blood sample was drawn as marked as '–5 min' sample and drug formulation (in 0.5% sodium caboxymethyl cellulose, Na-CMC) was administered orally at the volume of 1ml/kg body weight.

5 min after drug administration, blood sample was drawn as '0 min' sample and glucose load at the dose of 1g/kg body weight was administered orally. Blood samples were drawn subsequently at 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 180, 240 and 360 min time interval. Blood glucose is measured using glucometer.

Animals were divided into three groups: 1. Vehicle (0.5% Na-CMC) treated, 2. Compound no. 25 treated (22mg/kg) and 3. Compound no. 27 treated (22mg/kg).

**Calculation**

Change in blood glucose at various time points was measured as percent increase or decrease from basal glucose level. A graph was plotted using time (min) on X-axis and corresponding percent change in blood glucose on Y-axis as shown in Fig.1 of the drawings. Area under the curve (AUC) for glucose was calculated using WinNonlin software.

| Time (min) Treatment | Vehicle (0.5% Na CMC) | Compound no. 25 (22mg/kg) | Compound no. 27 (22 mg/kg) |
|---|---|---|---|
| 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 5.0 | 28.7 ± 0.0 | 21.97 ± 1.14 | 9.79 ± 4.13 |
| 10.0 | 44.62 ± 8.34 | 49.11 ± 11.7 | 29.41 ± 3.4 |
| 15.0 | 53.90 ± 0.0 | 64.67 ± 10.17 | 46.51 ± 5.23 |
| 20.0 | 92.27 ± 17.0 | 69.62 ± 12.73 | 56.51 ± 4.33 |
| 30.0 | 122.65 ± 24.01 | 90.29 ± 22.93 | 77.93 ± 12.12 |
| 45.0 | 157.07 ± 14.17 | 99.19 ± 17.87 | 87.25 ± 15.61 |
| 60.0 | 154.15 ± 16.26 | 82.76 ± 16.22 | 67.33 ± 11.49 |
| 75.0 | 146.0 ± 13.51 | --- | --- |
| 90.0 | 113.52 ± 18.03 | 34.3 ± 20.32 | 67.45 ± 9.8 |
| 120.0 | 65.27 ± 14.70 | -21.87 ± 25.68 | 31.4 ± 8.51 |
| 180.0 | -10.67 ± 14.03 | -45.71 ± 18.48 | -48.05 ± 5.75 |
| 240.0 | -17.67 ± 10.49 | -48.6 ± 13.58 | -58.87 ± 6.10 |
| 300.0 | -9.03 ± 18.08 | --- | --- |
| 360.0 | -13.05 ± 16.09 | -49.76 ± 10.31 | -63.57 ± 4.77 |

5

| Treatment | $AUC_{glucose}$ |
|---|---|
| Vehicle (0.5% Na-CMC) | 15623.0 ± 1019.0 (n = 4) |
| Comp. 25 (22mg/kg) | 6939.0 ± 1632.0 (n = 3) |
| Comp. 27 (22mg/kg) | 7554.0 ± 955.3 (n = 3) |

## CONCLUSION OF IN-VIVO STUDY

Administration of oral glucose load of 1g/kg caused increase in blood glucose in overnight fasted rats with glucose levels peaking at 45 min time interval (157.07 ± 14.17 % from basal level) in vehicle treated rats. In Comp. 25 and Comp. 27 treated rats, peak glucose levels were significantly lower (99.19 ± 17.87% and 87.25 ± 15.61% respectively) as compared to vehicle treated group. The area under the curve for glucose ($AUC_{glucose}$) for Comp. 25 and Comp. 27 treated rats (6939.0 ± 1632.0 and 7554.0 ± 955.3 respectively) was found to be significantly lower as compared to vehicle treated rats (15623.0 ± 1019.0).

Among the various laboratory markers analyzed, the most consistent predictor of non-insulin dependent diabetes mellitus (type II) has been a high fasting serum insulin concentration, closely followed by the fasting plasma glucose concentration and plasma glucose after an oral glucose tolerance test (OGTT). During OGTT, in response to increased blood glucose levels, beta cells of pancreas secrete insulin. When blood glucose levels are plotted against time, the area under the curve for glucose is found to be higher in diabetics as compared to non-diabetics. This is termed as glucose intolerance and attributed to inability of beta cells to respond to the rise in blood glucose levels. The results of the present investigation indicate improved glucose tolerance in rats treated with Comp. 25 and Comp. 27 as $AUC_{glucose}$ values for treated animals were significantly lower than in vehicle treated animals. The improved glucose tolerance in Comp. 25 and Comp. 27 pretreated rats could be due to increased glucose-stimulated insulin release. From the present investigation it is concluded that Comp. 25 and Comp. 27 can be good candidates for the management of hyperglycemia in type II diabetes.

**Free Radical Scavenging activity:**

1.**Aim:**

To determine the in-vitro free radical scavenging activity of compounds of general formula-I on 2,2,-diphenyl-1-picrylhydrazyl (DPPH)radical (Ref: W. Brand- Williams, M.E.Cuvelier, C. Berset "Use of a free radical method to evaluate antioxidant activity ", Lebensm.- Wiss.u.Technol.,1995,28,Nr.1:25-30).

**2.Principle involved:**

To evaluate the free radical scavenging activity of compounds they are allowed to react with stable radical DPPH$^{\bullet}$. In its radical form, DPPH$^{\bullet}$ absorbed at the characteristic wave length of 515nm, but upon reduction by an antioxidant or radical scavenger (AH), the absorption disappears.

Equation:

a. $DPPH^{\bullet} + AH \longrightarrow DPPH\text{-}H + A^{\bullet}$

(free radical) (antioxidant)

**3. Reagents and Chemicals:**

DPPH$^{\bullet}$ ( Sigma Aldrich)

Methanol (Merck)

**4. Instrument used:**

UV-visible spectrophotometer (Jasco)

Quartz Microcuvette (1ml capacity)

5. **Procedure:**

**Preparation of DPPH$^{\bullet}$ solution:**

$10^{-4}$M solution of DPPH$^{\bullet}$ was prepared in methanol.

**Preparation of drug solution:**

Various concentrations (10mM,1mM, 0.5mM,0.25mM and 0.125mM) of drug solutions were prepared in methanol.

**Preparation of Control solution:**

900µl of DPPH$^{\bullet}$ radical solution was added to an eppendorf tube. To it was added 100µl of methanol.

**Preparation of Test solution:**

900µl of DPPH$^{\bullet}$ radical solution was added to an eppendorf tube. To it was added 100µl of various concentrations of drug solutions in methanol.

**Measurement of absorbance (O.D):**

The absorbance of control and test samples was recorded after incubation at 30°C for 30 minutes, at 515nm taking methanol as blank.

6. **Calculation:**

The percent antioxidant activity was calculated according to the formula:

% Antioxidant activity = 100-[O.D of test sample/O.D of control*100]

**TABLE - 3**

IN-VITRO FREE RADICAL SCAVENGING ACTIVITY OF THE MOLECULES USING DPPH FREE RADICAL

| Compound No. | Concentration (μM) | % Activity |
|---|---|---|
| 2 | 100 | 53.44 |
| 4 | 100 | 54.93 |
| 5 | 100 | 55.03 |
| 6 | 100 | 57.13 |
| 15 | 100 | 56.37 |
| 16 | 100 | 57.73 |

The test compounds listed in the Table 3 above exhibit *invitro* (antioxidant) free radical scavenging activity. Excessive production of free radicals; reactive oxygen species (ROS) results in oxidative stress . Therefore, these molecules would be very effective in reducing oxidative stress by their ability to trap ROS. Antioxidants (free radicals scavengers) are reported to be effective in the management of various diseases linked with oxidative stress.

Also, the novel compounds show Free Radical Scavenging Activity which is useful for (a) Neurodegenerative disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Motor Neuron Disease, Prion Disease etc, (b) Diabetes and Diabetic Vascular Complications, (c) Intestinal Diseases such as Intestinal Ischemia, Radiation Enteritis, Inflammatory Bowel Disease, Gastric and Colorectal Cancers etc., (d) Liver Diseases such as Alcoholic Liver Disease, Chronic Hepatitis C etc., (e) Cancers such as Lung Cancer, Colorectal Cancer, Cervical Cancer, Breast Cancer, Malignant Melanoma etc., (f) Cardiac Diseases such as Atherosclerosis, Myocardial Infarction, Ischemic Stroke, Endothelial Dysfunction etc., (g) Opthalmic Disorders such as Cataract formation, Macular degeneration etc., (h) HIV Diseases, (i) Respiratory Diseases such as

Chronic Obstructive Pulmonary Diseases, Asthma etc., (j) Renal Diseases such as Glomerulonephritis, Acute Renal Failure etc.

**Discussion of Test Results :**

Oral glucose tolerance test is one of the methods to test pre-diabetic or diabetic condition and to evaluate insulin secretagogues and/or releasers. Glucose level in the body is mainly controlled by insulin although many other factors contribute to insulin release. Administration of glucose by oral route will increase the glucose level in the blood, which induces the release of insulin. This glucose stimulated insulin release is impaired in diabetes. By pretreatment with drugs that releases or stimulates insulin release before taking food/glucose, the rise in glucose level can be controlled.

Free radicals along with AGE formation contributes to macroangiopathic (atherosclerosis, coronary artery disease) and microangiopathic (neuropathy, retinopathy, nephropathy) complication of diabetes.

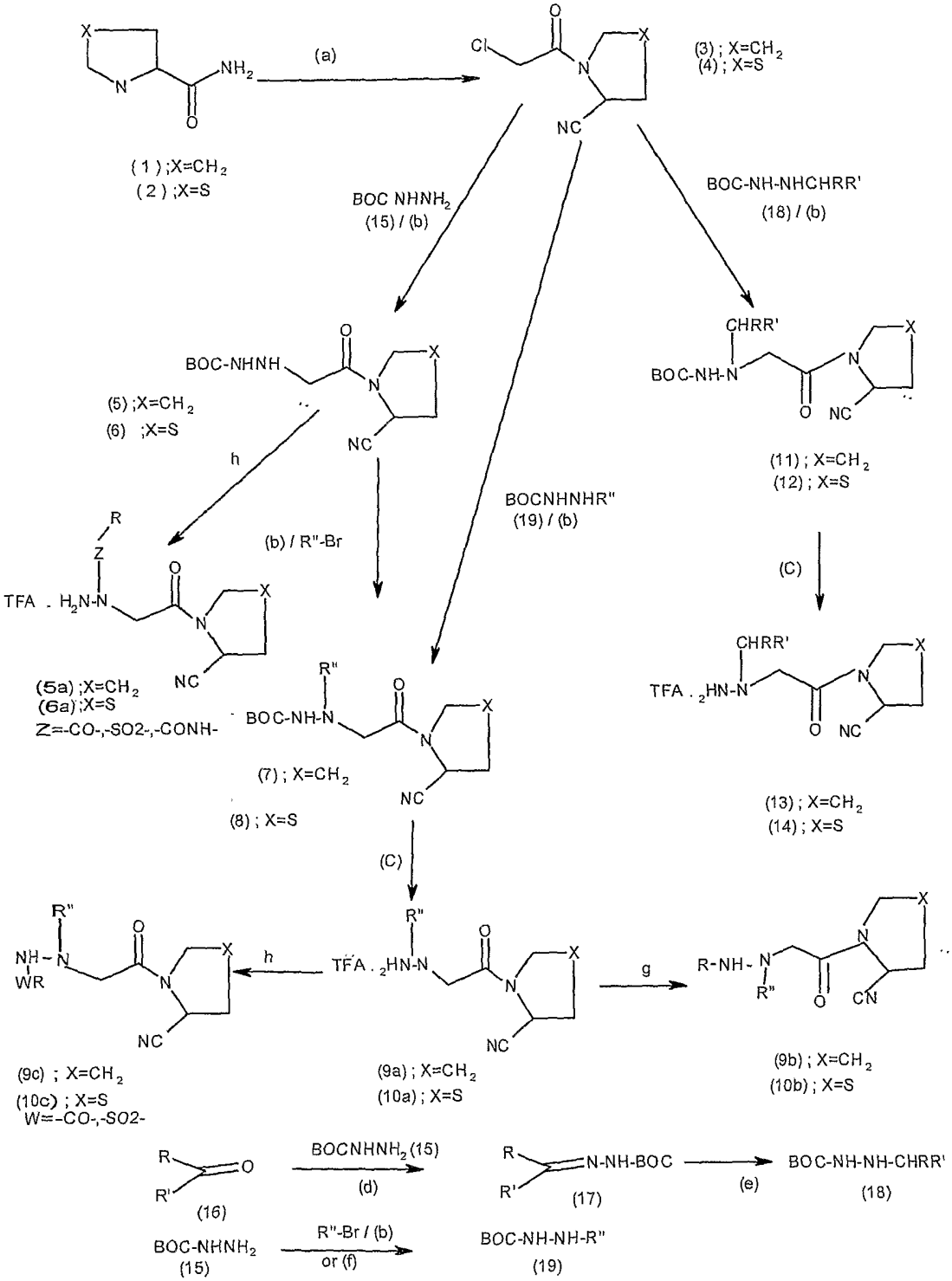
The test compounds listed in Table-3 exhibit in vitro (antioxidant) free radical scavenging activity. The novel compounds show free radical scavenging activity, which would be useful for treatment of diabetes and diabetic vascular complications (DVCs).

The DPP-IV inhibitors under study are preferably expected to not only control diabetes, but also to prevent diabetic complications by their antioxidant actions.

**Preparation of representaive compounds of the invention :**

The compounds of the invention may be prepared by alternative synthetic routes as per Scheme I, 1A, 2 or 3 as described below:

## SCHEME-I


(1) ;X=CH2
(2) ;X=S
(a)
(3) ; X=CH2
(4) ; X=S
BOC NHNH2
(15) / (b)
BOC-NH-NHCHRR'
(18) / (b)
(5) ;X=CH2
(6) ;X=S
h
(11) ; X=CH2
(12) ; X=S
(b) / R"-Br
BOCNHNHR"
(19) / (b)
(C)
(5a) ;X=CH2
(6a) ;X=S
Z=-CO-,-SO2-,-CONH-
(7) ; X=CH2
(8) ; X=S
(13) ; X=CH2
(14) ; X=S
(C)
h
g
(9c) ; X=CH2
(10c) ; X=S
W=-CO-,-SO2-
(9a) ; X=CH2
(10a) ; X=S
(9b) ; X=CH2
(10b) ; X=S
BOCNHNH2 (15)
(d)
(e)
(16)
(17)
(18)
BOC-NHNH2
(15)
R"-Br / (b)
or (f)
BOC-NH-NH-R"
(19)

Reagents and conditions for Scheme – 1 :

[a] (I) $Et_3N$, THF, $K_2CO_3$, $ClCH_2COCl$, 0-20$^0$C, 2.5-3.0 hrs. (II) (CF3CO)2O / THF ;

[b] K2CO3, KI, THF, Reflux, 6-20 hrs.

[c] CF3COOOH, Room Temp., 10-20 min.

[d] Hexane / Reflux, 2-4 hrs.

[e] NaBH4, MeOH, Reflux, 4-20 hrs.

[f] Neat, Reflux.

[g] (i) Aldehyde/ketone, MeOH, Reflux, (ii) $NaCNBH_3$, $TiCl_4$, MeOH

[h] (i) R8NHCOCl or $R8SO_2Cl$ or R8COCl , TEA, THF, 0-20$^0$C (ii) [c]

**Description:**

The compounds of present invention may be prepared by the general methods as depicted in Scheme (I). The starting amide compound of formula (1) i.e L – prolinamide is prepared in four steps from L-proline following the same methods as described in literature for the synthesis of (R)-(-)-thiazolidine-4-amide of formula (2) from the corresponding acid.

Ref. US pat-6110949 dated 29.8.00, Doreen M et al, Bio.Org. Med. Chem. Lett. 6(22), 1996, 2745-48]. L-prolinamide (1) is then converted to 1-chloroacetyl-2-cyanopyrrolidine of formula (3) in two steps which involves chloroacylation of the amide followed by dehydration [Ref. US pat –6124305 dated 26.09.00, WO- 0034241 dated 15.06.00 and US pat 6011155 dated 01.04.00].

In a similar manner, the another starting material 3-chloroacetyl-4-cyano thiazolidine of formula (4) is prepared by following two step reactions sequence. Step-1 involves the reaction of thiazolidine amide of formula (2) with chloroacetylchloride in presence of a base such as potassium carbonate and an inert organic solvent like tetrahydrofuran at a temperature of from 0 $^0$C to 20 $^0$C for 2.5 to 3 hrs. Step 2 involves the dehydration of 3-chloroacetyl-thiazolidine-4-amide prepared in step-1, with 2-equivalents of trifluroacetic anhydride conducted in presence of an inert organic solvent such as tetrahydrofuran at a temperature preferably at 20 $^0$C.

The second major component of the present invention i.e. N-2-substituted – tertbutyl carbazates of formulae (18) and (19), is prepared by the conventional manner. The tert-butyl alkylidine carbazates of formula (17) is prepared by refluxing hexane or tetrahydrofuran solution of tert-butyl carbazate (15) with appropriate aldehyde or ketone of formula (16) in 1:1 molar ratio for 2-4 hrs. [ Ref. Dutta Anand S et.al., J.Chem. Soc.Perkin I, 1975, 1712-1720. Ghali N.I et al, J.Org.Chem. 46, 1981, 5413-5414 ].

The alkylidine carbazates thus formed in the previous step is reduced to N-2 substituted –tert-butyl carbazates of formula (18) using metal hydrides like sodium borohydride or lithium aluminium hydride, preferably sodium borohydride and Sodium cyanoborohydride. The solvent used in the reaction is organic solvent like methanol or tetrahydrofuran at a temperature ranging from $25^0$C to $70^0$C for 4 to 20 hrs.

On the other hand, direct alkylation of tert-butyl carbazate with alkyl or aryl halides preferably with the corresponding chlorides or bromides either in neat reaction condition or in presence of an inorganic base such as potassium carbonate and a catalyst such as potassium iodide in presence of THF provides carbazate derivatives of formula (19). Coupling of chloroacyl derivatives of formula (3) or (4) with the tert-butyl carbazate derivatives (18) or (19) in presence of $K_2CO_3$ /KI in THF gives rise to hydrazinoacyl derivatives (11),(7),(12) or (8) which on deprotection using trifluoroaceticacid provides the final compounds (13), (9a),(14) or (10a) respectively as trifluoroacetate salts and further reaction of 9(a) or 10(a) with appropriate aldehyde followed by reduction using metal hydride like sodium borohydride or sodium cyanoborohydride in presence of catalytic compound of $TiCl_4$ (Titanium tetrachloride) gives rise to compounds 9(b) or 10(b).

Similar reaction of 9(a) or 10(a) with appropriate acid chloride or sulfonyl chloride gives rise to respective compounds 9(c) or 10(c).

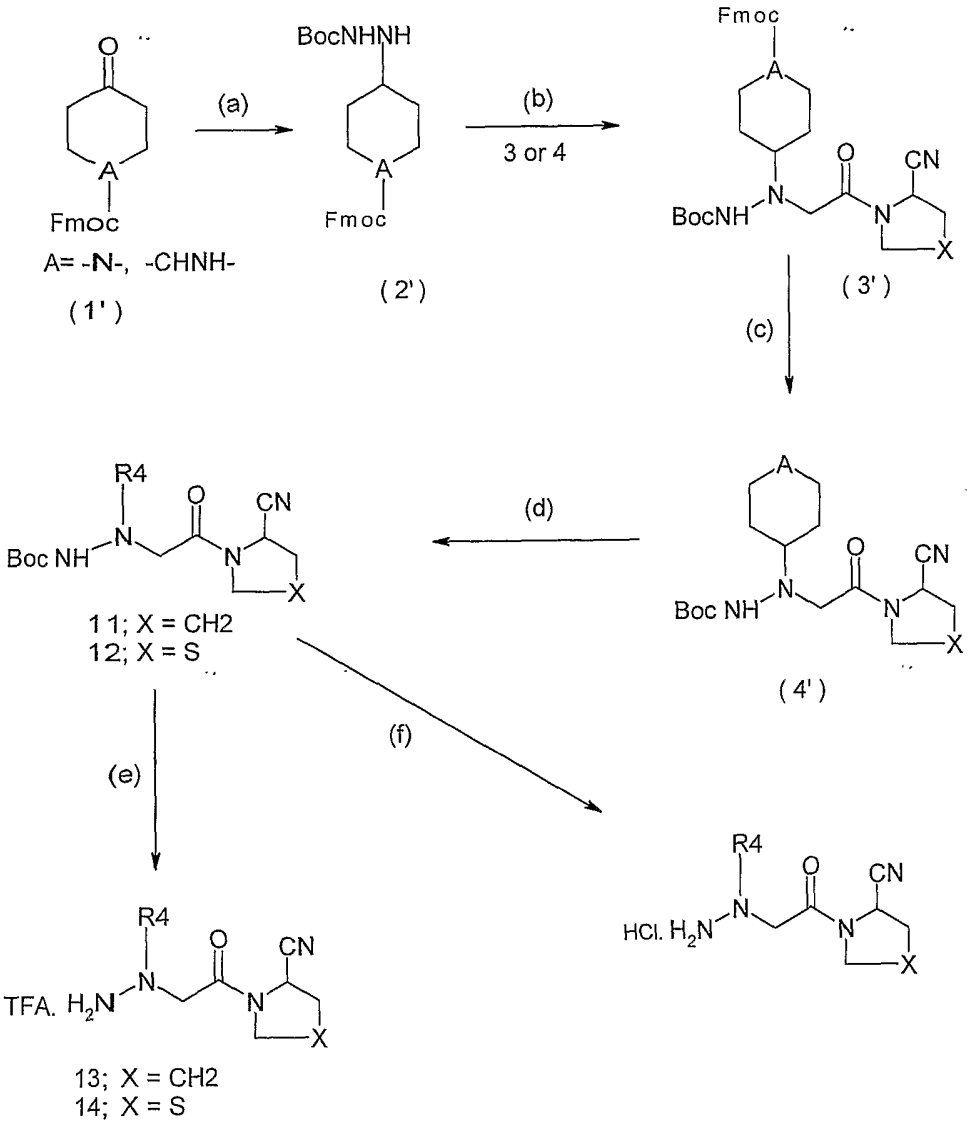
Alternatively, the hydrazino derivatives (5) or (6) can be prepared from the corresponding chloroacyl derivatives (3) or (4) by reaction with tert-butyl carbazate itself.

Alkylation of (5) or (6) with alkyl halides gives rise to penultimate intermediates (7) or (8) respectively.

Also, the reaction of compound (5) or (6) with appropriate carbamoyl chloride, sulphonyl chloride or acid chloride followed by deprotection with trifluroacetic acid gives rise to compound 5(a) or 6(a) respectively.

The compounds of the present invention can also be synthesized according to Scheme 1A given below in another embodiment.

**SCHEME-1A**



Fmoc : 9-fluorenyl methoxy carbonyl
Boc : tertary butoxy carbonyl
TFA : Trifluro acetic acid

(a) (i) Methanol, $BoCNHNH_2$, Reflux, 2-3 hrs.
(ii) Methanol, 0° C - Room Temp, $NaCNBH_3$, $TiCl_4$

(b) $K_2CO_3$, KI, THF, Reflux, 24 - 40 hrs.

(c) Morpholine

(d) Acid, EDCI, DIEA, THF or Acid Chloride, TEA, THF or Carbamonyl chloride, TEA, THF or Sulphonyl chloride, THF, TEA or Alkyl halide, $K_2$ $CO_3$, THF, reflux or N-substituted chloroacetamide, $K_2CO_3$, THF, reflux.

(e) $CF_3COOH$, Room temperature, 10-20 min.

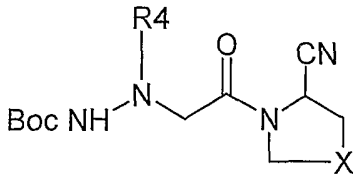
(f) 4N-HCl -Dioxane

The Scheme 1A covers the process wherein nitrogen protected cyclic ketone is used as starting material. The protection is by way of for example Fmoc group.

Compound of formula 11 or 12 can be prepared as a method depicted in Scheme-1A in which refluxing the solution of nitrogen protected ketone with tert-butyl carbazate (15) (as referred earlier) followed by reduction of schiffs base using metal hydrides like sodium cyano borohydride in presence of catalytic amount of titanium tetrachloride. The reaction of N-2-substituted tert-butyl carbazate obtained in previous step with corresponding chloroacyl derivatives (3) or (4) in presence of base like potassium carbonate in suitable solvent like tetrahydrafuran to give nitrogen protected coupled product. Removal of protecting group gives rise free amino group. Functionalizing the said free amino group gives rise to compound no. 11 or 12. Deprotection of Boc-group

by trifluoroacetic acid or by 4 N-HCl-Dioxane provide final compound as triflate or hydrochloride salt respectively.

More specifically, compounds of formula 11 or 12




11; X = CH2
12; X = S

where $R^4$ and X are as defined above,

can be prepared by the following process steps :

(a) reacting a N-protecting cyclic ketone ($1^1$) preferably Fmoc protection with $BocNHNH_2$ in alcoholic solvents under heating for 1-8 hours followed by reduction in alcoholic solvent at 0-35°C to obtain ($2^1$) N-2-substituted tert-butyl carbazate.

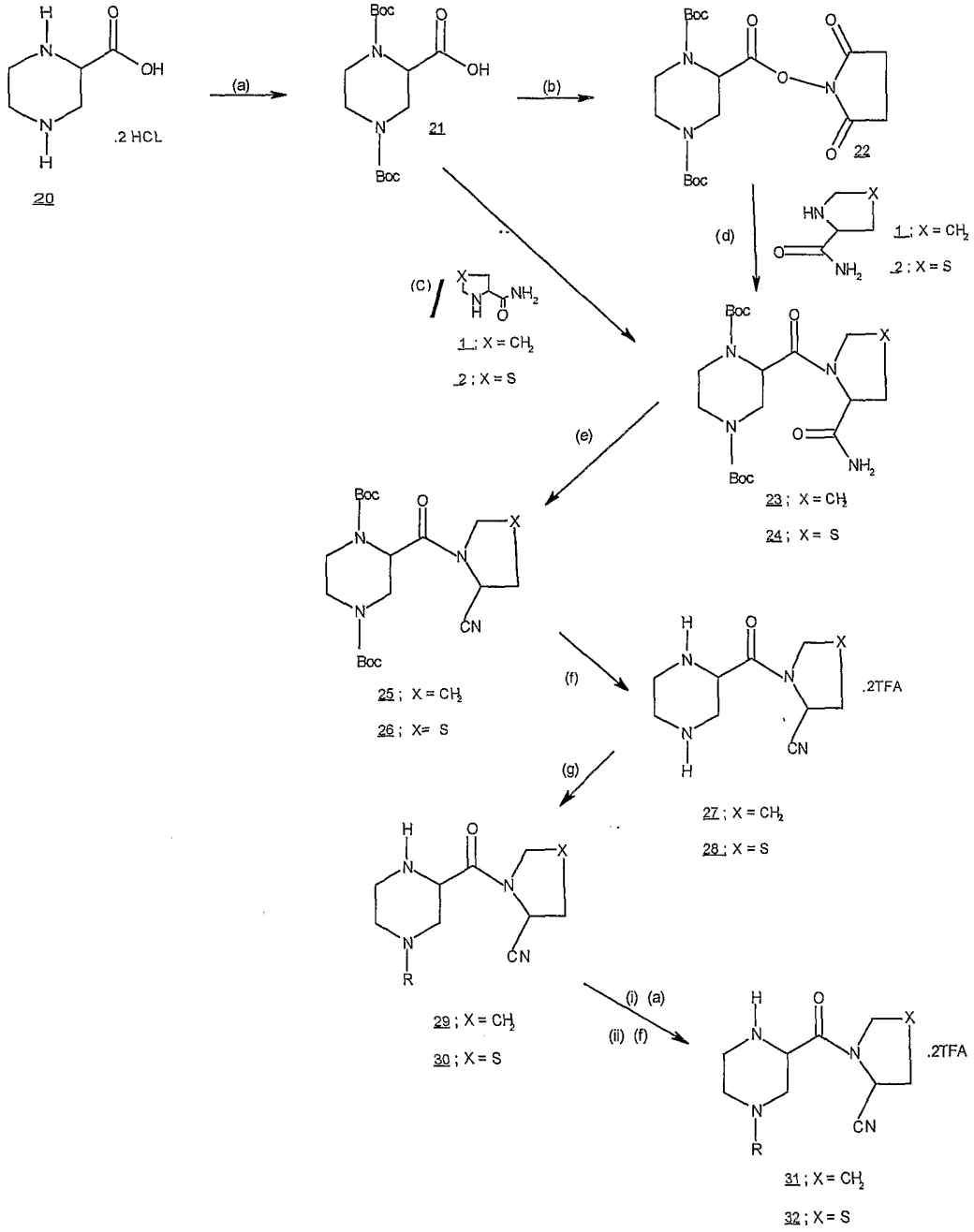
(b) coupling of said carbazate derivative with 3 or 4 in presence of base and in an organic solvent under heating 20 - 50 hrs. optionally in presence of potassium iodide to obtain coupled product ($3^1$).

(c) deprotection of $3^1$ obtained in above step (b) is carried out by using base, preferably morpholine at 10- 40°C for 1- 4 hrs. to obtain compound ($4^1$)

(d) functionalizing the deprotected product ($4^1$) to get compound of formula 11 or 12 with the substituent $R^4$ as desired.

Scheme 2 shown below is another route for synthesis of the compounds of the invention.

**Scheme- 2**



reagents and conditions for Scheme - 2:

[a] : $(Boc)_2O$, NaOH, Dioxan, $H_2O$, $0^0$-$25^0C$, 2-4 hrs;

[b] : NOSU, DCC, DCM, THF, $0^0$-$15^0C$, 3-5 hrs;

[c] : HOBT, DCC, DIEA, DCM, $-5^0$-$25^0C$, 6-16 hrs;

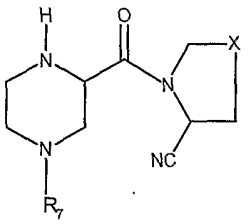
[d] : DCM or THF, $5^0$-$25^0C$, 12-22 hrs;

[e] : $(CF_3CO)_2O$, DCM or THF, Room Temp., 1-3 hrs;

[f] : $CF_3COOH$, $CH_3CN$, Room Temp., 3-4 hrs;

[g] : R7Br, $Et_3N$, $K_2CO_3$, THF, $CH_3CN$ or RBr, $Et_3N$, THF, $0^0$-$60^0C$, 1-25 hrs.

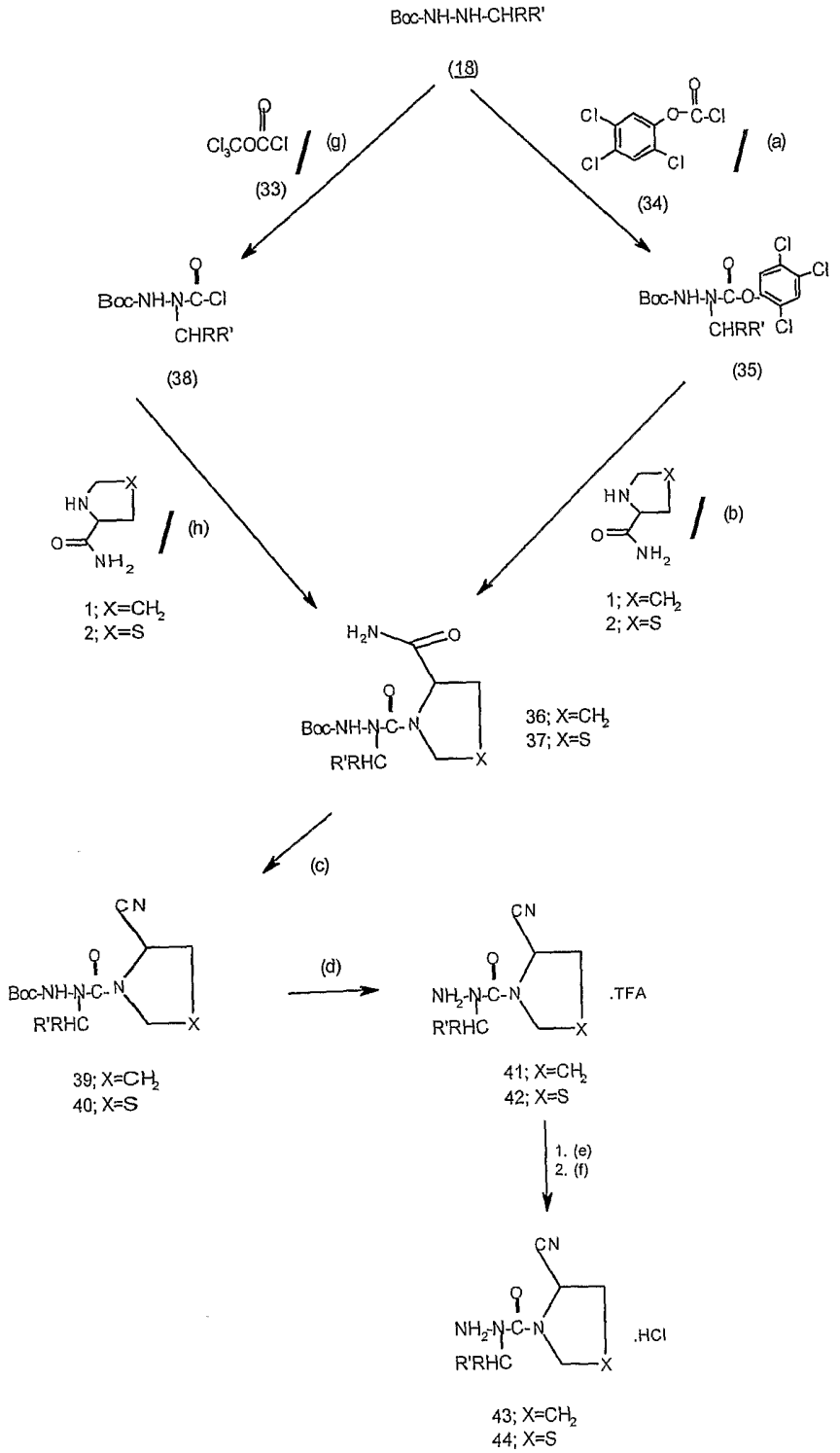
In an another embodiment of the present invention in which compounds, wherein the value of "k" mentioned in the general formula (I) is "null", then $R_4$ and $R_6$ together form optionally six or seven membered ring optionally containing two or three heteroatoms independently selected from O, S and $NR_7$, with $R_1$ is hydrogen, and $N_1$ is attached to hydrogen. As described represented by the formula (II), compounds may be prepared by the general methods as depicted in Scheme-2.




(II)

Piperazine-2-carboxylic acid dihydrochloride (20) is first protected by using usual protecting groups like Boc (tert-butyloxycarbonyl) or CBZ (benzyloxycarbonyl). The protected acid (21) is subjected to coupling with L-prolinamide (1) or (R)-(-)-thiazolidine-4-amide (2) to give the coupled products (23) or (24). This can either be done by first dicyclohexylcarbodiimide (DCC) mediated coupling of the acid (21) with N-hydroxysuccinimide (NOSU) to form the active ester (22) followed by its reaction with the amides ( 1 or 2 ), or by direct coupling of the protected acid (21) with the amides ( 1 or 2 ) in presence of 1-hydroxybenzotriazole (HOBT), DCC and the tertiaryamine like, diisopropylethyl amine (DIEA). Dehydration of the coupled products (23 or 24) using trifluroacetic anhydride as dehydrating agent provides the corresponding cyano derivatives (25 or 26). Deprotection of the compounds (25 or 26) in presence of trifluroacetic acid followed by regioselective functionalization of the deprotected compounds (27 or 28) at N-4 of piperazine ring using alkyl or aryl halides, or with acyl or sulphonyl halides yield the target compounds as represented by formula (29,30).They (29,30) can optionally be purified by reprotecting them at N-1 of the piperazine ring with a non-polar protecting group like Boc group, thereby, making these compounds more non polar, followed by deprotection of Boc group of this column purified intermediate using trifluroacetic acid results in the formation of final compounds as trifluroacetate salts (31,32).

## SCHEME-3


Boc-NH-NH-CHRR'
(18)
Cl3COCCl
(g)
(33)
(a)
(34)
Boc-NH-N-C-Cl
CHRR'
(38)
Boc-NH-N-C-O-
CHRR'
(35)
(h)
1; X=CH2
2; X=S
(b)
1; X=CH2
2; X=S
H2N
Boc-NH-N-C-N
R'RHC
36; X=CH2
37; X=S
(c)
(d)
Boc-NH-N-C-N
R'RHC
39; X=CH2
40; X=S
NH2-N-C-N
R'RHC
.TFA
41; X=CH2
42; X=S
1. (e)
2. (f)
NH2-N-C-N
R'RHC
.HCl
43; X=CH2
44; X=S

Reagents and Conditions for Scheme -3:

a)$Et_3N$, THF or DCM, -25$^0$ to 4$^0$C, $N_2$, 10-16 hrs.

b)$Et_3N$, THF or DCM, Reflux, 6-10 hrs.

c)$(CF_3CO)_2O$, THF, Room temp. 2-4 hrs.,

d)$CF_3COOH$, THF, 5$^0$C to Room temp. 0.5 to 2 hrs.,

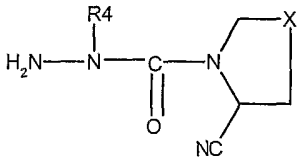
e)Aqueous $NaHCO_3$,

f)MeOH.HCl

g)$Et_3N$,THF, -5$^0$ to 0$^0$C, 1-2 hrs., $N_2$,

h)$Et_3N$, THF, 5$^0$ to 60$^0$C, 12-18 hrs.

In a yet another embodiment of the present invention in which compounds described represented by the formula (III), wherein the value of "n" mentioned in the formula (I) is "null", may be prepared by the general methods as depicted in Scheme-3.




(III)

N-2-substituted tert-butyl carbazate (18) on reaction with 2,4,5-trichlorophenyl chloroformate (34), prepared from 2,4,5-trichlorophenol and trimethyl chloroformate (33) by the method as described in the literature, in presence of triethylamine as base results in the formation of carbazate derivatives (35). [Ref. Konakahara T et al, Synthesis, 1993, 103-106.]

The carbazate derivatives (35) on coupling with L-prolinamide (1) or thiazolidine amide (2) in presence of a tertiary amine as a base preferably triethylamine in an organic solvent like THF under reflux for 4-10 hrs. give the coupled products (36,37). These amide derivatives (36,37) can also be obtained by chlorocarbonylation of tert-butyl-

carbazates (18) with trichloromethyl chloroformate (33) in presence of $Et_3N$ at a low temperature(-5$^0$ to 0$^0$ C), followed by coupling of the amides (1,2) with the chlorocarbonyl derivative of carbazates (38) in presence of $Et_3N$ / THF at a temperature ranging from 25$^0$ to 60$^0$C for 8-12 hrs.

Subsequently usual dehydration of the amide derivatives (36,37) with trifluoroacetic anhydride in THF at a temperature from 5$^0$ to 30$^0$C for 2-4 hrs. followed by deprotection of the corresponding cyano derivatives (39,40) with a deprotecting agent like trifluoroacetic acid at a temperature in the range of 5$^0$C to 30$^0$C for 0.5 to 2 hrs, results in the formation of the final compounds (41,42) as trifluoroacetate salts. They can optionally be purified by neutralizing with an aqueous alkali like sodium bicarbonate (aqueous), purifying the free base thus obtained by column chromatography followed by converting to hydrochloride salts (43,44) by treating with methanolic hydrochloric acid at 10$^0$C to 20$^0$C for 1 to 2 hrs.

***Representative example of Scheme IA*:**

**Example-1**

3-[1-oxo-2-(1-(1-(2-oxo-2-( 4-sulphonylaminophenyl)aminoethyl) piperidine-4-yl))hydrazine] ethyl-4-cyanothiazolidine, dihydrochloride (Compound no. 27)

**Step-1**

To the stirred solution of 4-piperidone monohydrochloride hydrate (30g, 0.2 mol) and sodium carbonate (22g, 0.207 mol) in 300 ml water, added dropwise solution of 9-Fluorenylmethoxysuccinimide (74g, 0.22 mole) in 300 ml dioxane at 0°C over 30 min period. After 7 hrs. of stirring at room temperature,1000 ml of chilled water was added under continuous stirring. Separated solid was filtered, washed with water (500ml) and dried at 60°C for 6 hrs. to give 60 gm of 9-Fluorenylmethoxy carbonyl-4-piperidone (yield: 93%).

**Step-2**

Product obtained in step-1 (60gm, 0.20 mol) was refluxed with tert-butyl carbazate (27 gm, 0.204 mol) in methanol (300 ml) for 3 hrs. Reaction mixture was evaporated to dryness, treated with 200 ml diethyl ether and filtered to get schiff's base (white solid). To the stirred solution of obtained solid in 500 ml. methanol, added solution of sodium cyanoborohydride (23g, 0.37 mol) in 100 ml. methanol in portion-wise at 0°C, followed by catalytic amount of titanium tetrachloride (4ml) at 0°C. Reaction mixture was stirred at room temperature for 2 hrs, evaporated, treated with water (1000 ml) and filtered. Obtained precipitate was dissolved in 1000 ml. dichloromethane & washed with water, dried over sodium sulphate and distilled off to give a crude desired product (63 g, yield 80%).

**Step-3**

Crude product obtained in step-2 (20g, 0.045 mol) was refluxed with chloroacetyl-4-cyano thiazolidine (10.4g, 0.052 mole) in dry tetrahydrofuran (300 ml) in presence of potassium cabonate (7.5g, 0.052 mole) and potassium iodide (0.8g, 0.005 mole) for 24 hrs. Reaction mixture was then filtered, distilled off and purified by column chromatography (eluent: 40% ethylacetate – Hexane) to give 8g of required product (yield: 30%).

**Step-4**

Product obtained in step-3 was stirred in 25 ml. morpholine for 1.5 hrs. Reaction mixture was then poured into 100 ml chilled water and filtered. Filtrate was extracted with dichloromethane. Organic layer was dried, distilled to give a solid (3.5 g, yield: 70%).

**Step-5**

Product obtained in step – 4 (6g, 0.016 mole) was dissolved in 100 ml. tetrahydrafuran and added N-[4-sulphonylaminophenyl] chloroacetamide (4.7 g, 0.019 mole), potassium carbonate (2.8g, 0.02 mole). Reaction mixture was then refluxed for 18 hrs., filtered,

evaporated and residue was purified by column chromatography (eluent: ethylacetate : hexane, (70 : 30) 1.8g, Yield: 20%).

**Step-6**

Product obtained in step-5 (1.2g, 0.002 mole) was stirred in 4-N-dioxane.HCl (8ml) at room temperature for 3 hrs. To the reaction mixture added 20 ml. methanol and 50 ml diethylether. The separated solid was filtered and washed with diethyl ether and suck dried and finally crude product was purified using methanol-diethylether mixture (1:1) to yielded title compound, 3-[1-oxo-2-(1-(1-(2-oxo-2-( 4-sulphonylaminophenyl) aminoethyl) piperidine-4-yl))hydrazine] ethyl-4-cyanothiazolidine, dihydrochloride (Compound no. 142), (770mg, yield: 70%).

**The following representative compounds may be prepared by following the synthetic route of Scheme IA.**

**Example-2**

3-[1-oxo-2-(1-(1-(2-oxo-2-(5-chloropyridin-2-yl)aminoethyl) piperidin-4-yl))hydrazino]ethyl(s)-(+)-4-cyanothiazolidine tris-trifluoroacetate (Compound no. 1)

Yield : 87%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 8.32 - 8.33 (d, 1H), 8.16-8.18 (d,1H), 7.84 - 7.86 (dd,1H), 5.32-5.33 (d,1H), 4.72-4.74 (d,1H), 4.64-4.66 (d, 1H), 4.03 - 4.15 (m, 4H), 3.67 - 3.69 (m, 2H), 3.54 - 3.57 (m, 1H), 3.38 - 3.39 (d, 2H), 3.31 - 3.33 (m, 2H), 2.21-2.24 (m, 2H), 1.98 - 2.02 (m, 2H)

Mass (m/z) : 438 ($M^+$+1)

IR (KBr, $Cm^{-1}$) : 2952, 2249, 1663

$[\alpha]_D$: +37.9° (C=0.5, MeOH)

**Example-3**

3-[1-oxo-2-(1-(1-(2-oxo-2-(5-bromothiazol-2-yl)aminoethyl)piperidine-4-yl))hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 2)

Yield : 70%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 7.46 (s, 1H), 5.32 - 5.34 (t, 1H), 4.65 - 4.67 (d, 1H), 4.75 - 4.77 (d, 1H), 3.99-4.14 (m, 4H), 3.58 - 3.62 (m, 2H), 3.45 - 3.47 (m, 1H), 3.38 - 3.39 (d, 2H), 3.12 - 3.14 (m, 2H), 2.19 - 2.22 (m, 2H), 1.98 - 2.10 (m, 2H)

Mass (m/z) : 488 ($M^+$+1), 512 ($M^+$+Na)

IR (KBr, $Cm^{-1}$) : 2940, 2248, 1667

**Example-4**

3-[1-oxo-2-(1-(1-(2-oxo-2-amino)ethyl)piperidine-4-yl))hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate. (Compound no. 3)

Yield : 50%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 5.30 - 5.32 (t, 1H), 4.64 - 4.66 (d, 1H), 4.54 - 4.56 (d, 1H), 3.93 - 4.12 (m, 4H), 3.64 - 3.69 (m, 2H), 3.48 - 3.50 (m, 1H), 3.37 - 3.38 (d, 2H), 3.18 - 3.20 (m, 2H), 2.48 - 2.50 (m, 2H), 2.17 - 2.20 (m, 2H)

Mass (m/z) : 327($M^+$+1)
IR (KBr, $Cm^{-1}$) : 2941 , 2246, 1671

**Example-5**

3-[1-oxo-2-(1-(1-(2-oxo-2-(4,5-dimethylthiazole-2-yl) aminoethyl)piperidin-4-yl)) hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 4)

Yield : 50%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 5.32 - 5.34 (t, 1H), 4.72 - 4.75 (d, 1H), 4.63 - 4.66 (d, 1H), 4.03 - 4.18 (m, 4H), 3.65 - 3.67 (m, 2H), 3.51 - 3.53 (m, 1H), 3.38 - 3.39 (d, 2H), 3.10 - 3.15 (m, 2H), 2.29 (s, 3H), 2.23 - 2.28 (m, 2H), 2.22 (s, 3H), 1.98 - 2.12 (m, 2H)

Mass(m/z): 438 ($M^+$+1), 460 ($M^+$+Na)

IR (KBr, $Cm^{-1}$): : 2939, 2248, 1671

$[\alpha]_D$: -36.15° (C=0.5, MeOH)

**Example-6**

3-[1-oxo-2-(1-(1-(2-oxo-2(5-cyanopyridin-2-yl)aminoethyl)piperidin-4-yl))hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 5)

Yield : 40%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 8.70 (s, 1H), 8.29 - 8.30 (m, 1H), 8.16 - 8.17 (dd, 1H), 5.32 - 5.33 (t, 1H), 4.72 - 4.74 (d, 1H), 4.64 - 4.66 (d, 1H), 4.02 - 4.20 (m, 4H), 3.66 - 3.70 (m, 2H), 3.48 - 3.50 (m, 1H), 3.38 - 3.39 (d, 2H), 3.14 - 3.17 (m, 2H), 2.23 - 2.29 (m, 2H), 2.03 - 2.09 (m, 2H)

Mass (m/z) : 429 ($M^+$+1)

IR (KBr, $Cm^{-1}$) : 2950, 2232, 1672

**Example-7**

3-[1-oxo-2-(-1-(-1-(2-oxo-2-(2-chloropyridyl-3-yl)aminoethyl) piperidin-4-yl))hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 6)

Yield : 50%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 8.44 - 8.46 (d, 1H), 8.22 - 8.23 (dd, 1H), 7.43 - 7.46 (dd, 1H), 5.32 - 5.35 (t, 1H), 4.72 - 4.74 (d, 1H), 4.64 - 4.66 (d, 1H), 4.04 - 4.16 (m, 4H), 3.58 - 3.62 (m, 2H), 3.48 - 3.50 (m, 1H), 3.38 - 3.39 (d, 2H), 3.13 - 3.17 (m, 2H), 2.20 - 2.24 (m, 2H), 2.00 - 2.04 (m, 2H)

Mass (m/z) : 438 ($M^+$+1)

IR (KBr, $Cm^{-1}$) : 2955, 2251, 1671

$[\alpha]_D$: -40.01° (C=0.5, MeOH)

**Example-8**

3-[1-oxo-2-(1-(1-(2-oxo-2-(2-flurobenzyl)amino ethyl)piperidine-4-yl))hydrazino]ethyl-4-cyanothiazolidine, bis-trifluoroacetate (Compound no.7)

Yield : 45%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 7.31 - 7.41 (m, 2H), 7.09 - 7.18 (m, 2H), 5.32 - 5.33 (t, 1H), 4.71 - 4.73 (d, 1H), 4.66 - 4.68 (d, 1H), 4.51 (s, 2H), 4.01 - 4.17 (m, 2H), 3.98 (s, 2H), 3.61 - 3.65 (m, 2H), 3.48 - 3.52 (m, 1H), 3.37 - 3.38 (d, 2H), 3.18 - 3.22 (m, 2H), 2.20 - 2.24 (m, 2H), 1.84 - 1.88 (m, 2H)

Mass (m/z) : 457 ($M^+$+Na)

IR (KBr, $Cm^{-1}$) : 2578, 1668, 1639

**Example-9**

3-[1-oxo-2-(1-(1-phenoxyethyl)piperidin-4-yl)hydrazino] ethyl-4-cyano-thiazolidine, bis-trifluoroacetate. (Compound no. 8)

Yield : 70%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 7.31 - 7.35 (t, 2H), 7.01 - 7.04 (m, 3H), 5.32 - 5.34 (t, 1H), 4.71 - 4.74 (d, 1H), 4.63 - 4.66 (d, 1H), 4.38 - 4.40 (t, 2H), 4.02 - 4.18 (m, 2H), 3.75 - 3.81 (m, 2H), 3.46 – 3.63 (m, 3H), 3.37 - 3.38 (d, 2H), 3.13 - 3.16 (m, 2H), 2.23-2.27 (m, 2H), 1.93 - 1.96 (m, 2H)

Mass (m/z) : 390 ($M^+$+1) 412 ($M^+$+Na)

IR (KBr, $Cm^{-1}$) : 2249, 1667, 1594

**Example-10**

3-[1-oxo-2-(1-(1-(2-oxo-2-(5-chloropyridin-2-yl)amino ethyl) piperidin-4-yl))hydrazino] ethyl-2-cyanopyrrolidine, tris-trifluoroacetate. (Compound no.9)

Yield : 70%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 8.32 (bs, 1H), 8.16 - 8.18 (d, 1H), 7.84 - 7.86 (dd, 1H), 5.32 - 5.33 (d, 1H), 4.10 - 4.43 (m, 4H), 3.60 - 3.97 (m, 3H), 3.49 - 3.52 (m, 2H), 3.18 - 3.20 (m, 2H), 1.96 - 2.36 (m, 8H)

Mass (m/z) : 420 ($M^+$+1), 442 ($M^+$+Na)

IR (KBr, $Cm^{-1}$) : 2984, 2245, 1668

**Example-11**

3-[1-oxo-2-(1-(1-(2-oxo-2-cyclohexyl)aminoethyl)piperidin-4-yl)hydrazino]ethyl-4-cyanothiazoline, bis-trifluoroacetate. (Compound no. 10)

Yield : 75%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 5.32 - 5.34 (t, 1H), 4.73 - 4.76 (d, 1H), 4.62 - 4.65 (d, 1H), 4.04 - 4.20 (m, 2H), 3.91 (s, 2H), 3.63 - 3.74 (m, 4H), 3.47 - 3.51 (m, 1H), 3.37 - 3.38 (d, 2H), 2.98 - 3.12 (m, 2H), 2.22 - 2.25 (m, 2H), 1.98 - 2.03 (m, 2H), 1.88 - 1.91 (m, 2H), 1.76 - 1.79 (m, 2H), 1.64 - 1.68 (m, 1H), 1.17 - 1.43 (m, 4H),

Mass (m/z) : 409 ($M^+$+1), 431 ($M^+$+Na)

IR (KBr, $Cm^{-1}$) : 2934, 2249, 1666

**Example-12**

3-[(1-oxo-2-(1-(1-(2-oxo-2-(3-isopropoxy propan-1-yl)amino ethyl) piperidine-4-yl))hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate. (Compound no. 11)

Yield : 70%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 5.32 - 5.34 (t, 1H), 4.73 - 4.75 (d, 1H), 4.60 - 4-62 (d, 1H), 4.04 - 4.19 (m, 2H), 3.94 (s, 2H), 3.56 - 3.62 (m, 4H), 3.47-3.50 (m, 3H), 3.36 - 3.38 (m, 3H), 2.98 - 3.12 (m, 2H), 2.22 - 2.25 (m, 2H), 1.97 - 2.11 (m, 2H), 1.74 - 1.80 (m, 2H), 1.14 - 1.16 (d, 6H)

Mass (m/z) : 426 ($M^+$+1), 449 ($M^+$+Na)

IR (KBr, $Cm^{-1}$) : 2941, 2248, 1666

**Example-13**

3-[1-oxo-2-(1-(1-(2-oxo-2-(2-(thiophene-2-yl) ethyl) aminoethyl) piperidine-4-yl))hydrazino]ethyl - 4- cyanothiazolidine, bis-trifluoroacetate. (Compound no. 12)

Yield: 70%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 7.23 - 7.24 (d, 1H), 6.94 - 6.96 (t, 1H), 6.89 - 6.90 (d, 1H), 5.32 - 5.34 (t, 1H), 4.71 - 4.73 (d, 1H), 4.59 - 4.61 (d, 1H), 4.03 - 4.15 (m, 2H), 3.91 (s, 2H), 3.48 - 3.58 (m, 5H), 3.37 - 3.38 (d, 2H), 3.15 - 3.18 (m, 2H), 3.05 - 3.09 (t, 2H), 2.20 - 2.23 (m, 2H), 1.95 - 2.03 (m, 2H)

Mass (m/z) : 437 ($M^+$+1), 459 ($M^+$+Na)

IR (KBr, $Cm^{-1}$) : 2939, 2248, 1666

**Example-14**

3-[1-oxo-2-(1-(1-(2-oxo-2-(3-chloro-4-fluoro-phenyl)aminoethyl)piperidine-4-yl)) hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate. (Compound no. 13)

Yield : 60%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 7.89 - 7.91 (dd, 1H), 7.45 - 7.47 (m, 1H), 7.23 - 7.25 (t, 1H), 5.32 - 5.33 (t, 1H), 4.73 - 4.75 (d, 1H), 4.65 - 4.67 (d, 1H), 3.97 - 4.10 (m, 4H), 3.66 - 3.69 (m, 2H), 3.46 - 3.50 (m, 1H), 3.37 - 3.38 (d, 2H), 3.12 - 3.16 (m, 2H), 2.20 - 2.24 (m, 2H), 2.00 - 2.03 (m, 2H)

Mass (m/z) : 477 ($M^+$+Na), 479

IR (KBr, $Cm^{-1}$) : 2947, 2249, 1674

**Example-15**

3-[1-oxo-2-(1-(1-(2-oxo-2-(4-ethoxycarbonyl methyl thiazole-2-yl) amino ethyl) piperidine-4-yl)) hydrazino] ethyl -4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 14)

Yield : 60%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 7.00 (s, 1H), 5.32 - 5.34 (t, 1H), 4.73 - 4.75 (d, 1H), 4.65 - 4.67 (d, 1H), 4.01 - 4.20 (m, 6H), 3.73 (s, 2H), 3.61 - 3.64 (m, 2H), 3.47 - 3.50 (m, 1H), 3.38 - 3.39 (d, 2H), 3.15 - 3.18 (m, 2H), 2.21 - 2.24 (m, 2H), 1.99 - 2.01 (m, 2H), 1.25 - 1.28 (t, 3H)

Mass (m/z) : 496 ($M^+$+1)

IR (KBr, $Cm^{-1}$) : 2943, 2250, 1677

**Example-16**

3-[1-oxo-2-(1-(1-(2-oxo-2-(3,4-methelenedioxyphenyl) amino ethyl ) piperidine -4-yl)) hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate. (Compound no. 15)

Yield : 75%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 7.28 (d, 1H), 6.93 - 6.95 (dd, 1H), 6.79 - 6.81 (d, 1H), 5.96 (s, 2H), 5.33 - 5.35 (t, 1H), 4.72 - 4.74 (d, 1H), 4.64 - 4.66 (d, 1H), 4.04 - 4.16 (m, 4H), 3.68 - 3.70 (m, 2H), 3.48 - 3.51 (m, 1H), 3.38 - 3.39 (d, 2H), 3.14 - 3.16 (m, 2H), 2.25 - 2.28 (m, 2H), 2.01 - 2.03 (m, 2H)

Mass (m/z) : 447 ($M^+$+1), 469 ($M^+$+Na)

IR (KBr, $Cm^{-1}$) : 2900, 2250, 1563

**Example-17**

3-[1-oxo-2-(1-(1-(2-oxo-2-(4-aminosulphonylphenyl) aminoethyl)piperidin-4-yl)) hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate. (Compound no.16)

Yield : 50%

$^1$HNMR ($d_4$-DMSO, 400 MHz): δ 7.74 - 7.79 (d, 2H), 7.72 - 7.75 (d, 2H), 5.28 - 5.30 (t, 1H), 4.76 - 4.79 (d, 1H), 4.61 - 4.64 (d, 1H), 3.70 - 4.03 (m, 3H), 3.37 - 3.47 (m, 4H), 3.17 - 3.26 (m, 2H), 2.67 - 2.83 (m, 2H), 1.88 - 1.99 (m, 2H), 1.78 - 1.82 (m, 2H)

Mass (m/z) : 482 ($M^+$+1)

IR (KBr, $Cm^{-1}$) : 2940, 2247, 1674

$[\alpha]_D$: -33.10° (C=0.5, MeOH)

**Example-18**

3-[1-oxo-2-(1-(1-(3-oxo-3-cyclopropyl)amino propyl)piperidin-4-yl)) hydrazino]ethyl-4-cyanothiazolidine, bis-trifluoroacetate (Compound no. 17)

Yield : 50%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 5.32 - 5.33 (t, 1H), 4.72 - 4.74 (d, 1H), 4.64 - 4.66 (d, 1H), 4.01 - 4.12 (m, 2H), 3.68 - 3.71 (m, 2H), 3.50 - 3.53 (m, 3H), 3.37 - 3.38 (d, 2H), 3.06 - 3.08 (m, 2H), 2.69 - 2.71 (m, 3H), 2.22 - 2.25 (m, 2H), 2.01 - 2.03 (m, 2H), 0.72 - 0.77 (m, 2H), 0.50 - 0.55 (m, 2H)

Mass (m/z) : 381 ($M^+$+1)

IR (KBr, $Cm^{-1}$) : 2946, 2248, 1674

**Example-19**

3-[1-oxo-2-(1-(1-(2–oxo-2-(5-chloropyridin-2-yl) amino ethyl )piperidine-4-yl)-2-methoxycarbonyl) hydrazino] ethyl-4-cyanothiazolidine, trihydrochloride. (Compound no. 18)

Yield : 80%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 8.32 - 8.33 (d, 1H), 8.16 - 8.18 (d, 1H), 7.84 - 7.86 (dd, 1H), 5.24 - 5.28 (t, 1H), 4.67 - 5.00 (m, 2H), 4.16 - 4.19 (m, 2H), 3.75 - 3.97 (m, 4H), 3.53 (s, 3H), 3.46 - 3.48 (m, 1H), 3.38 - 3.39 (d, 2H), 3.15 - 3.21 (m, 2H), 2.10 - 2.22 (m, 2H), 1.88 - 1.91 (m, 2H)

Mass (m/z) : 496 ($M^+$+1), 518 ($M^+$+Na)

IR (KBr, $Cm^{-1}$) : 2946, 2244, 1702

**Example-20**

3-[1-oxo-2-(1-(1-(2-oxo-2-(thiazole-2-yl)-aminoethyl)piperidin-4-yl))hydrazino] ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 19)

Yield : 60%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 7.47 - 7.48 (d, 1H), 7.20-7.21 (d, 1H), 5.33 - 5.35 (t, 1H), 4.72 - 4.74 (d, 1H), 4.64 - 4.66 (d, 1H), 4.24 (s, 2H), 4.06 - 4.13 (m, 2H), 3.65 - 3.70 (m, 2H), 3.61 - 3.63 (m, 1H), 3.38 - 3.39 (d, 2H), 3.28 - 3.32 (m, 2H), 2.25 - 2.28 (m, 2H), 2.01 - 2.05 (m, 2H)

Mass (m/z) : 410 ($M^+$+1), 432 ($M^+$+Na)

**Example-21**

3-[1-oxo-2-(1-(1-(2-oxo-2-(2-methoxyethyl)aminoethyl)piperidin-4-yl))hydrazino]ethyl-4-cyanothiazolidine, bis -trifluoroacetate. (Compound no. 20)

Yield : 50%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 5.32 - 5.33 (t, 1H), 4.72 - 4.74 (d, 1H), 4.61 - 4.63 (d, 1H), 4.02 - 4.19 (m, 2H), 3.94 (s, 2H), 3.61-3.64 (m, 2H), 3.46 - 3.51 (m, 5H), 3.37 - 3.38 (d, 2H), 3.33 (s, 3H), 3.01 - 3.06 (m, 2H), 2.21 - 2.24 (m, 2H), 2.01 - 2.03 (m, 2H)

Mass (m/z) : 385 ($M^+$+1), 407 ($M^+$+Na)

IR (KBr, $Cm^{-1}$) : 2936, 2246,1681

**Example-22**

3-[1-oxo-2-(1-(1-(2-oxo-2-(pyridin-2-yl)aminoethyl)piperidine-4-yl))hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 21)

Yield : 50%

$^1$HNMR ($d_4$-MeOH, 400 MHz) δ 8.34 - 8.35 (d, 1H), 8.12 (bs, 1H), 7.81 - 7.84 (t, 1H), 7.17 - 7.20 (t, 1H), 5.33 - 5.34 (t, 1H), 4.72 - 4.74 (d, 1H), 4.64 - 4.66 (d, 1H), 4.05 - 4.18 (m, 4H), 3.62 - 3.76 (m, 3H), 3.38 - 3.39 (d, 2H), 3.11 - 3.15 (m, 2H), 2.19 - 2.28 (m, 2H), 2.01 - 2.04 (m, 2H)

Mass (m/z) : 404 ($M^+$+1), 426 ($M^+$+Na)

IR (KBr, $Cm^{-1}$) : 2937,2247,1671

**Example-23**

3-[1-oxo-2-(1-(1-(3-pyridylacetyl) piperidine-4-yl))hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate. (Compound no.22 )

Yield : 60%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 8.76 (bs, 2H), 8.43 - 8.45 (d, 1H), 7.99 - 8.03 (t, 1H), 5.33 - 5.36 (t, 1H), 4.73 - 4.75 (d, 1H), 4.62 - 4.64 (d, 1H), 4.06 - 4.17 (m, 4H), 3.48 - 3.56 (m, 2H), 3.38 - 3.39 (d, 2H), 3.25 - 3.27 (m, 2H), 2.81 - 2.88 (m, 1H), 2.02 - 2.12 (m, 2H), 1.63 - 1.69 (m, 1H), 1.48 - 1.52 (m, 1H)

Mass (m/z) : 389 ($M^+$+1)

IR (KBr, $Cm^{-1}$) : 2929,1713,1646

**Example-24**

3-[1-oxo-2-(1-(1 -(2-oxo-2-(benzothiazole-2-yl)piperidine-4-yl))hydrazino] ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 23)

Yield : 70%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 7.89 - 7.91 (d, 1H), 7.75-7.77 (d, 1H), 7.47 - 7.50 (t,1H), 7.35 - 7.39 (t, 1H), 5.33 - 5.35 (t, 1H), 4.73 - 4.75 (d, 1H), 4.61 - 4.63 (d, 1H), 4.18 (s, 2H), 4.00 - 4.12 (m, 2H), 3.63 - 3.65 (m, 2H), 3.47 - 3.50 (m, 1H), 3.38 - 3.39 (d, 2H), 3.07 - 3.09 (m, 2H), 2.21 - 2.24 (m, 2H), 2.00 - 2.03 (m, 2H)

Mass (m/z) : 460 ($M^+$+1), 482 ($M^+$+Na)

IR (KBr, $Cm^{-1}$): 2939,2339,1663

$[\alpha]_D$: -12.47° (C=0.5, MeOH)

**Example-25**

3-[1-oxo-2-(1-(1-(2-oxo-2-(5-cyano pyridin-2-yl)aminoethyl)piperidin-4-yl))hydrazino]ethyl-4-cyano thiazolidine, tris-trihydrochloride. (Compound no.25 )

Yield :80%

$^1$HNMR ($d_4$-Methanol, 400 MHz): δ 8.70 (s, 1H), 8.30 (d, 1H), 8.15 - 8.18 (d, 1H), 5.36 - 5.37 (t, 1H), 4.73 - 4.77 (d, 1H), 4.71 - 4.73 (d, 1H), 4.19 - 4.40 (m, 4H), 3.81-3.85 (m, 2H), 3.67-3.69 (m, 1H), 3.50 - 3.54 (m, 2H), 3.38 - 3.41 (d, 2H), 2.25 - 2.35 (m, 2H), 2.01 - 2.06 (m, 2H)

Mass (m/z) : 428 ($M^+$+1)
IR (KBr, $Cm^{-1}$) : 2936,2231,1663

**Example-26**

3-[1-oxo-2-(1-(1-(2-oxo-2-(2-chloro pyridin-3-yl)aminoethyl)piperidin-4-yl))hydrazino]ethyl-4-cyano thiazolidine, tris-trihydrochloride. (Compound no. 26)

Yield:

$^1$HNMR ($d_4$-Methanol, 400 MHz): δ 8.38 - 8.40 (d, 1H), 8.24 - 8.25 (d, 1H), 7.44 - 7.47 (t, 1H), 5.34 - 5.36 (t, 1H), 4.76 - 4.78 (d, 1H), 4.64 - 4.66 (d, 1H), 4.04 - 4.17 (m, 4H), 3.78 - 3.83 (m, 2H), 3.49 - 3.53 (m, 1H), 3.14-3.18 (m, 2H), 3.37 - 3.39 (d, 2H), 2.23 - 2.31 (m, 2H), 2.00 - 2.03 (m, 2H)

Mass (m/z) : 438 ($M^+$+1)

IR (KBr, $Cm^{-1}$): 2941,2247,1665

**Example-27**

3-[1-oxo-2-(1-(1-(2-oxo-2-(4-amino sulphonyl phenyl)aminoethyl) piperidin-4-yl))hydrazino]ethyl-4-cyano thiazolidine, dihydrochloride. (Compound no.27 )

$^1$HNMR ($d_4$-Methanol, 400 MHz): δ 7.88 - 7.91 (d, 2H), 7.82 - 7.84 (d, 2H), 5.36 - 5.37 (t, 1H), 4.73 - 4.77 (d, 1H), 4.66 - 4.68 (d, 1H), 4.11 - 4.35 (m, 4H), 3.77 - 3.86 (m, 2H), 3.67-3.69 (m, 1H), 3.50 - 3.53 (m, 2H), 3.37 - 3.39 (d, 2H), 2.24 - 2.32 (m, 2H), 2.01 - 2.06 (m, 2H),

| | | |
|---|---|---|
| Mass (m/z) | : | 504 ($M^+$+Na) |
| IR (KBr, $Cm^{-1}$) | : | 2981,1694,1648 |

**Example-28**

3-[1-oxo-2-(1-(1-(2-oxo-2-(4-chlorophenyl)aminoethyl)piperidin-4-yl)) ydrazino]ethyl-4-cyano thiazolidine, drihydrochloride. (Compound no. 28)

$^1$HNMR ($d_4$-Methanol, 400 MHz): δ 7.63 - 7.65 (d, 2H), 7.34 - 7.36 (d, 2H), 5.36 - 5.37 (t, 1H), 4.75 - 4.77 (d, 1H), 4.69 - 4.71 (d, 1H), 4.10 - 4.24 (m, 4H), 3.77 - 3.83 (m, 2H), 3.49-3.52 (m, 1H), 3.40 - 3.42 (m, 2H), 3.35 - 3.37 (d, 2H), 2.23 - 2.33 (m, 2H), 2.01 - 2.04 (m, 2H)

| | | |
|---|---|---|
| Mass (m/z) | : | 437 ($M^+$+1) |
| IR (KBr, $Cm^{-1}$) | : | 2930,2353,1730 |

**Example-29**

3-[1-oxo-2-(1-(1-(2-oxo-2-(benzothiazole-2-yl)aminoethyl)piperidine-4-yl)) hydrazine]ethyl-4-cyanothiazolidine, trihydrochloride. (Compound no.29)

$^1$HNMR ($d_4$-Methanol, 400 MHz): δ 7.89 - 7.91 (d, 1H), 7.76 - 7.78 (d, 1H), 7.47 - 7.51 (t, 1H), 7.35 - 7.39 (t, 1H), 5.34 - 5.36 (t, 1H), 4.74 - 4.76 (d, 1H), 4.68 - 4.70 (d, 1H), 4.38 (s, 2H), 4.11 - 4.17 (m, 2H), 3.78 - 3.83 (m, 2H), 3.61 - 3.63 (m, 1H), 3.42 - 3.44 (m, 2H), 3.39 - 3.41 (d, 2H), 2.30 (m, 2H), 2.00 - 2.04 (m, 2H),

Mass (m/z) : 458 ($M^+$+1)

IR (KBr, $Cm^{-1}$) : 2931,2342,1652

**Example-30**

3-[1-oxo-2-(1-(1-(2-(4,5-dimethylthiazole-2-yl)aminoethyl)piperidin-4-yl)) hydrazino]ethyl-4-cyano thizolidine, trihydrochloride. (Compound no. 30)

$^1$HNMR ($d_4$-Methanol, 400 MHz): δ 5.36 - 5.37 (t, 1H), 4.78 - 4.80 (d, 1H), 4.70 - 4.72 (d, 1H), 4.51(s, 2H), 4.11 - 4.23 (m, 2H), 3.74 - 3.77 (m, 2H), 3.59-3.61 (m, 1H), 3.36 - 3.40 (m, 2H), 3.31 - 3.33 (d, 2H), 2.38 (s, 3H), 2.36(s, 3H), 2.31-2.33(m, 2H), 2.12-2.14 (m, 2H)

Mass (m/z) : 438 ($M^+$+1)

IR (KBr, $Cm^{-1}$) : 2920,2342,1718

**Example-31**

3-[1-oxo-2-(1-(1-(2-cyclopropyl-1-yl)aminoethyl)piperidin-4-yl)) hydrazino]ethyl-4-cyano thiazolidine, dihydrochloride. (Compound no. 31)

$^1$HNMR ($d_4$-Methanol, 400 MHz): δ 5.34 - 5.36 (t, 1H), 4.73 - 4.75 (d, 1H), 4.61 - 4.63 (d, 1H), 4.09 - 4.22 (m, 2H), 3.97 (s, 2H), 3.72 - 3.77 (m, 2H), 3.61 - 3.63 (m, 1H), 3.43 - 3.46 (m, 2H), 3.32 - 3.34 (d, 2H), 2.75 - 2.77 (m, 1H), 2.24 - 2.30 (m, 2H), 2.19 (m, 2H), 0.76 - 0.81 (q, 2H), 0.56 (q, 2H)

Mass (m/z) : 367 ($M^+$+1), 389 ($M^+$+Na)

IR (KBr, $Cm^{-1}$) : 2934 , 2246,1731

**The following representative compounds may be prepared by following the synthetic route of Scheme I.**

**Example-32**

3-[1-oxo-2-(1-(1-(5-methylpyrazine-2-ylcarbonyl)amino-4-cyclohexyl))hydrazino] ethyl-4-cyanothiazolidine, tris-trifluoroacetate. (Compound no. 24)

Yield : 50%

$^1$HNMR ($d_4$-MeOH, 400 MHz): δ 9.08 (bs, 1H), 8.58 (bs, 1H), 5.33-5.35 (t, 1H), 4.72 - 4.74 (d, 1H), 4.64 - 4.66 (d, 1H), 4.05 - 4.17 (m, 2H), 3.92 - 3.99 (m, 1H), 3.49 - 3.53 (m, 1H), 3.38 - 3.40 (d, 2H), 2.64 (s, 3H), 2.09 - 2.13 (m, 4H), 1.55 - 1.59 (m, 4H)

Mass (m/z) : 404 ($M^+$+1), 426 ($M^+$+Na)

IR (KBr, $Cm^{-1}$): 2951,2246,1644

**Example-33**

3-[-oxo-2- (2-ter-butyloxycarbonyl) hydrazino] ethyl-4-cyanooxazolidine. (Compound no. 32)

Yield: 5%

$^1$HNMR ($d_4$-CHCl3, 400 MHz): δ 3.41- 5.12 (m, 7H)

Mass (m/z): 293 ($M^+$+Na)

IR (KBr, $Cm^{-1}$): 2923, 2852,1676

The compounds of the invention can also be prepared by the method of Schemes 2 and 3 as described above.

**Pharmaceutical Compositions**

Pharmaceutical compositions may be prepared with a pharmaceutically effective quantity of compounds of general formula I, individually or in combination. It is common practice to administer the compounds in the form of pharmaceutical dosage forms comprising pharmaceutically acceptable excipient(s) and at least one active ingredient. These dosage forms may be administered by a variety of routes including oral, topical, transdermal, subcutaneous, intramuscular, intravenous, intranasal, pulmonary etc. Administration of the agents according to the instant invention may take place over an extended period of time at a dosage level of, for example, up to about 30 mg/kg. The pharmaceutical composition can be in the range of 0.5% to 90% by weight of the compound.

The following pharmaceutical formulations suggested are by way of example alone and in no way restrict the forms in which they can be used.

**Oral formulations**

Oral formulations may be administered as solid dosage forms for example pellets, powders, sachets or discreet units such as tablets or capsules and like. Other orally administered pharmaceutical preparations include monophasic and biphasic liquid dosage forms either in ready to use form or forms suitable for reconstitution such as mixtures, syrups, suspensions or emulsions. The preparations in addition may contain diluents, dispersing agents, buffers, stabilizers, solubilizers, surfactants, preservatives, chelating agents and/ or other pharmaceutical additives as are used. Aqueous or non-aqueous vehicle or their combination may be used and if desired may contain suitable sweetener, flavoring agent or similar substances. In case of suspension or emulsion a suitable thickening agent or suspending agent or emulsifying agent may be present in addition. Alternatively, the compounds may be administered as such in their pure form unassociated with other additives for example as capsules or sachets. It may also be administered with a vehicle. Pharmaceutical preparations can have a slow, delayed or controlled release of active ingredients as is provided by a matrix or diffusion controlled system.

When the present invention or its salts or suitable complexes is presented as a discrete unit dosage form like tablet, it may contain in addition medically inert excipients as are used in the art. Some example of suitable excipients include lactose, cellulose and its derivatives such as microcrystalline cellulose, methylcelulose, hydroxy propyl methyl cellulose, ethylcellulose, dicalcium phosphate, mannitol, starch, gelatin, polyvinyl pyrolidone, various gums like acacia, tragacanth, xanthan, alginates & its derivatives, sorbitol, dextrose, xylitol, magnesium stearate, talc, colloidal silicon dioxide, mineral oil, glyceryl mono stearate, glyceryl behenate, sodium starch glycolate, Cross Povidone, crosslinked carboxymethylcellulose, various emulsifiers such as polyethylene glycol, sorbitol, fattyacid esters, polyethylene glycol alkylethers, sugar esters, polyoxyethylene

polyoxypropyl block copolymers, polyethoxylated fatty acid monoesters, diesters and mixtures thereof.

**Preparation of oral dosage form:**

A typical tablet can have the following compositions:

**Oral formulation**

A tablet formulation may be prepared as per the following compositions.

**Example - 34**

| Ingredients | Qty. (mg / tablet) |
|---|---|
| Active ingredient* | 20.0 mg |
| Microcrystalline Cellulose | 200.0 mg |
| Starch | 50.0 mg |
| Magnesium Stearate | 5.0 mg |
| Talc | 2.0 mg |

*Any one or more of compound Nos. 1-32

**Example - 35**

| Ingredients | Qty. (mg / tablet) |
|---|---|
| Active ingredient * | 10 mg |
| Lactose | 75 mg |
| Starch | 50 mg. |
| Polyvinyl pyrolidone (10% solution in water) | 5 mg |
| Sodium starch glycolate | 5 mg |
| Magnesium Stearate | 2 mg |
| Colloidal Silicon-dioxide | 5 mg |

*Any one or more of compound Nos.1-32

**Example 36**

| Ingredients | Qty. (mg / tablet) |
|---|---|
| Active ingredient* | 5.0 mg |
| Microcrystalline Cellulose | 80.5 mg |
| Starch | 8.0 mg. |
| Talc | 3.3 mg |
| Magnesium Stearate | 1.6 mg |
| Colloidal Silicon-dioxide | 1.6 mg |

*Any one or more of compound Nos.1-32

Active ingredient, lactose and starch are screened through 40 # sieve and blended. The blend is then granulated with polyvinyl pyrolidone solution. Resultant mass is screened through number 16 sieve. The granules produced are then dried at 50 - 60 °C and passed through 16-mesh sieve. Sodium starch glycolate, magnesium Stearate and colloidal silicon dioxide are sifted through 60-mesh sieve and blended with the granules. The resultant blend is then compressed into tablets.

The above ingredients may be blended into tablets by any other conventional materials.

**Parenteral Formulations**

For parenteral administration, the compounds or their salts or suitable complexes thereof may be present in a sterile vehicle which may be an aqueous or non-aqueous vehicle or a combination thereof. The examples of vehicles are water, ethyl oleate, oils and derivatives of polyols, glycols and their derivatives. It may contain additives common in injectable preparations like stabilizers, solubilizers, pH modifiers, buffers, antioxidants, cosolvents, complexing agents, tonicity modifiers, etc.

Some suitable additives are for example tartrate, citrate or similar buffers, alcohol, sodium chloride, dextrose and high molecular weight polymers. Another alternative is sterile powder reconstitution. The compound may be administered in the form of injection for more than once daily administration, or intravenous infusion/ drip or suitable depot preparation.

For injectable administration, the active ingredient or its salt is dissolved or dispersed in a sterile vehicle. The vehicle may be aqueous or non-aqueous and may contain suitable surfactants, solubilizers, buffers, stabilizers, surfactants, antioxidants, cosolvents, chelating agents, tonicity modifiers etc. Various excipients commonly used include propylene glycol, polythene glycol, mannitol, sodium chloride, ethyloleate, polyethylene glycol fatty acid esters, polyethylene glycol castor oil, polyethylene glycol sarbitan fatty acid esters, sugar esters, various buffers such as phosphate, succinate, citrate, borate, antioxidants such as sodium metabisulphite etc.

An injectable formulation containing the following ingredient may be prepared:

**Example 37**

| Ingredients | Qty. |
|---|---|
| Active ingredient * | 1 mg |
| Polythylene glycol | 0.1 ml |
| Isotonic Saline / WFI | to 1 ml |
| Sodium metabisulphite | |

*Any one or more of compound Nos.1-32

## OTHER FORMULATIONS

For the dermatological application and for the buccal delivery, the recommended formulations are gel, ointment, creams, patches, liniment, lotions, oral rinse, gurgles and toothpaste containing appropriate compounds of the compounds of the general formula I.

The above examples are presented by way of illustration alone and in no way limit the scope of the invention.

# CLAIMS

1. A heterocyclic compound selected from the group consisting of :

a) 3-[1-oxo-2-(1-(1-(2-oxo-2-(5-chloropyridin-2-yl)aminoethyl) piperidin-4-yl)) hydrazino]ethyl(s)-(+)-4-cyanothiazolidine tris-trifluoroacetate

b) 3-[1-oxo-2-(1-(1-(2-oxo-2-(5-bromothiazol-2-yl)aminoethyl) piperidine-4-yl))hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate.

c) 3-[1-oxo-2-(1-(1-(2-oxo-2-amino)ethyl)piperidine-4-yl))hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate.

d) 3-[1-oxo-2-(1-(1-(2-oxo-2-(4,5-dimethylthiazole-2-yl) aminoethyl) piperidin-4-yl)) hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate.

e) 3-[1-oxo-2-(1-(1-(2-oxo-2(5-cyanopyridin-2-yl)aminoethyl) piperidin-4-yl))hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate.

f) 3-[1-oxo-2-(-1-(-1-(2-oxo-2-(2-chloropyridyl-3-yl)aminoethyl) piperidin-4-yl))hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate.

g) 3-[1-oxo-2-(1-(1-(2-oxo-2-(2-flurobenzyl)amino ethyl)piperidine-4-yl))hydrazino]ethyl-4-cyanothiazolidine, bis-trifluoroacetate

h) 3-[1-oxo-2-(1-(1-phenoxyethyl)piperidin-4-yl)hydrazino] ethyl-4-cyano-thiazolidine, bis-trifluoroacetate.

i) 3-[1-oxo-2-(1-(1-(2-oxo-2-(5-chloropyridin-2-yl)amino ethyl) piperidin-4-yl))hydrazino] ethyl-2-cyanopyrrolidine, tris-trifluoroacetate.

j) 3-[1-oxo-2-(1-(1-(2-oxo-2-cyclohexyl)aminoethyl)piperidin-4-yl)hydrazino]ethyl-4-cyanothiazoline, bis-trifluoroacetate.

k) 3-[(1-oxo-2-(1-(1-(2-oxo-2-(3-isopropoxy propan-1-yl)amino ethyl) piperidine-4-yl))hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate.

l) 3-[1-oxo-2-(1-(1-(2-oxo-2-(2-(thiophene-2-yl) ethyl) aminoethyl) piperidine-4-yl))hydrazino]ethyl - 4- cyanothiazolidine, bis-trifluoroacetate.

m) 3-[1-oxo-2-(1-(1-(2-oxo-2-(3-chloro-4-fluoro-phenyl)aminoethyl) piperidine-4-yl)) hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate.

n) 3-[1-oxo-2-(1-(1-(2-oxo-2-(4-ethoxycarbonyl methyl thiazole-2-yl) amino ethyl) piperidine-4-yl)) hydrazino] ethyl -4-cyanothiazolidine, tris-trifluoroacetate.

o) 3-[1-oxo-2-(1-(1-(2-oxo-2-(3,4-methelenedioxyphenyl) amino ethyl ) piperidine -4-yl)) hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate.

p) 3-[1-oxo-2-(1-(1-(2-oxo-2-(4-aminosulphonylphenyl) aminoethyl) piperidin-4-yl)) hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate.

q) 3-[1-oxo-2-(1-(1-(3-oxo-3-cyclopropyl)amino propyl)piperidin-4-yl)) hydrazino]ethyl-4-cyanothiazolidine, bis-trifluoroacetate

r) 3-[1-oxo-2-(1-(1-(2-oxo-2-(5-chloropyridin-2-yl) amino ethyl ) piperidine-4-yl)-2-methoxycarbonyl) hydrazino]ethyl-4-cyanothiazolidine, trihydrochloride.

s) 3-[1-oxo-2-(1-(1-(2-oxo-2-(thiazole-2-yl)-aminoethyl)piperidin-4-yl))hydrazino] ethyl-4-cyanothiazolidine, tris-trifluoroacetate.

t) 3-[1-oxo-2-(1-(1-(2-oxo-2-(2-methoxyethyl)aminoethyl)piperidin-4-yl))hydrazino]ethyl-4-cyanothiazolidine, bis -trifluoroacetate.

u) 3-[1-oxo-2-(1-(1-(2-oxo-2-(pyridin-2-yl)aminoethyl)piperidine-4-yl))hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate.

v) 3-[1-oxo-2-(1-(1-(3-pyridylacetyl) piperidine-4-yl))hydrazino] ethyl-4-cyanothiazolidine, bis-trifluoroacetate.

w) 3-[1-oxo-2-(1-(1-(2-oxo-2-(benzothiazole-2-yl)piperidine-4-yl)) hydrazino] ethyl-4-cyanothiazolidine, tris-trifluoroacetate.

x) 3-[1-oxo-2-(1-(1-(5-methylpyrazine-2-ylcarbonyl)amino-4-cyclohexyl))hydrazino]ethyl-4-cyanothiazolidine, tris-trifluoroacetate.

y) 3-[1-oxo-2-(1-(1-(2-oxo-2-(5-cyano pyridin-2-yl)aminoethyl)piperidin-4-yl))hydrazino]ethyl-4-cyano thiazolidine, trihydrochloride.

z) 3-[1-oxo-2-(1-(1-(2-oxo-2-(2-chloro pyridin-3-yl)aminoethyl)piperidin-4-yl))hydrazino]ethyl-4-cyano thiazolidine, trihydrochloride.

aa) 3-[1-oxo-2-(1-(1-(2-oxo-2-(4-amino sulphonyl phenyl)aminoethyl) piperidin-4-yl))hydrazino]ethyl-4-cyano thiazolidine, dihydrochloride.

bb) 3-[1-oxo-2-(1-(1-(2-oxo-2-(4-chlorophenyl)aminoethyl)piperidin-4-yl)) ydrazino]ethyl-4-cyano thiazolidine, dihydrochloride.

cc) 3-[1-oxo-2-(1-(1-(2-oxo-2-(benzothiazole-2-yl)aminoethyl)piperidine-4-yl)) hydrazine]ethyl-4-cyanothiazolidine, trihydrochloride.

dd) 3-[1-oxo-2-(1-(1-(2-(4,5-dimethylthiazole-2-yl)aminoethyl)piperidin-4-yl)) hydrazino]ethyl-4-cyano thizolidine, trihydrochloride.

ee) 3-[1-oxo-2-(1-(1-(2-cyclopropyl-1-yl)aminoethyl)piperidin-4-yl)) hydrazino]ethyl-4-cyano thiazolidine, dihydrochloride and

ff) 3-[-oxo-2- (2-ter-butyloxycarbonyl) hydrazino] ethyl-4-cyanooxazolidine,

their stereoisomers or their pharmaceutically acceptable solvates or salts.

2. A pharmaceutical composition comprising one or more compounds as claimed in claim 1, their stereoisomers, or one or more pharmaceutically acceptable salts in association with a pharmaceutically acceptable carrier, diluent or excepient.

3. A method of inhibiting the enzyme DPP-IV in the body tissue of a mammal including human being which comprises administering an effective amount of one or more compounds as claimed in claim 1, their stereoisomers, or pharmaceutically acceptable salts in association with a pharmaceutically acceptable carrier, diluent or excepient to a mammal in need thereof.

4. A method of scavenging free radical from the body tissue of a mammal including human being which comprises administering an effective amount of one or more compounds as claimed in claim 1, their stereoisomers or pharmaceutically acceptable salts in association with a pharmaceutically acceptable carrier, diluent or excepient to a mammal in need thereof.

5. A method for treatment and/or prophylaxis of glucose intolerance in a mammal including human being which comprises administering an effective amount of one or more compounds as claimed in claim 1, their stereoisomers, or pharmaceutically acceptable salts in association with a pharmaceutically acceptable carrier, diluent or excepient to a mammal in need thereof.

6. A method for treatment and/or prophylaxis of disorders associated with DPP-IV in a mammal including human being by administering an effective amount of one or

more compounds as claimed in claim 1, their stereoisomers, or pharmaceutically acceptable salts to a mammal in need thereof, wherein the said disorders are selected from the group consisting of

a) Cushing's syndrome;
b) Hyperthyroidism;
c) Obesity;
d) Hyperglucagonemia;
e) Diseases including ulcers and HIV infection;
f) Disorders related to increased gastric emptying, acid secretion and hunger;
g) Autoimmune disorders including multiple sclerosis;
h) Rheumatoid arthritis;
i) Grave's disease ;
j) Diarrhea;
k) Mucosal regeneration in patients with intestinal disease;
l) Growth hormone deficiency;
m) Neurological and neuropsychological disorders and
n) Cancers and tumors.

7. A method of treating a mammal including human being in disease conditions caused by accumulation of free radicals in the body cells which comprises administering an effective amount of one or more compounds as claimed in claim 1, their stereoisomers or pharmaceutically acceptable salts in association with a pharmaceutically acceptable carrier, diluent or excepient to a mammal in need thereof.

8. The method as claimed in claim 7 wherein the said disease condition is selected from the group consisting of (a) Neurodegenerative disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Motor Neuron Disease and Prion Disease, (b) Diabetes and Diabetic Vascular Complications, (c) Intestinal Diseases such as Intestinal Ischemia, Radiation Enmteritis, Inflammatory Bowel Disease, Gastric and Colorectal Cancers, (d) Liver Diseases such as Alcoholic Liver Disease and Chronic Hepatitis C etc, (e) Cancers such as Lung Cancer, Colorectal Cancer,

Cervical Cancer, Breast Cancer and Malignant Melanoma, (f) Cardiac Diseases such as Atherosclerosis, Myocardial Infarction, Ischemic Stroke and Endothelial Dysfunction, (g) Opthalmic Disorders such as Cataract formation and Macular degeneration, (h) HIV Diseases, (i) Respiratory Diseases such as Chronic Obstructive Pulmonary Diseases and Asthma and (j) Renal Diseases such as Glomerulonephritis and Acute Renal Failure.

9. Use of the compounds as claimed in claim 1, their stereoisomers pharmaceutically acceptable solvates or salts in the manufacture of a medicament useful for inhibiting the enzyme DPP-IV in the body tissue of a mammal including human being.

10. Use of the compounds as claimed in claim 1, their stereoisomers pharmaceutically acceptable solvates or salts in the manufacture of a medicament useful for scavenging free radical from the body tissue of a mammal including human being.

11. Use of the compounds as claimed in claim 1, their stereoisomers pharmaceutically acceptable solvates or salts in the manufacture of a medicament useful for treatment and/or prophylaxis of glucose intolerance in a mammal including human being.

12. Use of the compounds as claimed in claim 1, their stereoisomers pharmaceutically acceptable solvates or salts in the manufacture of a medicament useful for treatment and/or prophylaxis of disorders associated with DPP-IV in a mammal including human being.

13. The use as claimed in claim 12, wherein said disorder is selected from the group consisting of :

a) Cushing's syndrome;
b) Hyperthyroidism;
c) Obesity;
d) Hyperglucagonemia;
e) Diseases including ulcers and HIV infection;

f) Disorders related to increased gastric emptying, acid secretion and hunger;

g) Autoimmune disorders including multiple sclerosis;

h) Rheumatoid arthritis;

i) Grave's disease ;

j) Diarrhea;

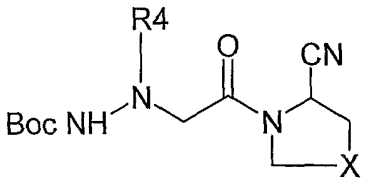
k) Mucosal regeneration in patients with intestinal disease;

l) Growth hormone deficiency;

m) Neurological and neuropsychological disorders and

n) Cancers and tumors

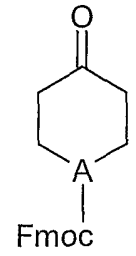
14. A process for the preparation of compounds of formula 11 or 12.




11; X = CH2
12; X = S

where $R^4$ and X are as defined in Claim 1, which comprises the steps of :

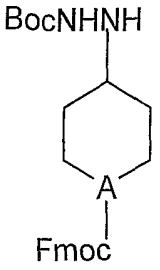
(a) reacting a N-protecting cyclic ketone ($1^1$)




A= -N-, -CHNH-

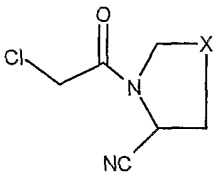
(1')

with $BocNHNH_2$ in alcoholic solvents under heating for 1-8 hours followed by reduction in alcoholic solvent at 0-35°C to obtain ($2^1$) N-2-substituted tert-butyl carbazate.




( 2' )

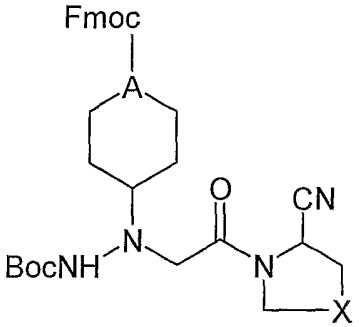
(b) coupling of said carbazate derivative with 3 or 4




(3) ;X=$CH_2$

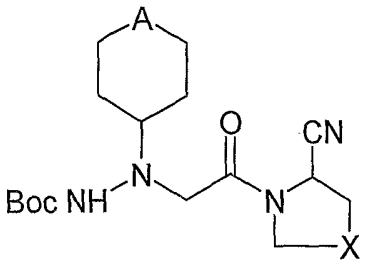
(4) ;X=S

in presence of base and in an organic solvent under heating 20 - 50 hrs. to obtain coupled product ($3^1$).




( 3' )

(c) deprotection of $3^1$ obtained in above step (b) is carried out by using a base, at 10- 40°C for 1- 4 hrs. to obtain compound ($4^1$)




( 4' )

(d) functionalizing the deprotected product ($4^1$) to get compound of formula 11 or 12 with the substituent $R^4$ as desired.

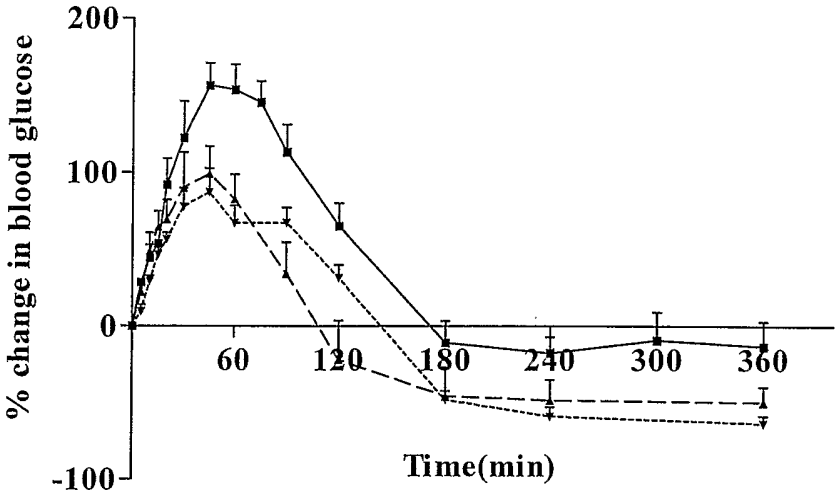
15. The process as claimed in claim 37, wherein (i) protection of cyclic ketone (i) in step (a) is Fmoc protection (ii) coupling reaction in step(b) is optionally carried out in presence of potassium iodide and (iii) the base used in step (c) is morpholine.

16. Heterocyclic compounds such as herein described particularly with reference to the examples.

17. Process for preparation of heterocyclic composition such as herein described particularly with reference to the examples.

18. Pharmaceutical composition such as herein described particularly with reference to the examples.

**Effect of Comp. No. 25 and Comp. No. 27**




**on blood glucose during OGTT**

**Key:** Vehicle (0.5% Na-CMC); (-■-), Comp. No. 25 (-▲-) and Comp. No. 27 (-▼-).

*Fig.1*

# INTERNATIONAL SEARCH REPORT

PCT/IB 03/04377

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D417/14 C07D417/12 C07D401/14 C07D263/04 A61K31/4439
A61K31/454 A61K31/497 A61K31/421 A61P3/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 95/15309 A (FERRING B.V.)<br>8 June 1995 (1995-06-08)<br>the whole document | 1,3 |
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☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 28 May 2004 | 09/06/2004 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31–70) 340–2040, Tx. 31 651 epo nl,<br>Fax: (+31–70) 340–3016 | Allard, M |

# INTERNATIONAL SEARCH REPORT

PCT/IB 03/04377

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 3-8 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 16-18
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Continuation of Box I.1

Although claims 3-8 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

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Continuation of Box I.2

Claims Nos.: 16-18

The scope of claims 16-18 is so unclear (Article 6 PCT) that a meaningful international search is impossible with regard to these claims.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

# INTERNATIONAL SEARCH REPORT

PCT/IB 03/04377

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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