Role of protein kinases in signal transduction and their inhibitors

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Summary

Protein kinase is a kinase enzyme that modifies other proteins by chemically adding phosphate groups to them (i.e. phosphorylation). The enzyme is involved in many biochemical signaling pathways within cells (i.e. signal transduction) and effectors in cellular functions, such as cell proliferation and necrosis. Protein kinases act as the third messenger system and activity of most of its isoforms often depends upon second messengers such as cAMP and calcium. Overexpression of protein kinase is a frequent cause of disease. Presently there are certain numbers of protein kinase inhibitors available which mediate the protein kinase activity. They can be used to control the cellular responses produced by protein kinase activity. How protein kinases functions with respect to signal transduction, effects of overexpression of it and medicinal roles of diverse protein kinase inhibitors are reviewed here.

Keywords**:** Protein kinase, Signal transduction, Overexpression of protein kinases, Protein kinase inhibitors

Introduction

Enzymes that biochemically modifies other proteins through the chemical addition of phosphate groups (i.e. phosphorylation) to receptor proteins are known as the protein kinases[57]. Signal transduction can be defined as any biochemical communication between one part of a cell to another[7,55,61]. Signal transduction at the cellular level refers to the movement of signals from outside the cell to inside (see figure 2)[7] and vice-versa. Protein kinases play a central key role in producing cellular responses like cell proliferation and death[42,57]. They are implicated in the etiology of many pathological conditions like cancer[7,24,26], central nervous system disorders (alzheimer's disease)[63] cardiovascular disorders (hypertension)[7,43], skin disorders (inflammation)[5,12] and diabetes mellitus[7,55]. Evidence of implied pathology relates to their ability to mediate abnormal or excess protein phosphorylation[57]**.** Many cellular events are regulated by phosphorylation[42,54,56]. The mechanism of phosphorylation in signal transduction is fundamental to the process. Proteins or lipids in the presence of ATPs (Adenosine triphosphates) and kinases undergo phosphorylation. The modified protein may subsequently have functional outcomes such as 1) Binding together with new protein 2) Stabilization or degradation of particular target 3) Movement of protein to new location such as nucleus or mitochondria. The human genome contains about 500 protein kinase genes; they constitute about 2% of all eukaryotic genes**.** Up to 30% of all proteins may be modified by kinase activity, especially those involved in signal transduction, the transmission of signals within the cell[57].

The aim of this review is to determine and discuss the relevance of protein kinase inhibitors in the aforementioned pathologies according to evidence present in current literature. To understand the multitude of extant protein kinases and their biochemical role they are classified according to their structure and function indicated by Table 1 and 2 respectively.

Table 1: A table summarizing the types of protein kinases (According to the amino acid residue)

Sr.no.	Types	Example	Functions
1.	Serine/threonine-specific protein kinases	Calcium/calmodulin-dependent protein kinase II (CaMKII)[57]	Phosphorylate the –OH (hydroxyl) functional group of serine or threonine.
2.	Tyrosine-specific protein kinases	Platelet derived growth factor (PDGF) receptor [63] Epidermal growth factor (EGF) receptor[1] Insulin growth factor (IGF1) receptor [55] Stem cell factor (scf) receptor[23]	Implicated in: processing of alzheimer's amyloid precursor protein epithelial cell migration and carcinoma invasion osmoregulation and antiaging survival factor of spermatogonia
3.	Histidine-specific protein kinases	Histidine kinase[56]	The pyruvate dehydrogenase family of kinases in animals is structurally related to histidine kinases.
4.	Mixed kinases	Muscle action potential kinase (MAPK)[42]	Involved in the muscle action potential kinase cascade

Table 2:- Summary of classes of protein kinases

Types	Functions
Protein kinase A (PKA)[4,45]	Acts as a catalyst enabling catalyzes the activity of intracellular proteins. Regulaties of glycogen, sugar and lipid metabolism. In adipocytes, myocytes and hepatocytes - phosphorylates acetyl-CoA carboxylase and pyruvate dehydrogenase- acetyl-CoA- lipogenesis. In nucleus accumbens neurons- translates the dopamine signal into cells.
Protein kinase B (PKB) or "Akt" [18,65]	Glucose metabolism, cell proliferation, apoptosis, transcription and cell migration. Cell survival - promote growth factor-mediated cell survival both directly and indirectly. Metabolism – promotes the glycogen synthesis. Angiogenesis –is implicated in angiogenesis and tumor development.
Akt 1[10]	Is implicated in: cellular survival pathways, by inhibiting apoptosis processes. skeletal muscle hypertrophy and general tissue growth. the transforming retrovirus as the oncogene.
Akt 2[44]	Is implicated in: the insulin signaling pathway. the induction of glucose transport.
Akt 3[9,18]	It appears to be predominantly expressed in brain. Mice lacking Akt 3 have small brains.
Protein kinase C (PKC)[29,42,49]	Catalyses phosphorylation of intracellular proteins & alter their activities. Controls the growth and cellular differentiation.

1.1 Signal Transduction Phenomenon:

The transduction of information from membrane (outside of the cell) to internal targets (inside the cell) leads to a cascade of molecular events that translate into the ultimate biological response to the affector molecule (figure 1). Development in biochemical and molecular biological techniques in the past decade has enabled the identification of key enzymes involved in the transduction process and fabrication of several natural and synthetic modulators of biological processes[7]. These tools have helped to elucidate molecular events under normal and pathological conditions. Recent information concerning the molecular interactions that regulate cellular responses, the potential for design and develop new drugs to treat cancer, central nervous system disorders (alzheimer's disease), cardiovascular disorders (hypertension), skin disorders (inflammation), diabetes mellitus and other debilitating diseases has become most intriguing.

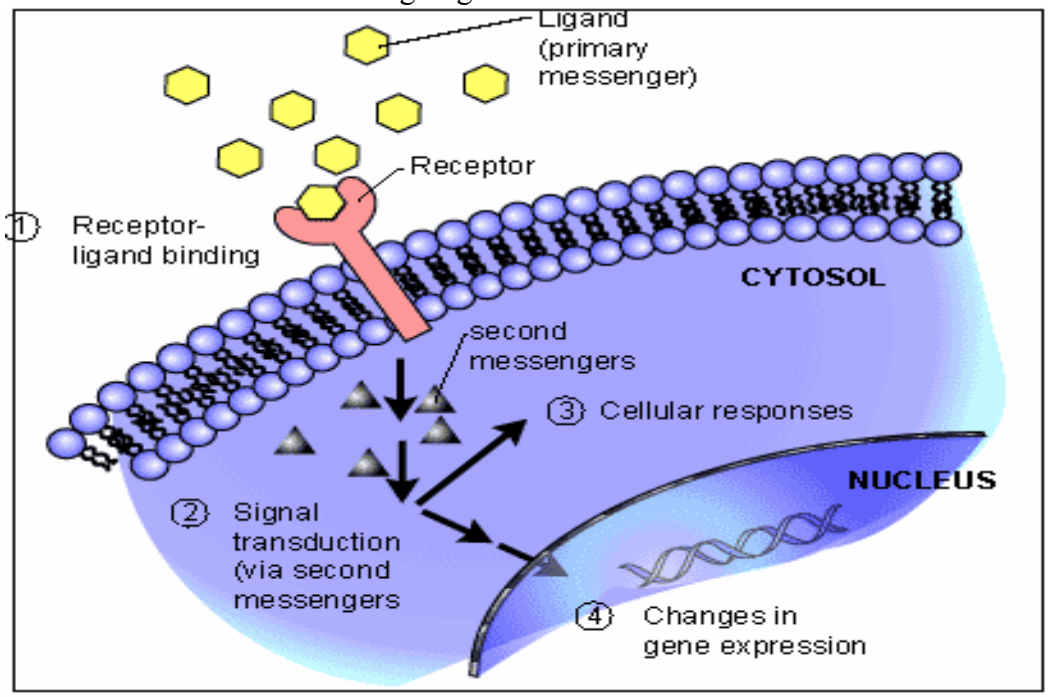


Figure 1:- Computer generated diagram showing signal transduction phenomenon

1.2 Protein kinase in signal transduction[4,42]:

The activity of most isoforms of these enzymes depends on second messengers such as cyclic AMP (adenosine monophosphate) and calcium. Given these facts, it is reasonable to refer to kinases and phosphatases as "third messengers". Signal transduction is the process by which an extracellular primary signal is converted into an intracellular second messenger. In the case of ligand-gated (i.e. ion channel) receptors, the influx of ions serves as a second messenger. G-protein-linked receptors, once activated, will not only activate a second messenger, but a third and fourth messenger as well. The ultimate end-point may be regulation of gene transcription to produce messenger RNA (mRNA) and the subsequent translation of mRNA to produce a protein specific to that gene. A receptor is a protein that binds a signaling molecule (hormone or transmitter) becomes activated and activates a signal transduction pathway. There are four messenger systems that are listed as follows -

1. First messengers (neurotransmitters)
2. Second messengers (G-protein-linked)
3. Third messengers (kinases and phosphatases)
4. Fourth messenger (phospho-calcium/cyclic AMP response element binding protein-CREB).

1.3. Sequence of events in the Signaling Process - An Example [7]:

As there are many signaling pathways it is best to demonstrate the sequence of events using a flow-diagram as shown in figure 2.This is a representative example of hundreds of pathways involved in cell signaling.

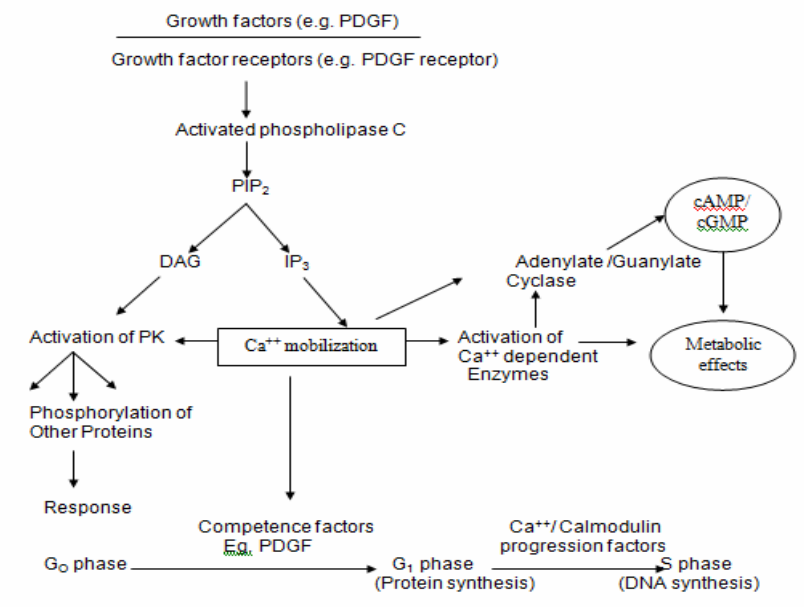


Figure 2:- The sequence of events during the signaling process {Where PIP_2: Phosphotidyl inositol biphosphate; IP_3: Inositol triphosphate; DAG: Di-acyl-glycerol; PK: protein kinase}

1.4 Some of the physiological actions of protein kinases[27,54]:

All kinds of different events in the cell are regulated by protein kinases (phosphorylation). All protein kinases have different physiological actions, some of which discussed below.

1) On cardiovascular system[54]

Activation of PKA results into activation of troponin which causes better excitation contraction coupling. It also causes increased cardiac contractility.

2) On smooth muscles[9,42]

Activation of PKC Phosphorylates other proteins while cytosolic Ca^{++} combines with calmodulin (CAM) to activate myosin light chain kinase- inducing contraction of smooth muscles.

3) On kidney[25]

- Protein kinase A mediates protein phosphorylation which triggers increased rate of exocytosis of water containing vesicles (WCV's) into the apical membrane and decreased rate of endocytosis of water containing vesicles (WCV's) from the apical membrane leads to increase water permeability of membrane.
- Angiotensin I receptors (At1) present in kidney stimulates cell growth especially vascular and cardiac cells by activation of mitogen activated protein (MAP) kinase pathway. It results into increased number of proton changes particularly C-fos and C-jun that regulates transcription of number of genes involved in cell growth[27].
- Activation of At1 results into stimulation of Protein kinase A which causes phosphorylation of various proteins which are involved in
    - Aldosterone synthesis
    - Facilation of neurotransmission
    - CNS effects
    - Renal effects

1.5 Overexpression of Protein Kinases [7, 24,29]

Protein kinase undergoes phosphorylation which ultimately leads to cellular responses. Overexpression of protein kinases activities do result into certain kinds of pathological conditions such as cancer, central nervous system disorders (alzheimer's disease), cardiovascular disorders (hypertension), skin disorders (inflammation) and diabetes mellitus etc. due to abnormal or over- protein phosphorylation.

1) The role of protein kinase C in cancer or tumor promotion:

When cells are stimulated, protein kinase C is transiently activated by diacylglycerol which is produced in the membrane during the signal-induced turnover of inositol phospholipids[36]. Tumour-promoting phorbol esters, when intercalated into the cell membrane, may substitute for diacylglycerol and permanently activate protein kinase C. This results into permanent phosphorylation of protein which causes change in activity of number of functional and structural protein resulting into uncontrollable growth of cell or tumour[24,29,54].

2) The role of protein kinase A in Hypertension :

The PKA phosphorylates many functional proteins including troponin and phospholamban, so that they interact with Ca^{++}, respectively resulting in increased force of contraction and faster relaxation[7,54]. Ca^{++} is made available by entry from outside as well as from intracellular stores. If ability of PKA to phosphorelate these functional proteins is increased then Ca^{++} influx into the cell from L-type Ca^{++}- channel would be increased leading to increase release of calcium by the sarcoplasmic reticulum. Increase calcium release would increase excitation-contraction coupling thereby increasing ionotropy i.e. force of contraction leading to hypertension[13,54].

3) The role of Tyrosine kinase in Hypoglycemia :

Insulin regulates whole-body glucose homoeostasis by modulating the activities of Tyrosine kinases in its target tissues like muscle, liver and fat. It results into phosphorylation and stimulation of enzymes or proteins involved in rapid metabolic action of insulin. Increase in insulin secretion from pancreas may results into increased ability of insulin to modulate tyrosine kinase activity producing hypoglycemia[7,50,54].

4) The role of protein kinase C in Vascular complications in Diabetes mellitus :

Vascular complications in diabetes mellitus are known to be associated with the activation of the protein kinase C (PKC) pathway through the de novo synthesis of diacylglycerol (DAG) from glycolytic intermediates. Indeed specific PKC isoforms, mainly the b- and d-isoforms, have been shown to be persistently activated in diabetic mellitus[55]. Multiple studies have reported that the activation of PKC leads to increased production of extracellular matrix and cytokines, enhances contractility, permeability and vascular cell proliferation, induces the activation of cytosolic phospholipase A_2 and inhibits the activity of Na^+-K^+-ATPase[27]. These events are not only frequently observed in diabetes mellitus but are also involved in the mode of actions of vasoactive agents or oxidative stress. Accordingly, it results into vascular complications[54,55].

5) The role of protein kinase C induces Keratinocyte Apoptosis and Intraepidermal inflammation [30, 31]:

Skin keratinocytes are major mediators of host immune responses. The skin is also a target for immunologically based inflammation in many pathological states. Activation of protein kinase C (PKC) can induce cutaneous inflammation, but the precise role of each of six cutaneous PKC isoforms (alpha, delta, epsilon, eta, zeta, μ) that regulate normal skin homeostasis or contribute to skin pathology has not been clarified. From different experiments it was observed that transgenic mice that overexpresses PKC in the basal layer of the epidermis and the outer root sheath of hair follicles under the regulation of the bovine keratin 5 (K5) promoter[36]. K5-PKC exhibits severe intraepidermal neutrophilic inflammation and disruption of the epidermis and upper hair follicles when treated topically with 12-O-

tetradecanoylphorbol-13-acetate (TPA). TPA causes apoptosis in transgenic skin which also evokes intraepidermal inflammation[41]. TPA also induces apoptosis in cultured transgenic keratinocytes and this is prevented by an AP-1 dominant negative construct. Thus, PKC activation induces keratinocyte apoptosis via an AP-1-dependent pathway and mediates chemokine induction and intraepidermal inflammation independently. This model system will be useful to define specific chemokines regulated by PKC that promote intraepidermal neutrophilic inflammation, a condition that characterizes several human cutaneous diseases such as pustular psoriasis and acute generalized exanthematous pustulosis[36].

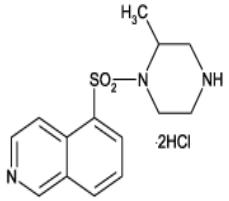
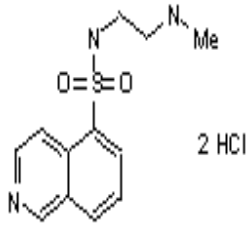
1.6 Protein Kinase Inhibitors:

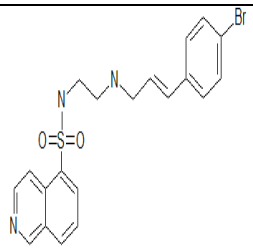
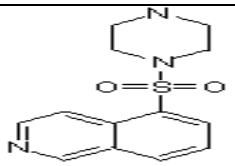
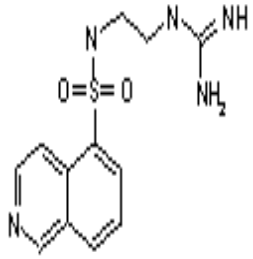
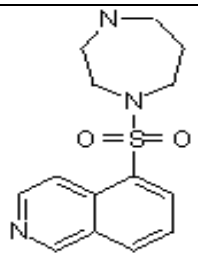
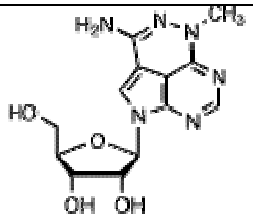
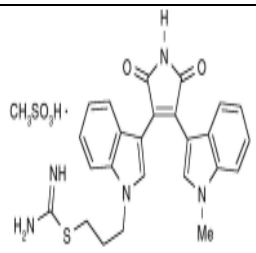
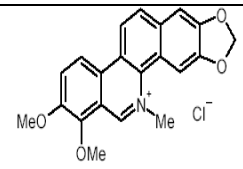
A protein kinase inhibitor is a type of enzyme inhibitor which specifically blocks the action of one or more protein kinases; hence they can be subdivided or characterized by the amino acids whose phosphorylation is inhibited as follows:

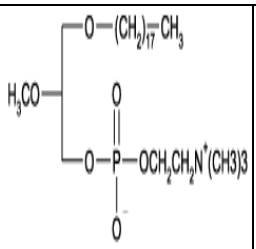
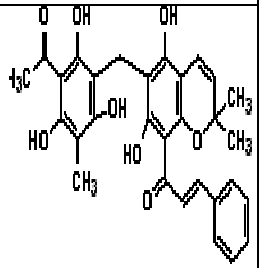
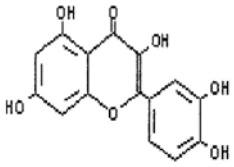
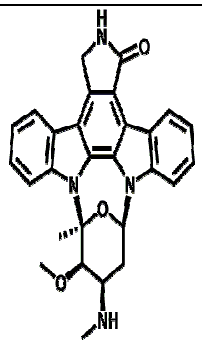
1. Serine/threonine-specific protein kinases inhibitors
2. Tyrosine-specific protein kinases inhibitors
3. Dual protein kinases inhibitors

Protein kinases modulate intracellular signal transduction by catalyzing the phosphorylation of specific proteins, and regulate many aspects that control cell growth, movement and death[57]. Overexpression of protein kinases is a frequent cause of disease. Molecules that can inhibit the activity of protein kinases are protein kinase inhibitors (PKI), can be used both in investigating the function of a specific kinase in a particular signaling pathway, as well as in preventing the aberrant action of protein kinases in pathophysiological conditions. Protein kinase inhibitors belong to a class of chemotherapy that disrupts the signal transduction within the cell[27,54]. To understand the multitude of extant protein kinase inhibitors, they are classified according to their structure and function indicated by Table 3.

Table 3: A table summarizing selected protein kinase inhibitors

Protein kinase inhibitor	Structure	IUPAC (International Union of Pure and Applied Chemistry)	Inhibitor of	Use
H-7 [25,30,33]		(5-Isoquinolinesulfonyl) -2-methylpiperazine . 2HCl	PKC, PKA, PKG, MLCK	In cancer.
H-8 [17,21]		N-[2-(Methylamino)ethyl]-5-isoquinolinesulfonamide. 2 HCl	PKG, PKC, PKA, MLCK	In cancer.

H-89 [10,18]		N-[2-(p Bromocinnamyl amino)ethyl]-5-isoquinolin esulfonamide . 2HCl	PKG, PKC, PKA, MLCK. CaMKII	Induces apoptosis. In cancer.
HA-100 [22]		1-(5-Isoquinolinesulfonyl)-piperazine dihydrochloride	PKC, PKA, MLCK.	In cancer.
HA-1004 [13]		N-(2'-Guanidinoethyl)-5-isoquinolinesulfonamide dihydrochloride	PKG, PKC, PKA, MLCK. CaMKII	Causes selective pulmonary vasodilation during pulmonary hypertension.
HA-1077 [3,37,48]		1-(5-Isoquinolinesulfonyl)-1H-hexahydro-1,4-diazepine dihydrochloride	PKG, PKC, PKA, MLCK. CaMKII	In Cardiovascular disorder.
Triciribine [41,59]		6-Amino-4-methyl-8-(β-D-ribofuranosyl) 4H, 8H pyrrolo[4,3,2-de]pyrimido [4,5-c] pyridazine	PKB	In case of cancer. It is active against HIV type 1.
Bisindolylma-leimide IX [13,14]		2,3-Bis(1H-indol-3-yl) maleimide; 3,4-Di-1H-indol-3-yl-1H-pyrrole-2,5-dione	PKC	In cancer. Inducer of apoptosis in chronic lymphocytic leukaemic cells.
Chelerythrine [16,36,62]		1,2-Dimethoxy-N-methyl (1,3)benzodioxolo(5,6-c) phenanthridinium chloride	PKC	In case of cancer.

Edelfosine **32,64**		rac-2-methyl-1-octadecyl-glycero-(3)-phosphocholine	PKC	In case of cancer. Induces expression of c-fos, c-jun and transcription factor AP-1. Induces apoptosis. Decreases multi- drug resistance (MDR).
Rottlerin **2,50**		1-[6-[(3-acetyl-2,4,6-trihydroxy-5-methyl-phenyl) methyl]-5,7-dihydroxy-2,2-dimethyl-chromen-8-yl]-3-phenyl-prop-2-en-1-one	PKC, PKA	Colon carcinoma.
Quercetin **6,40**		-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyrano-4-one	PKC, PKA	Cataracts. Pancreatic cancer. Heart diseases. Inflammation.
Staurosporine **15,40,58**		(*9S,10R,11R,13R*)-2,3,10,11, 12,13-Hexahydro-10 methoxy-9-methyl-11 (methylamino)-9,13-epoxy-1*H*,9*H*-diindolo[1,2,3gh: 3',2',1'-lm]pyrrolo[3,4-j] [1,7]benzodiazonin-1-one	PKA, PKG, MLCK, CaMKII	Used to induce apoptosis.

1.7 Selective Protein Kinase Inhibitors under Clinical Development:

There are several protein kinase inhibitors under clinical development. Some of them are listed below in Table 4

Table 4: A table summarizing selective protein kinase inhibitors under clinical development

Target	Compound	Manufacturer	Development Phase
A) Growth factor receptor inhibitors			
EGFR[20,28]	IMC-C225 Cetuximab	Imclone	III
	ABX-EGF	Abgenix	II
	EMD72000	Merck KgaA Darmstadt	I
	RH3	YMB Inc.	II
	MDX447	Merck KgaA	I
	ZD1839 gefitinib	Astra Zeneca	III

	OSI774	OSI Pharm.	III
	CI1033	Pfizer	II
	EKB569	Wyeth Ayerst	I
	GW2016	GlaxoSmithKline	I
HER-2[34]	Trastuzumab	Genentech	Registered
	MDX210	Novartis	I
	2C4	Genentech	I
	17-AAG	Kosan	I
PDGFR[28]	Imatinib	Novartis	Registered
B) Ras inhibitors			
Ras[61]	ISIS2503	Isis Pharm.	II
	R115777	Johnson and Johnson	III
	SCH66336	Schering Plough	II
	BMS214662	Bristol-Myers Squibb	I
C) Raf inhibitors			
Raf[52]	ISIS5132	Isis Pharm.	II
	L-779,450	Merck	II
	BAY439006	Bayer	II
D) MEK inhibitors			
MEK[39]	PD184352	Pfizer	II
	U-0126	Promega	I
E) mTOR inhibitors			
mTOR[35]	CCI779	Wyeth	II
	RAD0001	Novartis	I as cancer therapeutics III as immunosuppressant
	Rapamycin	Wyeth	Registered
F) Cyclin dependent protein kinase inhibitors			
CDK[8]	Flavopirodol	Aventis	II
	E7070	Easai	I
	CYC202	Cyclacel	I
	BMS 387032	Bristol-Myers Squibb	I
G) Other targets and agents			
PKC[30,31]	ISIS3521	ISIS Pharm	III
	CGP41251	Novartis	II
	Bryostatin-1	GPC Biotech	II
	UCN-01	Kyowa Hakko Kogyo	II
PKB[17,39]	LY333531	Eli Lilly	I oncology III diabetic neuropathy
PDK1	UCN-01	Kyowa Hakko Kogyo	II

Conclusion

The present review has given an overview approach on the protein kinase in signal transduction as well as their overexpression and glimpse on their inhibitors. Protein kinase play an important role in many signaling pathways and are well known as key effectors of cellular function such as cell proliferation and necrosis. However, overexpression of protein kinase leads to life threatening disorders like cancer, cardiovascular disorders (hypertension), central nervous system disorders, skin disorder (inflammation), diabetes mellitus etc. Hence, it is potential for design and development of new drugs which inhibits overexpression of protein kinases for prevention and treatment of associated disorders. Thus more extensive research on the protein kinase is essential to develop more potent and efficacious prophylactics for a solution to disease.

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