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REVIEW ARTICLE

Carbazole Derivatives as Kinase-Targeting Inhibitors for Cancer Treatment

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Abstract: Protein Kinases (PKs) are a heterogeneous family of enzymes that modulate several biological pathways, including cell division, cytoskeletal rearrangement, differentiation and apoptosis. In particular, due to their crucial role during human tumorigenesis and cancer progression, PKs are ideal targets for the design and development of effective and low toxic chemotherapeutics and represent the second group of drug targets after G-protein-coupled receptors. Nowadays, several compounds have been claimed to be PKs inhibitors, and some of them, such as imatinib, erlotinib and gefitinib, have already been approved for clinical use, whereas more than 30 others are in various phases of clinical trials. Among them, some natural or synthetic carbazole-based molecules represent promising PKs inhibitors due to their capability to interfere with PK activity by different mechanisms of action including the ability to act as DNA intercalating agents, interfere with the activity of enzymes involved in DNA duplication, such as topoisomerases and telomerases, and inhibit other proteins such as cyclin-dependent kinases or antagonize estrogen receptors. Thus, carbazoles can be considered a promising this class of compounds to be adopted in targeted therapy of different types of cancer.

Keywords: Protein kinases, kinases inhibitors, carbazoles, anticancer drugs, targeted therapy, cancer.

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1. INTRODUCTION

In the last years, the validation and the clinical application of targeted therapies towards specific proteins or genes, involved in cancer emergence and development, have prolonged the survival and improved quality of life of cancer patients [1]. This approach allows a more rational clinical use of conventional anticancer drugs in order to limit their high toxicity and consequential severe side effects [2]. The action of commonly used antineoplastic drugs is based on their capability to interfere with the replication of rapidly growing cells, such as cancer cells. However, the use of these drugs unavoidably leads to serious side effects due to the action of these chemotherapeutics also on cells healthy with a rapid turnover, including immune system and mucous membranes cells.

In contrast, targeted therapies are designed through a rational approach, selectively directed toward molecular components that are altered in tumor cells, leading to a substantial reduction of side effects [3]. Nowadays, targeted therapies, although combined with conventional treatments, represent the most promising therapeutic strategies to be adopted during antineoplastic clinical protocols.

A number of experimental evidences suggest that during the development of cancer, several signaling pathways are altered and, as a consequence, unhealthy cells are no longer subject to the normal replication control systems inducing altered growth, survival, neovascularization and invasion.

To become cancerous, cells must accumulate mutations of the genes that control proliferation. The deletion and/or amplification of products encoded by these genes lead to the alteration of key regulatory molecules, such as protein kinases, that are main control factors of various cellular pathways [4].

Protein-tyrosine kinases (PTKs), whose activity is finely tuned in physiological conditions, are amongst the major regulators of intracellular signal transduction pathways. Genetic modifications altering the PTKs-mediated signal are closely related to the malignant transformation of cells [5].

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The key role of PTKs during tumorigenesis and cancer progression makes them ideal targets for the development of highly selective anticancer drugs. Imatinib is the first member acting as a selective Bcr-Abl tyrosine kinase inhibitor, approved by FDA in 2001 for the treatment of chronic myeloid leukemia as an alternative drug for conventional cell cycle inhibitors. This result prompted further studies focusing on a more comprehensive knowledge of the structure, function and role of PTKs during carcinogenesis and metastasis, leading to the identification of a number of small molecules endowed with inhibitory activity against such proteins, and thus is potentially useful during the treatment of different types of cancer [6].

Several receptors with tyrosine kinase activity (RTK), in particular Tie-2, IGF1R, Her-2/ Neu, EGFR and VEGFR1-3, have been recognized as important regulators of cell growth and differentiation and a number of drugs and prodrugs of natural or synthetic origin have been identified as reversible or irreversible PTK inhibitors [7, 8].

In particular, carbazoles closely related to previously studied indole derivatives [9-16], represent an important and heterogeneous class of anticancer agents [17-19], used also for other many biological properties [20]. The activity of these compounds seems to be related to different mechanisms of action including the ability to act as DNA intercalating agents and interfere with specific DNA-dependent enzymes, such as topoisomerases and telomerase, or inhibit other proteins such as cyclin-dependent kinases and estrogen receptors [21-26].

This survey represents an overview of natural or synthetic carbazole-based molecules claimed as kinase inhibitors. thus representing promising tools to be adopted in targeted therapy of different types of cancer and, in particular, for the treatment of hormone-dependent cancers including breast or ovarian cancer.

2. PROTEIN KINASES: STRUCTURE, CLASSIFICATION AND FUNCTION

As in the general drug discovery pathway [27, 28], the availability of the crystallographic structures of different PKs has allowed, through docking studies, to obtain important information to better understand the mechanism of action of these proteins and has led to the discovery of new rationale ligands. PKs are a heterogeneous family of enzymes that share a highly conserved catalytic site, although they differ in structure and size, cellular localization, activation mechanism and substrate specificity. The conserved residue sequences of the active site have provided important information on the mechanism of action of this class of enzymes. In particular, their ability to transfer the phosphate group of a purine nucleotide triphosphate to a hydroxyl group of their protein substrates was well established. In general, the overall structure of PKs is very flexible, and these proteins are characterized by a two-lobed structural arrangement surrounding a deep cleft with the active site at its upper region.

The smaller amino terminal lobe is constituted by a five-stranded antiparallel β-sheet and a long α-helix, and is usually involved in protein-protein interactions. On the other hand, the larger helical C-terminal lobe includes the active site of the enzyme and a metal binding loop. The active site residues play a pivotal role in the interaction with the ATP triphosphate group, which is essential for the catalytic activity, whereas the metal-binding loop is involved in binding with a bivalent ion essential for the ATP interaction also affecting the affinity and selectivity of PK towards the substrate [29].

More than one PKs classification is possible, but conventionally these enzymes are classified according the specific OH-residue of substrate or according the aminoacid sequence of their catalytic domain. In a first classification, the PKs are divided into two subgroups: serine-threonine kinases and tyrosine kinases (TKs), which catalyze the phosphorylation of the OH group of serine and threonine or tyrosine residues, respectively. The catalytic domain of both PK classes is characterized by a glycine rich N-terminal region involved in ATP binding and a central key residue consisting in a conserved aspartic acid, necessary for the catalytic activity [30]. TKs are in turn classified into receptor TKs and non-receptor TKs. The first ones are trans-membrane proteins showing an extracellular ligand-binding domain and an intracellular catalytic domain. Non-receptor TKs lack of transmembrane domains and are located in the cytosol, nucleus or are adherent to the inner surface of the cytoplasmic membrane [31].

According to the residue sequences in the catalytic domains, PKs can be classified into at least seven groups:

- PK A, G and C (AGC);
- Ca^{2+}/CAM-dependent PK (CAMK);
- Casein kinase 1(CK1);
- CDK, MAPK, GSK3 and CLK (CMGC);
- Homologues of yeast sterile 7, 11, 20 kinases (STE);
- Tyrosine kinases (TKs);
- Tyrosine kinase-like PKs (TKL) [32].

PKs modulate several biological pathways, including cell division, cytoskeletal rearrangement, differentiation and apoptosis.

The general kinase phosphorylation mechanism involves the transfer of a gamma phosphate group of ATP onto hydroxyl groups of different substrates including sugars, proteins or lipids [5, 33].

More in detail, the reaction catalyzed by protein kinases is the following [34]:

$$\mathrm{MgATP^{-1}} + \text{target molecule} - \mathrm{O{:}\,H} \rightarrow \text{target molecule} - \mathrm{O{:}\,PO_3^{-2}} + \mathrm{MgADP} + \mathrm{H^+}$$

These phosphorylation reactions, necessary for most cellular pathways, are reversed by the action of phosphatases, which catalyze the removal of phosphoric groups from the target molecules [35]. The interaction between a ligand and the outer region of a surface PKs receptor promotes the activation of the intracellular domain of the kinase, auto-

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phosphorylation and therefore a series of cascade reactions in the cytoplasm and in the nucleus and, subsequently, the translation of both intracellular and extracellular signal [36, 37]. The activation of genes and cellular response is therefore finely controlled through a balanced, but independent action of protein kinases and phosphatases and by the phosphorylation rate of their protein substrates. The coordinated action of these enzymes represents the base of the deep modulation of protein-protein interactions, and therefore of cell differentiation, growth, functions and death [38-40].

In conclusion, due to their crucial role during human tumorigenesis and cancer progression, kinases are ideal targets for the design and development of effective and low toxic chemotherapeutics [41, 42] and nowadays, represent the second group of drug targets after G-protein-coupled receptors [41, 43].

3. PKS INHIBITORS

PKs represent ideal therapeutic targets for the development of new and effective antineoplastic chemotherapeutics, since their altered activity has been widely demonstrated in different forms of human cancer [40], and is strictly associated with uncontrolled proliferation and inhibition of apoptosis of tumor cells [44]. As a result, PKs inhibition could represent a plausible therapeutic strategy [6, 45, 46].

Nowadays, a number of compounds have been claimed to be PKs inhibitors, and some of them, such as imatinib, erlotinib and gefitinib, have already been approved for clinical use [40], whereas more than 30 others are in various phases of clinical trials [39]. Considering the high number of identified PKs inhibitors, their classification has become necessary and the most plausible mode is by taking into consideration the specific mechanism of action.

Type I inhibitors are chemically characterized by the presence of a heterocyclic system in their structure that mimics purine of ATP adenine and binds to the catalytic site in the active phosphorylated form of PKs and several side chains occupying the adjacent hydrophobic regions [47].

Among this class of inhibitors, several compounds, active at millimolar concentrations, have been already approved by the FDA, including gefitinib (2003), dasatinib (2006), sunitinib (2006), lapatinib (2007), pazopanib (2009), erlotinib (2016), crizotinib (2016), bosutinib (2017), vemurafenib (2017) and ruxolitinib (2019) [48]. A major limitation to clinical use of such drugs is represented by a low selectivity due to the high analogy of the pocket of ATP with binding sites of other proteins. For example, a recurrent side effect of these compounds includes cardiotoxicity associated with deterioration of cardiac function [49, 50]. Tofacitinib, approved by the FDA in 2018, is a highly selective inhibitor of the JAK type I family. This drug has promoted the beginning of a new era for high selective PKs inhibitors [51].

A new subclass of PKs inhibitors, known as type I½ inhibitors, has recently been described [52]. Similarly to type I inhibitors, these compounds are able to reversibly interact with the ATP binding pocket in the active conformation of the target PKs. However, they are also able to reach the deeper region of the crevice in between the lobes of the protein, allowing these compounds to interact with residues that define the type II pharmacophore model.

Type II kinase inhibitors are able to selectively interact with the catalytic site of PKs in the non-phosphorylated and inactive protein conformation [53, 54]. As a consequence of such interaction, generally mediated by an H-bond between these inhibitors and the "hinge region" of the enzyme, the inactive conformation of the PKs results is stabilized, preventing its activation [53, 55].

Generally, type II inhibitors have a high affinity and specificity compared to those of type I [47, 55, 56].

Type II inhibitors are further subdivided into two subclasses: IIA drugs able to reach the deeper region of the cleft and IIB drugs, which specifically interact with the frontal area of the slit and the gate between the protein lobes. The first PK inhibitor binding the inactive form of the protein, imatinib, was discovered by serendipity [57-59]. Several experimental evidences have confirmed that this drug is able to bind the ABL, c-Kit and PDGFR kinases in the non-phosphorylated form. Successively, several type-II PKs inhibitors were co-crystallized with their own target protein, including the complexes BIRB796-p38MAPK [59], Sorafenib-B-raf [60] and AAL993-VEGFR [61].

Sunitinib, a IIB inhibitor of Kit and VEGFR2, is a drug approved by FDA for the treatment of renal cell carcinoma (RCC), gastrointestinal stromal tumor (GIST) and progressive pancreatic neuroendocrine tumors. More recently, it has been shown that type II inhibitors can interact with more than one kinase [47], and this makes them non-selective agents; therefore, the discovery of new inhibitors with high affinity and selectivity remains an important goal of this field of research.

Many other compounds have been shown to be able to modulate the activity of PKs by acting as allosteric inhibitors. In this case, both ATP and the allosteric inhibitor can bind simultaneously to the protein.

In particular, type III inhibitors are small molecules exclusively interacting with a region close to the catalytic domain/ ATP binding site, without interacting with the hinge region of the ATP pocket. CI-1040, targeting MEK, represents the lead compound of type III PKs inhibitors [62]. The interaction of this compound with the target kinase induces a conformational change of the protein three-dimensional structure, forcing it to adopt an inactive conformation that prevents the binding of glutamate in the ATP pocket, which is essential for the catalytic activity [63]. Type IV allosteric inhibitors interact with protein regions far from the substrate binding site. Since allosteric inhibitors exploit binding sites and modulate physiological mechanisms that are exclusive for a specific kinase, they show very high selectivity towards the target enzyme [64]. GNF-2, is a type IV inhibitor able to occupy the myristoyl binding site of BCR-Abl, stabilizing the inactive form of the protein [65]. TAK-733, another allosteric PKs inhibitor, within the MEK1-ATP complex accommodates into a region close to the gate and the deeper cleft area next to the ATP pocket [66]. Trametinib is a non-competitive inhibitor of MEK1/2, recently approved for the treatment of melanoma expressing mutant BRAFV600E/K

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[67]. Further examples of allosteric inhibitors are RO0281675 and its analogues [68, 69].

Overall, it would seem that targeting PKs with allosteric inhibitors is one of the most promising approach to overcoming the main limitations of inhibitors in clinical use, including inadequate selectivity, off-target side effects and emergence of drug-resistance.

Finally, covalent kinase inhibitors (type V) establish an irreversible interaction with the target kinase [70, 71]. In general, these compounds are small molecules containing weak electrophile groups able to covalently and selectively bind a nucleophilic key cysteine residue in the catalytic site of the enzyme. The design of novel type V PKs inhibitors is based on the chemical features of the side chain of the ATP pocket. In particular, the presence of an electrophilic acceptor group in the inhibitor structure is crucial since it can establish a covalent bond with the key cysteine residue [72-77].

Neratinib (also known as HKI-272) is the lead compound of irreversible PKs inhibitors [78]. This compound covalently binds the key cysteine residue in the ATP binding site of the EGFR kinase inhibiting Herceptin-2 (HER-2), thus preventing relapses in patients with early-stage HER2-positive breast cancer [78]. CL-387785 is another example of covalent inhibitor. The major advantage of such a compound is its capability to overcome drug resistance due to a T790 M mutation of the epidermal growth factor receptor (EGFR) [79].

Several other compounds have been approved by FDA as PKs inhibitors, including afatinib, a mutant-selective EGFR inhibitor (targeting EGFR (ErbB1), ErbB2 and ErbB4) that showed a very favorable toxicity profile, approved for the treatment of NSCLC [80] and ibrutinib (also targeting mutant-EGFR kinase, but interacting with a distinct enzyme conformation), approved for the treatment of mantle cell lymphoma, chronic lymphocytic leukemia, and Waldenström's macroglobulinemia [81]. Compared to other PKs inhibitors, covalent type V ligands show several advantages, including a prolonged dissociation half-life that, together with high potency and selectivity, significantly reduces off-target side effects [73, 74].

In conclusion, considering the key role of PKs during the emergence and progression of different types of cancer and the positive results obtained especially with selective inhibitors, the discovery of novel and potent inhibitors of this family of proteins, remains one of the major goals of oncological scientific research.

4. CARBAZOLES AS SERINE/THREONINE KINASES INHIBITORS

Serine/threonine protein kinases are enzymes able to phosphorylate several proteins involved in important molecular pathways that play a key role in cancer progression. Different authors highlighted that molecules with a carbazolic core are capable of inhibiting a wide class of these kinases and among them, proto-oncogene serine/threonine-protein kinase Pim and checkpoint kinase 1 (Chk1) represent their preferential targets [82-95].

4.1. Pim Kinases Inhibitors

Pim kinases are a sub-family of CAMK (calmodulin-dependent protein kinase-related), comprising three isoforms Pim-1, Pim-2 and Pim-3. Over-expression of these proteins has been observed in different types of cancer. In particular, Pim-1 and Pim-2 are highly expressed in leukemia, lymphoma, prostate cancer and multiple myeloma [96-99], whereas Pim-3 isoform is over-expressed in solid tumors such as pancreas, colon and gastric cancers [100-102]. For these reasons, Pim kinases are deemed important biological targets for the discovery and development of novel antitumor agents. In particular, pyrrolocarbazoles represent promising Pim kinases inhibitors.

Akué-Gédu *et al.* in 2009 synthesized a series of pyrrolo[2,3-*a*]- and [3,2-*a*]carbazole derivatives [82]. They screened these molecules against 66 different protein kinases, identifying two compounds as low nM selective Pim inhibitors. The best results were obtained for two pyrrolo[2,3-*a*]carbazole: compound **1**, substituted at C-3 position with a formyl group and its analogue **2,** bearing a bromine at C-7 position. Compound **1** exhibited a good and selective inhibitory activity against Pim kinase, especially on isoform Pim-3 with a IC_{50} value of 0.01 μM.

In molecular docking studies, carried out on the crystallographic structure reported in the Protein Data Bank (PDB) as 3JPV, this inhibitor and its analogues were observed to interact with Pim-1 in a non-ATP mimetic binding mode since they are not able to form a hydrogen bond with the hinge region. In particular, compound **1** was able to interact with the target through a polar interaction between the aldehyde group and the lysine 67 residue, several aromatic interactions with the glycine rich loop residue Phe49 and several hydrophobic interaction with Leu44, Ile104 and Leu120. The planar pyridocarbazole scaffold perfectly fitted into the ATP-binding cleft, avoiding the orientation of NH groups into hinge region. Moreover, both **1** and **2** have been tested for their anti-proliferative activity toward prostate (PC3 and DU145) and ovarian (PA1) cancer cells as well as against fibroblast primary culture. Compound **2** showed the best action as cytotoxic agents, particularly against PA1 and PC3 cell lines with an IC_{50} value of 2.8 and 5.9 μM, respectively (Fig. **1**).

Successively, the same authors reported a library of pyrrolo[2,3-*a*]carbazole formylated at C-3 as new scaffolds for the preparation of Pim kinase inhibitors [83]. The derivatives differently substituted at C-6 - C-9 positions (**3-9**) showed a promising PKs inhibitory activity and, with respect to non-substituted compound **1**, they exhibited an improved antitumor effect. Particularly, analogues **3**, bearing a bromine atom at the C-6 and a hydrogen atom at C-7/C-9 positions, showed the best inhibitory profile against Pim kinases and a 17-fold increased potency toward Pim-1, compared to compound **1** (IC_{50}= 0.0068μM). This improving effect could be explained by the repulsive effect of bromine atom at the C-6 position towards the oxygen of the Glu121 carbonyl backbone as deducted after molecular docking studies on the 3JPV PDB structure of the protein. The consequence regards the positioning of ligand in the top of the binding pocket, encouraged the formation of a hydrogen bond with HOH360

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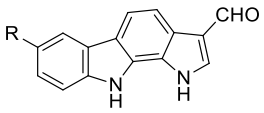
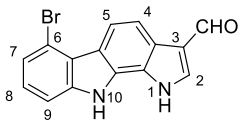


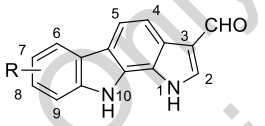
1 R= H
2 R= Br

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*			*Anti-proliferative activities* IC_{50} *(μM)*			
	Pim-1	**Pim-2**	**Pim-3**	**PC3**	**DU145**	**PA1**	**Fibroblast**
1	0.12 ± 0.01	0.51 ± 0.23	0.01 ± 0.00	9.5 ± 0.5	26 ± 2	4.5 ± 0.4	21 ± 1
2	0.57 ± 0.04	/	0.04 ± 0.01	5.9 ± 0.4	12.2 ± 0.8	2.8 ± 0.2	11 ± 1

Fig. (1). Structure and activity of pyrrolo[2,3-*a*]carbazole derivatives (**1** and **2**). IC_{50} values are the concentrations for 50% inhibition of Pim Kinases, or the drug concentration causing a 50% decrease in the cell lines population.



3



4 R8= Br
5 R9= Br
6 R9= Et
7 R8= Me
8 R9= CO_2Me
9 R9= CF_3

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*			*Anti-proliferative activities* IC_{50} *(μM)*			
	Pim-1	**Pim-2**	**Pim-3**	**PC3**	**DU145**	**PA1**	**Fibroblast**
3	0.0068 ± 0.0003	0.131 ± 0.003	0.0124 ± 0.0005	2.2 ± 0.2	1.42 ± 0.9	< 0.8	1.94 ± 0.04
4	/	/	0.09 ± 0.02	1.7 ± 0.2	1.15 ± 0.8	< 0.8	1.13 ± 0.5
5	0.031 ± 0.03	/	0.024 ± 0.04	3.3 ±0.2	2.7 ± 0.4	< 0.8	3.4 ± 0.2
6	0.066 ± 0.004	/	0.06 ± 0.03	8.6 ± 0.7	5.8 ± 0.5	1.1 ± 0.09	5.0 ± 0.3
7	/	/	0.117 ± 0.02	4.1 ± 0.4	2.5 ± 0.1	< 0.8	2.4 ± 0.2
8	0.030 ± 0.004	/	0.04 ± 0.001	6.3 ± 0.3	2.1 ± 0.1	0.89 ± 0.03	8 ± 1
9	0.018 ± 0.005	/	0.006 ± 0.02	2.99 ± 0.45	4.47 ± 0.21	1.96 ± 0.19	3.24 ± 0.32

Fig. (2). Structure and activity of pyrrolo[2,3-*a*]carbazole derivatives (**3-9**). IC_{50} values are the concentrations for 50% inhibition of Pim Kinases, or the drug concentration causing a 50% decrease in the cell lines population.

molecule which interacted itself with Glu121 backbone carbonyl. Furthermore, the bromine atom at C-6 could interfere, with a repulsive effect, with the oxygen of the Glu121 carbonyl backbone of Pim-1 kinase (Fig. **2**).

In 2012, Giraud *et al.* prepared a series of 4-substituted pyrrolo[2,3-*a*]carbazoles functionalised or not at 6-position with an amino group [84]. Both Pim kinase inhibitory and anti-proliferative activities were evaluated and compound **10**, bearing a methoxycarbonyl group at the 4-position without a formyl at position 3, was found to be the most efficient in the Pim-1 and Pim-3 inhibition (IC_{50}= 3 μM and 0.5 μM, respectively). Moreover, the results reported a good cellular growth inhibitory activity against the prostate cancer cells PC3 (IC_{50}= 6 μM) and fibroblast primary culture (IC_{50}= 8 μM). Furthermore, the corresponding 6-amino analog **11** showed an IC_{50} value of 0.41 μM toward Pim-3. In both cases, the corresponding carboxylic acids were less efficient than methyl esters. Docking experiments were performed for compound **10** using Pim-3 model. The compound interacted within Pim-3 ATP-binding site *via* two hydrogen bonds and a hydrophobic interaction between the methyl ester group and Phe51. Furthermore, the presence of the methyl ester group at position 4 in compound 10 allowed a better accommodation of the NH groups of the pyrrolocarbazole scaffold in the bottom of the protein pocket, thus making it selective for Pim-3. These results have been reached using Q86V86 structure of the protein (Fig. **3**).

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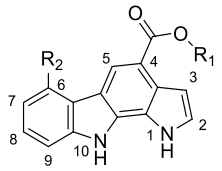
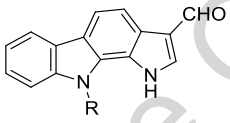


10 R_1= CH_3 R_2= H
11 R_1= CH_3 R_2= NH_2

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*			*Anti-proliferative activities* IC_{50} *(μM)*			
	Pim-1	**Pim-2**	**Pim-3**	**PC3**	**DU145**	**PA1**	**Fibroblast**
10	3 ± 1	/	0.5 ± 0.1	6 ± 4	> 50	27 ± 5	8 ± 4
11	/	/	0.41 ± 0.06	/	/	/	/

Fig. (3). Structure and activity of pyrrolo[2,3-*a*]carbazole derivatives (**10** and **11**). IC_{50} values are the concentrations for 50% inhibition of Pim Kinases, or the drug concentration causing a 50% decrease in the cell lines population.



12 R= $(CH_2)_4CN$
13 R= $(CH_2)_4NMe_2$

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*			*Anti-proliferative activities* IC_{50} *(μM)*			
	Pim-1	**Pim-2**	**Pim-3**	**PC3**	**DU145**	**PA1**	**Fibroblast**
12	0.046 ± 0.0003	/	0.041 ± 0.009	16 ± 2	20 ± 1	8 ± 2	145 ± 0.8
13	0.075 ± 0.015	/	0.08 ± 0.00	0.65 ± 0.02	0.96 ± 0.06	0.486 ± 0.003	0.63 ± 0.02

Fig. (4). Structure and activity of pyrrolo[2,3-*a*]carbazole derivatives (**12** and **13**). IC_{50} values are the concentrations for 50% inhibition of Pim Kinases, or the drug concentration causing a 50% decrease in the cell lines population.

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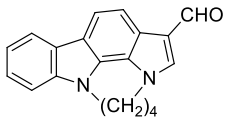
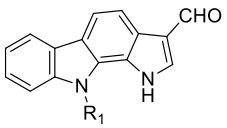
Akué-Gédu *et al.* in 2012 [85] modified the chemical structure of pyrrolo[2,3-*a*]carbazoles, adding different aminoalkylated side chains at N-10 position, with the aim to enhance the hydrophilic/hydrophobic balance. The outcome underlined that compounds **12** and **13**, functionalised with a cyanobutyl and a dimetylaminobutyl chain at N-10 position, resulted in more potent inhibitors of Pim-1 isoform compared to the not substituted analogue (**1**), with IC_{50} values of 0.046 and 0.075 μM, respectively. **12** and **13** were less active than compound **1** on Pim-3 and no activity was measured when tested against Pim-2 (Fig. **4**). The cyanobutyl analogue exhibited a similar anti-proliferative profile of compound **1** in *in vitro* assays, with IC_{50} values in the range of 8-20 μM. On the other hand, the introduction of a *N,N*-dimethylaminobutyl group increased cytotoxic effect against three human solid cancer cell lines (PA1, PC3, DU145) and fibroblast primary culture.

In 2012, Letribot *et al.* [86] studied a pyrrolo[2,3-*a*]carbazole-3-carbaldehydes substituted at N-10 position. SAR studies indicated that substitution at both nitrogen atoms in the pyrrolocarbazole moiety enhanced the inhibitor activity of compound **14** against Pim-1 isoform with respect to **1**, increasing selectivity toward Pim-2. Though the substitution at only N-10 reduced the potency, in fact, compounds **15-18** proved less active towards Pim-2, compared to compound **1,** even if the introduction of the different alkyl-1,2,3-triazole groups on the pyrrolocarbazole scaffold of these compounds still allows the Pim-1 inhibition. Furthermore, the presence of the dansyl fluorescent group in **18** was useful to demonstrate that this compound localized into the cytoplasm of the cells but not in the nuclei, making it a useful tool to distinguish the nuclear from the cytoplasmic functions of Pim (Fig. **5**).

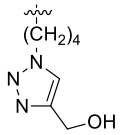
Successively, Suchaud *et al.* [87] prepared and tested new 1,6-dihydropyrazolo[4,3-*c*]carbazoles and 3,6-dihydropyrazolo [3,4-*c*]carbazoles for their Pim kinase inhibitor activity and anti-proliferative activities against two prostatic cancer cell lines (PC3 and LNCaP). In both regioisomer series, 5-nitro derivatives proved more potent inhibitors compared to the 5-amino analogues. 5-Nitro-1,6-dihydropyrazolo[4,3-*c*]carbazole **19** performed the best inhibitory potency on all the Pim kinase isoforms, mostly on isoforms 1 and 3 (IC_{50}= 0.04 and 0.10 μM, respectively) and also exhibited a noteworthy anti-proliferative activity against PC3 prostate cancer

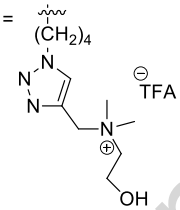


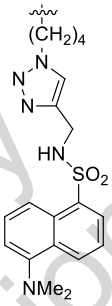
14



15 R_1= 

16 R_1= 

17 R_1= 

18 R_1= 

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*		
	Pim-1	**Pim-2**	**Pim-3**
14	0.008 ± 0.01	0.35 ± 0.01	0.013 ± 0.002
15	0.105 ± 0.001	/	0.108 ± 0.037
16	0.315 ± 0.154	/	0.235 ± 0.0023
17	0.327 ± 0.01	/	0.49 ± 0.04
18	/	/	/

Fig. (5). Structure and activity of pyrrolo[2,3-*a*]carbazole derivatives (**14**-**18**). IC_{50} values are the concentrations for 50% inhibition of Pim Kinases.

cells (IC_{50}= 3 μM). Interestingly, 9-chloro-5-nitro-3,6-dihydropyrazolo[3,4-*c*]carbazole (**20**) strongly inhibited Pim-3 with IC_{50} values of 0.09 μM, without any anti-proliferative effect on all the investigated cell lines. Molecular modeling experiments were performed for the study of the possible binding mode of compound **19** in the ATP-binding pocket of Pim-1 and Pim-3 kinases (PDB code=3JPV and 1XWS, respectively), demonstrating a similar binding mode with both protein isoforms (Fig. **6**). In particular, the indazole group perfectly fitted into the ATP binding cleft while the pyrazole ring accommodated near the hinge region. The complex was stabilized by the formation of a hydrogen bond between the hydrogen of N-1 and the carbonyl oxygen of Glu121 or Glu124 in Pim-1 and Pim-3, respectively.

4.2. Checkpoint kinase 1 inhibitors

Other interesting targets for anticancer drug discovery are different PKs involved in the cell cycle regulation. In particular, Chk1 kinases play a significant role in G2 checkpoint modulating G2-M transition in response to DNA damage, in order to repair it [103-105]. Several literature studies suggest that molecules with carbazolic moiety have the ability to interfere with Chk1 kinases inducing cell cycle arrest and selective cancer cell death, making them promising tools for targeted therapy.

Henon *et al.* in 2006 [88] synthesized a series of di-pyrrolo[3,4-*a*:3,4-*c*]carbazole-1,3,4,6-tetraones with saturated and unsaturated side chains at 10-position, as new analogues of Granulatimide (**21**). This latter is a natural product isolated from an ascidian and is a Chk1 inhibitor with an IC_{50}value of 2 μM [106]. The ChK1 inhibitory capability of the new library was evaluated. Data highlighted that the best inhibitory efficiency was provided by compound **22**, characterized by the presence of a methyl group in C-10 position in its chemical structure (IC_{50}= 0.008 μM). When this substituent was replaced by an ethyl group, the inhibitory activity was drastically reduced. The cytotoxicity profile was evaluated against four tumor cell lines: a murine leukemia L1210, and four human tumor cell lines (DU145 prostate carcinoma, A549 non-small cell lung carcinoma, and HCT116 and HT29 colon carcinoma cells). According to the Chk1 inhibitory activity, compound **22** exhibited the most promising cytotoxicity profile toward all the tested cell lines (IC_{50}= 1.8, 2.2, and 3.29 μM against L1210, HCT116 and HT29, respectively).

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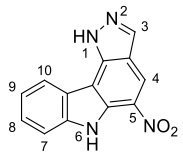
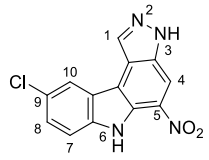


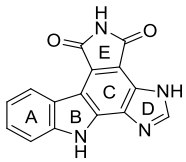
19



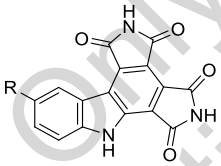
20

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*			*Anti-proliferative activities* IC_{50} *(μM)*	
	Pim-1	**Pim-2**	**Pim-3**	**PC3**	**LNCaP**
19	0.04 ± 0.03	/	0.10 ± 0.06	3 ± 1	/
20	/	/	0.09 ± 0.05	/	/

Fig. (6). Structure and mechanism of action of pyrazolo[3,4-*c*] and [4,3-*c*]carbazole derivatives (**19** and **20**). IC_{50} values are the concentrations for 50% inhibition of Pim Kinases, or the drug concentration causing a 50% decrease in the cell lines population.



Granulatimide (**21**)



22 R= CH_3
23 R= CH=CHPh

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*	*Anti-proliferative activities* IC_{50} *(μM)*				
	Chk-1	**L1210**	**DU145**	**A549**	**HCT116**	**HT29**
21	> 2.54	2.8	2.8	11.4	6.1	5.7
22	0.008	1.8	/	/	2.2	3.29
23	/	2.2	/	/	1.7	4.7

Fig. (7). Structure and activity of Granulatimide (**21**) and pyrrolo[3,4-*a*:3,4-*c*]carbazole derivatives (**22** and **23**). IC_{50} values are the concentrations for 50% inhibition of Chk-1 Kinase, or the drug concentration causing a 50% decrease in the cell lines population.

Moreover, compound **23**, bearing a styryl substituent at 10 position, showed a promising cancer cell growth inhibition (IC_{50}= 2.3, 1.7 and 4.7 μM against L1210, HCT116 and HT29, respectively), even if with a lower Chk1 inhibitory percentage (Fig. **7**).

In 2007, Henon *et al.* [89] synthesized a series of bis-imides carbazole analogues, analogs of Granulatimide, in which imidazole moiety was replaced by an imide heterocycle, (**21**). Several compounds substituted on one or both imide heterocycles, on the benzene ring and on the nitrogen of the indolic nucleus have been described. The indole moiety was also replaced by a 7-azaindole obtaining aza-bis-imide analogues leading to not active compounds (**24** and **25**). Analogues that had a substitution as regards the indole moiety demonstrated a powerful inhibitory activity toward Chk1, except for those with $–CH_3$ or -OBn at 8 position. The best Chk1 inhibitory activity was measured for compound **26** functionalized with a hydroxyl group at 10 position (IC_{50}= 0.002 μM).

Docking studies showed a similar binding mode of this last molecule and the reference compound Ganulatimide, including a dynamical hydrogen-bonded network that fine-tuning the function of the protein (PDB code= 1NVR). In particular, the most crucial hydrogen bonds for kinase activity are those that are established between the NH group of indole moiety and the COO^- of Glu91; the carbonyl group and the imide NH of ring E with Cys87 and Glu85 residues, respectively; one nitrogen atom of ring D with the NH_2 group of Lys132 and the COO^- of Glu91 *via* the intermediate of a water molecule. Furthermore, four more hydrogen bonds are formed *via* a water molecule between the C=O of the E heterocycle with NH of Asp148, the imide NH of ring D with the COO^- of Asp148, the second C=O with NH of Lys132 and COO^- of Glu91. The stronger Chk1 inhibitory activity of 26 could be due to the presence of a further hydrogen bond between the OH at position 10 and the carboxylic group of Cys87.

Additionally, the research team tried to synthesize bis-imide analogues functionalized with a sugar moiety, in order to increase the solubility by forming supplementary hydrogen bonds in the ATP binding pocket [107]. The anti-proliferative effect of these new compounds was investigated toward five tumor cell lines: murine L1210, human DU145,

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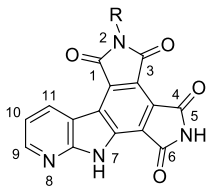
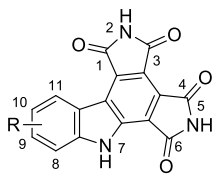


24 R= H
25 R= CH_3



26 R10= OH
27 R9= Cl
28 R9= Br

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*	*Anti-proliferative activities* IC_{50} *(μM)*				
	Chk-1	**L1210**	**DU145**	**A549**	**HCT116**	**HT29**
24	5.0	17.9	/	/	7.0	10.6
25	/	21.7	12.2	/	/	9.0
26	0.002	54.7	71.9	62.9	/	/
27	0.0345	0.9	/	/	1.5	9.8
28	0.011	0.7	3.06	/	/	3.1

Fig. (8). Structure and activity of bis-imide Granulatimide analogues (**24-28**). IC_{50} values are the concentrations for 50% inhibition of Chk-1 Kinase, or the drug concentration causing a 50% decrease in the cell lines population.

A549, HCT116 and HT29. The best results were obtained for compounds **27** and **28,** bearing a halogen atom in 9-position (9-Cl and 9-Br, respectively), especially on murine leukemia L1210 cells (IC_{50}= 0.9 μM and 0.7 μM, respectively). The other compounds did not show good efficacy (Fig. **8**).

Other Granulatimide analogues were prepared by Conchon *et al.* in 2006 [95]. The research group modified the imidazole moiety with a maleimide and they added a lactam function as well, as in staurosporine (indolocarbazole isolated from cultures of Streptomyces). The dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,4,6-triones and dipyrrolo[3,4-*a*:3,4-*c*]carbazole-3,4,6-triones synthesized were also substituted at 10-position. The results revealed that compound **29**, bearing a hydroxyl group at 10-position, was the most active as Chk1 inhibitor (IC_{50}=0.01 μM). Likewise, compound **30**, that did not bear any substitution at this position, showed a good activity with an IC_{50}value of 0.05 μM. However, the anti-proliferative tests toward murine leukemic L1210 and human colon carcinoma HT29 and HCT116 cells did not give the expected results, maybe due to possible instability of these compounds in the biological medium (IC_{50}> 25 μM) (Fig. **9**).

The same research group successively synthesized a further series of pyrrolo[3,4-*c*]-carbazoles [90]. The best Chk1 inhibitors of the 20 newly synthesized compounds were **31** and **32**, bearing a hydroxyl group at 11 position (IC_{50} = 0.027 μM and 0.023 μM, respectively). Docking studies on 1NVR structure pointed out the importance of the 11-OH group that establishes a hydrogen bond with the carbonyl of Cys87. *In vitro* anti-proliferative assays toward L1210 (murine leukemia cells), HT29 and HCT116 (human colon carcinoma cells) highlighted that there was no close relationship between Chk1 inhibitory activities and cytotoxicity. In fact, compound **31** did not show any cytotoxicity against all the tested cell lines. On the other hand, compounds **32**, **33**, **34**, **35** and **36** exhibited a good anti-proliferative effect in an IC_{50} range of 0.6 - 26.4 μM (Fig. **10**).

In order to evaluate the importance of D or E heterocycles on carbazolic nucleus of Granulatimide (**21**), Conchon *et al.* in 2008 [91] synthesized several substituted pyrrolo [3,4-*a*]carbazole-1,3-diones (**37-40**), in which the imidazole ring E was removed, leading to pyrrolo[3,4-*c*]carbazole-1,3-diones (**41** and **42**). Furthermore, they also prepared 2-aminopyridazino[3,4-*a*]pyrrolo[3,4-*c*]carbazole-1,3,4,7-tetraone (**43**) with 6-membered pyridazine dione ring replacing the D heterocycle of the reference compound, however, the low solubility of this compounds did not allow the following biological evaluation. Data reported that E ring was necessary for Chk1 inhibitory activity: in fact, compounds **37-40** did not show any inhibitory effect. Instead, D ring resulted not essential, in fact, compound **41**, in which D ring was missing, proved to be the best Chk1 inhibitor. Molecular modelling studies suggested that these compounds are able to interact with the active site of Chk1. Several hydrogen bonds are involved in the stabilization of the ligand-protein complex. The most crucial are those formed between the CH_2OH group of compound **41** or the imide moiety of compound **42** with the C=O of Glu85, together with the one formed between the carbonyl group of E ring and the NH of Cys87. *I In vitro* studies focusing on the evaluation of anti-proliferative profile of such class of compounds demonstrated that compound **41** displayed the higher antitumor activity against L1210 murine leukemic cells, HT29 and HCT116 human colon carcinoma cells (IC_{50}= 5.5, 3.9 and 2.9 μM, respectively). On the other hand, compounds **40** and **42**, both inactive against Chk1, were also able to inhibit cellular growth in the same micromolar range of compound **41** (Fig. **11**).

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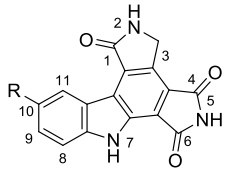
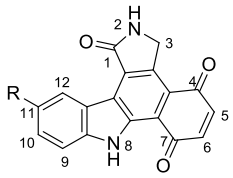


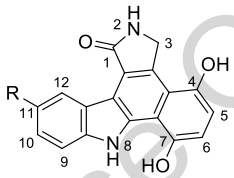
29 R= OH
30 R= H

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*	*Anti-proliferative activities* IC_{50} *(μM)*		
	Chk-1	**L1210**	**HCT116**	**HT29**
29	0.01	59.5	63.5	41.8
30	0.05	/	/	/

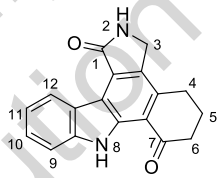
Fig. (9). Structure and mechanism of action of pyrrolo[3,4-*a*:3,4-*c*]carbazole derivatives (**29** and **30**). IC_{50} values are the concentrations for 50% inhibition of Chk-1 Kinase, or the drug concentration causing a 50% decrease in the cell lines population.



31 R= OH
33 R= CH_3



32 R= OH
34 R= H
35 R= CH_3



36

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*	*Anti-proliferative activities* IC_{50} *(μM)*		
	Chk-1	**L1210**	**HCT116**	**HT29**
31	0.027	8.4	15.4	50.6
32	0.023	1.4	2.7	26.4
33	0.107	0.75	0.70	20.6
34	0.311	1.1	3.1	5.6
35	0.161	0.6	1.6	3.8
36	/	3.7	1.1	15.5

Fig. (10). Structure and activity of pyrrolo[3,4-*c*]carbazole derivatives (**31**-**36**). IC_{50} values are the concentrations for 50% inhibition of Chk-1 Kinase, or the drug concentration causing a 50% decrease in the cell lines population.

Successively, the same research group [92] prepared a pyrrolo[3,4-*c*]carbazole (**44**) in which the imidazole ring was replaced with a five-membered lactam group. They also synthesized one analogue (**45**) with a sugar portion connected to the indole nitrogen and to D ring. Compound **44** possessed the higher Chk1 inhibitory activity with IC_{50} values of 0.024 μM. Docking studies showed the capability of compound **44** to interact through several hydrogen bonds with the binding pocket of the kinase (PDB code= 1NVR). As previously described for compounds **21**-**28** and **41**-**42,** the most determinant H-bonds in the ligand-protein complex were those formed between the NH group of indole ring with the COO^- of Glu91, the carbonyl group of ring E with Cys87 and the imide group with Glu85. Compound **44** was able to interact with the ATP-binding site of Chk1 by three further hydrogen bonds *via* a water molecule with Asp148, Lys132 and Glu91. The anti-proliferative evaluation was performed toward L1210 murine leukemic cells, HT29 and HCT116 human colon carcinoma cells. Results underlined that compound **44** exhibited a selective effect upon HT29 human colon carcinoma cells. Concerning the introduction of sugar unit, compound **45** also significantly inhibited Chk1 (IC_{50}= 1.92μM) without an important effect on cell lines. The presence of this sugar portion ensures the formation of two extra hydrogen bonds with Leu15 and Glu91 (Fig. **12**).

From literature studies, Smaill *et al*. in 2008 [93] concluded that 4-phenylpyrrolocarbazole **46** and its analogue **47,** with 20-Cl substituent on the 4-phenyl ring and a polar N-6 side chain, were powerful inhibitors of Wee1 and Chk1, although their poor solubility represented a limitation for successive biological experimentation [108]. In order to overcome

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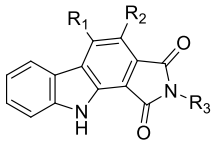
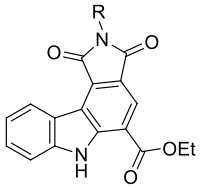
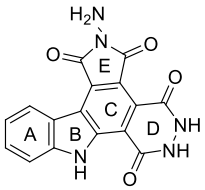


37 R_1= OH; R_2= H; R_3= H
38 R_1= OH; R_2= CO_2Et; R_3=CH_2OH
39 R_1= OH; R_2= CO_2Et; R_3= H
40 R_1= CO_2Et; R_2= H; R_3= H



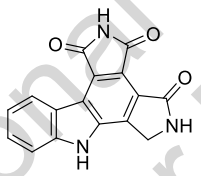
41 R= CH_2OH
42 R= H



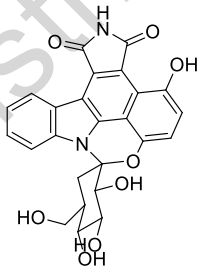
43

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*	*Anti-proliferative activities* IC_{50} *(μM)*		
	Chk-1	**L1210**	**HCT116**	**HT29**
37	/	54.4	34.4	46.7
38	/	10.6	11.5	31.7
39	/	57.5	33.6	84.9
40	/	4.9	3.8	6.4
41	0.21	5.5	2.9	3.9
42	> 5	5.8	2.3	4
43	Not tested for its low solubility			

Fig. (11). Structure and activity of pyrrolo[3,4-*a*] and [3,4-*c*]carbazole derivatives (**37**-**40** and **41**,**42**, respectively) and pyridazino[3,4-*a*]pyrrolo[3,4-*c*]carbazole derivative (**43**). IC_{50} values are the concentrations for 50% inhibition of Chk-1 Kinase, or the drug concentration causing a 50% decrease in the cell lines population.



44



45

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*	*Anti-proliferative activities* IC_{50} *(μM)*		
	Chk-1	**L1210**	**HCT116**	**HT29**
44	0.024	> 50	8.54	0.58
45	1.92	21.2	46.4	57.8

Fig. (12). Structure and mechanism of action of dipyrrolo[3,4-*a*:3,4-*c*]carbazole derivative (**44**) and pyrrolo[3,4-*c*]carbazole derivative with a sugar moiety (**45**). IC_{50} values are the concentrations for 50% inhibition of Chk-1 Kinase, or the drug concentration causing a 50% decrease in the cell lines population.

this drawback, the aim of their work was to investigate the activity of derivatives containing a hydrogen or a ($Me/(CH_2)_2OH$) side chain at N-6 and different hydrophilic side chains at C-8 position of ring A. Comparing the results for Wee1 and Chk1 inhibitory activity, it appeared that the best Chk1 inhibitors were the pyrrolidine analogues **48**, **49** and **50** (Chk1 IC_{50}= 0.004, 0.014 and 0.022 μM, respectively). The main characteristic of these compounds was strong basicity. Nevertheless, the same molecules were less active against Wee1 (IC_{50}= 0.036, 0.024 and 0.050 μM), whereas **51**, **52** and **53** resulted in good Wee1 inhibitor with a lower Chk1 potency (Wee1 IC_{50}= 0.015, 0.018, 0.050 μM and 0.290, 0.134, 0.374 μM against Wee1 and Chk1, respectively). Some of these compounds were tested in different cellular assays: compound **54** was observed to be able to interfere with G2/M checkpoint, increasing the cytotoxic effect of cisplatin on p53-negative cells (Fig. **13**).

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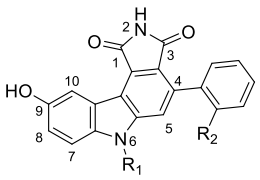
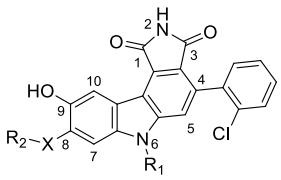


46 R_1= R_2= H
47 R_1= $(CH_2)_3OH$ R_2= Cl



48 R_1= CH_3 X= $(CH_2)_4$ R_2= Npyrrol
49 R_1= $(CH_2)_2OH$ X= $(CH_2)_4$ R_2= Npyrrol
50 R_1= H X= $(CH_2)_4$ R_2= Npyrrol
51 R_1= $(CH_2)_2OH$ X= $O(CH_2)_3$ R_2= Nmorph
52 R_1= $(CH_2)_2OH$ X= $O(CH_2)_3$ R_2= NMe_2
53 R_1= CH_3 X= $O(CH_2)_3$ R_2= Nmorph
54 R_1= CH_3 X= $O(CH_2)_3$ R_2= NMe_2

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*	
	Chk-1	**Wee1**
46	0.047	0.097
47	0.170	0.009
48	0.004	0.036
49	0.014	0.024
50	0.022	0.050
51	0.290	0.015
52	0.134	0.018
53	0.374	0.057
54	0.024	0.058

Fig. (13). Structure and mechanism of action of 4-(2-substituted-phenyl)pyrrolocarbazoles (**46** and **47**), C-8 and *N*-6-substituted 4-(2-chlorophenyl)pyrrolocarbazoles (**48**-**54**). IC_{50} values are the concentrations for 50% inhibition of Chk-1 and Weel Kinases.

Lefoix *et al.* in 2008 [94] designed and synthesized a small series of 5-azaindolocarbazoles Arcyriaflavin-A (**55**) analogues variously substituted at 9 and 10-positions and on the maleimide moiety (compounds **56-60, Fig. 14**).The anti-cancer profile of these molecules has been evaluated against two cancer cell lines (murine leukemia L1210 and human colon carcinoma HT29 cells). Compounds **56** and **57** resulted in the most cytotoxic compound, in particular against L1210 cells (IC_{50}= 0.195 and 0.495 μM, respectively). SAR studies on these compounds indicated that the presence of both a 9,10-methylendioxy group and a different substituent on the maleimide group (a methyl for **57** and a *N,N*-dimethylaminoethyl for **56**) is crucial for the activity of such a class of derivatives. The anti-proliferative activity was not related to Chk1 inhibition. In fact, **56** and **57** were not active as Chk1 inhibitors. The functionalization with a 10-benzyloxy group (**58**) or a hydroxylic group at 9 or 10 position (**59** and **60**) increased the Chk1 inhibition of these derivatives. In fact, compounds **58-60** exhibited the best inhibitory activity against Chk1 with IC_{50} values of 0.014, 0.027 and 0.072 μM, respectively (Fig. **14**).

5. CARBAZOLES AS TYROSINE KINASES INHIBITORS

Several literature studies reported that molecules with a carbazolic moiety are able to inhibit different TKs [109-114]. Gingrich D.E. *et al.* in 2003 [109] synthesized a series of 9-alkoxymethyl-12-(3-hydroxypropyl)indeno[2,1-*a*]pyrrolo [3,4-*c*]carbazole-5-ones (**61**). Through SAR studies, they highlighted that substitution at positions 9 and 12, with an alkoxy or a hydroxypropyl group~~s~~ respectively, was necessary for their activity. Compound **62**, named by authors as CEP-5214, was the most interesting and the most studied derivative. In particular, this carbazole was recognized as a potent, low-nanomolar inhibitor of human VEGF-R tyrosine kinases. In fact, it showed IC_{50} values of 16, 8 and 4 nM against VEGF-R1/FLT-1, VEGF-R2/KDR and VEGF-R3/FLT-4, respectively and a high selectivity against several tyrosine and serine/threonine kinases as PKC, Tie2, TrkA, CDK1, p38, JNK and IRK. The capability of compound **62** to interact with VEGF-R2/KDR tyrosine kinase was confirmed by molecular docking studies using the structure 1VR2 available on the PDB. Indeed, it establishes Van der Waals interactions with Val848, Leu1035, Cys1045 and Leu840. The ester derivative **63** (named CEP-7055) was designed in order to increase water solubility and oral bioavailability of CEP-5214 and has been used as a prodrug in phase 1 clinical trials. CEP-5214 exhibited *in vivo* anti-proliferative activity in several tumor models (eg in SVR murine angiosarcoma xenograft model) after oral administration of CEP-7055 ester (Fig. **15**).

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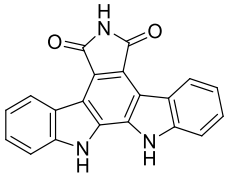
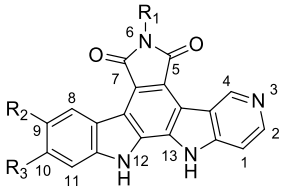


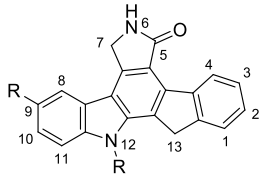
Arcyriaflavin-A (55)



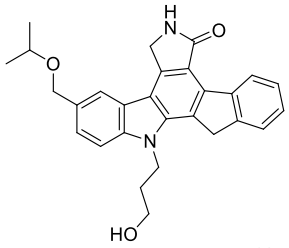
56 R_1= $(CH_2)_2N(CH_3)_2$ R_2, R_3= $-OCH_2O-$
57 R_1= CH_3 R_2, R_3= $-OCH_2O-$
58 R_1= H R_2= H R_3= OBn
59 R_1= H R_2= OH R_3= H
60 R_1= H R_2= H R_3= OH

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*	*Anti-proliferative activities* IC_{50} *(μM)*	
	Chk-1	**L1210**	**HT29**
56	/	0.195	1.76
57	/	0.495	3.345
58	0.025	1.1	32.4
59	0.027	80.7	> 100
60	0.014	3.3	> 100

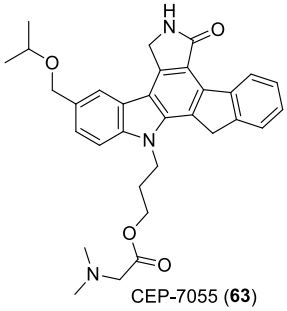
Fig. (14). Structure and activity of Arcyriaflavin-A (**55**) and pyrido[3',4':4,5]pyrrolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole derivatives (**56-60**). IC_{50} values are the concentrations for 50% inhibition of Chk-1 Kinase, or the drug concentration causing a 50% decrease in the cell lines population.



61



CEP-5214 (**62**)



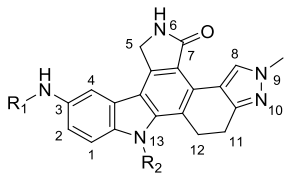
CEP-7055 (**63**)

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*							
	VEGF-R1	**VEGF-R2**	**VEGF-R3**	**Flt3**	**FGF-R1**	**PDGF-R*β***	**Kit**	**JNK1*β1***
62	0.016	0.008	0.004	0.0016	0.162	0.406	0.057	0.448
63	ND	0.018	ND	ND	ND	ND	ND	ND

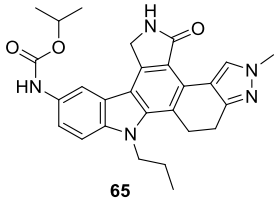
Fig. (15). Structure of 9-alkoxymethyl-12-(3-hydroxypropyl)indeno[2,1-*a*]pyrrolo[3,4-*c*]carbazole-5-ones(**61-63**) and relative inhibitory activity against a panel of Tyrosine Kinases.

Becknell N.C. *et al.* in 2006 [110] described a series of C-3 urea, amide, and carbamate fused dihydroindazolocarbazole derivatives (**64**) as dual inhibitors of TIE-2 and VEGF-R2 receptor tyrosine kinases. SAR revealed that the substitution of N-13 with *n*-propyl or *i*-butyl represented a good strategy to improve the activity. In fact, derivative **65** demonstrated strong inhibition of VEGF-R2 (IC_{50}= 5 nM) and of TIE-2 (IC_{50}= 11 nM) and then showed interesting pharmacokinetic (PK) properties in the rat with high oral bioavailability (44%). Compound **65** was also analyzed for dose-related oral antitumor capability against murine SVR angiosarcomas in nude mice: at oral doses of 0.3 mg/kg and 1.0 mg/kg *bis in die* from day 4 until day 10 of the study, showing a maximum inhibition of 62%. Anyways, at higher doses, toxicity was observed for **65** (Fig. **16**).

As reported by Gingrich D.E. *et al.* in 2003 [109], potent VEGF-R2 and moderate TIE-2 inhibitory activity of dihydroindazolocarbazole (DHI-carbazole) CEP-7055 (**63**) was due to two chemical features of the molecule: the indole N-13 and the aromatic C-3 positions.



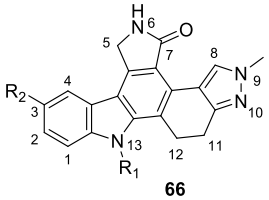
64



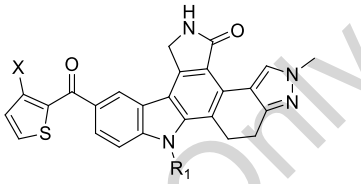
65

Compound	*Kinases inhibitory potencies IC_{50} (μM)*	
	TIE-2	**VEGF-R2**
65	0.011	0.005

Fig. (16). General structure of amino dihydroindazolocarbazole derivatives (**64**). Structure and Kinases inhibitory potencies of **65** against TIE-2 and VEGF-R2 Kinases. IC_{50} values are the concentrations for 50% enzymatic inhibition.



66



67 R_1= Et X=H
68 R_1= Pr X=H
69 R_1= *i*-Pr X=H
70 R_1= *i*-Bu X=H
71 R_1= *i*-Bu X=Cl

Compounds	*Kinases inhibitory potencies IC_{50} (μM)*	
	TIE-2	**VEGF-R2**
67	0.043	0.001
68	0.026	0.005
69	0.024	0.015
70	0.020	0.006
71	0.013	0.007

Fig. (17). General structure of dihydroindazolo[5,4-*a*]pyrrolo[3,4-*c*]carbazole derivatives (**66**). Structure and Kinases inhibitory potencies of compounds **67-71** against TIE-2 and VEGF-R2 Kinases. IC_{50} values are the concentrations for 50% enzymatic inhibition.

Because of this evidence, Underiner T. L. et al in 2008 [111], studied the TIE-2/VEGF-R2 SAR and *in vitro* activity of pyrrolodihydroindazolocarbazoles **66** functionalized at N-13 and C-3 positions. They confirmed that VEGF-R2 inhibitory activity was maintained for a variety of R-13 alkyl substituents and TIE-2 inhibitory activity increased with the length of the R-13 alkyl chain. Particularly, Pr and *i*-Bu groups proved to be the most favorable.

The derivatives substituted with 2-thiophenecarbonyl in R-3 (**67**-**70**) showed a potent dual of TIE-2 and VEGF-R2 kinases with VEGF-R2 cellular IC_{50} values between 10 and 50 nM. Therefore, the best dual TIE-2/VEGF-R2 inhibitor was the thiophene DHI-carbazole ketone (**70**) (TIE-2 IC_{50}= 20 nM; VEGFR2 IC_{50}= 6 nM). Molecular docking studies on this protein (PDB code = 1VR2) showed that lactam moiety of compound **70** perfectly fit into the VEGFR2 ATP pocket, and the stability of the complex was assured by the formation of hydrogen bonds between the NH of lactam moiety and the carbonyl group of Glu917 and between the lactam carbonyl group and the amide of Cys 919. Moreover, portion C3-thiophenecarbonyl established hydrogen interactions with Asp1046 and Lys868. Compound **70** was also able to interact with TIE-2 through hydrogen bonds of the NH and C=O of the lactam nucleo with the carbonyl of Glu903 and the amide of Ala905, respectively. Similar results were obtained for the halogen derivative **71** (TIE-2 IC_{50}= 13 nM; VEGFR2 IC_{50}= 7 nM). The same authors tested the *ex vivo* anti-angiogenic activity and the inhibition of microvessel growth of thiophene-DHI-carbazole ketones **70** and **71** in a rat aortic ring explant model. Compounds **70** and **71** significantly inhibited the rise of microvessels in a dose-dependent manner with estimated EC_{50} values around 20 nM (Fig. **17**).

Dandu R. *et al.* in 2008 [112] identified a series of dihydroindazolo[5,4-*a*]pyrrolo[3,4-*c*]carbazole oximes **72** as potent dual TIE-2/VEGF-R2 receptor kinase inhibitors. SAR studies pointed out that the ether group at C-8 and alkyl substitutions at N-11 are essential for selective dual TIE-2/VEGF-R2 inhibitors efficacy. Derivatives of **73** and **74** showed promising PK inhibitory activity and a favorable pharmacokinetic profile in *in vivo* assay on rats. Compound **73** was able to strongly inhibit VEGF-R1 and VEGF-R3 with IC_{50} values of 8 and 10 nM, respectively. In addition, **73** displayed potent dual enzyme and cellular activity, good rat pharmacokinetic properties, *in vitro* and oral *in vivo* anti-angiogenic activity and *in vitro* anti-proliferative efficiency against human tumor xenograft melanoma A375 cells (EC_{50}= 70 nM) (Fig. **18**).

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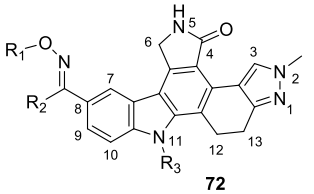
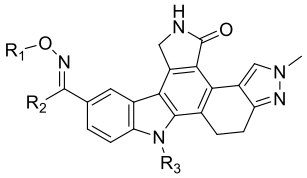


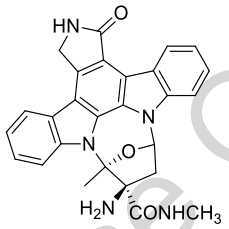
72



73 R_1= Me R_2= CH_3 R_3= *i*-Bu
74 R_1= Et R_2= CH_3 R_3= *i*-Pr

Compounds	*Kinases inhibitory potencies* IC_{50} *(μM)*	
	TIE-2	**VEGF-R2**
73	0.030	0.007
74	0.026	0.004

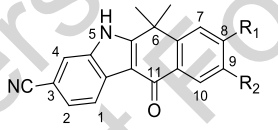
Fig. (18). General structure of dihydroindazolo[5,4-*a*]pyrrolo[3,4-*c*]carbazole oximes (**72**). Structure and Kinases inhibitory potencies of compounds **73** and **74** against TIE-2 and VEGF-R2 Kinases. IC_{50} values are the concentrations for 50% enzymatic inhibition.



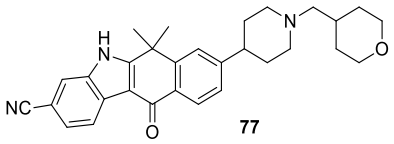
TTT-3002 (**75**)

Compounds	*Inhibition FLT3 autophosphorylation* IC_{50} *(pM)*	*Anti-proliferative activities* IC_{50} *(pM)*
		FLT3/ITD
75	100-250	490-920

Fig. (19). Structure and activity of TTT-3002 (**75**). IC_{50} values are the concentrations for 50% inhibition of FLT3 autophosphorylation, or the drug concentration causing a 50% decrease in the FLT3/ITD cell line population.



76



77

Cpds	*Kinases inhibitory potencies* IC_{50} *(μM)*		*Anti-proliferative activities* IC_{50} *(μM)*
	Wild-type ALK	**Gatekeeper-ALK**	**H3122 (ALK-driven cells)**
77	0.0034 ± 0.9	0.0039 ± 1	0.0179 ± 2.6

Fig. (20). General structure of benzo[*b*]carbazolone derivatives (**76**). Structure and Kinases inhibitory potencies of compound **77** against ALK Kinases. IC_{50} values are the concentrations for 50% enzymatic inhibition or the drug concentration causing a 50% decrease in the H3122 cell line population.

Ma H. *et al.* in 2014 [113] reported a tyrosine kinase inhibitor, TTT-3002 (**75**), as one of the most promising FMS-like tyrosine kinase-3 (FLT3) inhibitors. The IC_{50} values for **75** upon human FLT3/internal tandem duplication (ITD) mutant leukemia models were found to be in the range between 490 and 920 pM. Furthermore, **75** also displayed a potent activity toward the most commonly FLT3-activating point mutation, FLT3/D835Y. The obtained results were *in vivo* confirmed, employing a mouse model of FLT3-associated acute myeloid leukemia. Compound **75** was cytotoxic against leukemic blasts isolated from FLT3/ITD-expressing acute myeloid leukemia patients, exhibiting minimal toxicity to normal hematopoietic stem/progenitor cells from healthy blood and bone marrow donors (Fig. **19**).

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Jiang X. *et al.* in 2015 [114] developed a different a series of benzo[*b*]carbazolone derivatives and compound **76** was selected as a lead compound. SAR studies indicated its derivative **77**, with a 1-((tetrahydropyran-4-yl)methyl) piperidin-4-yl functionalization at C-8, showed a similar good activity toward both wild-type and gatekeeper mutant anaplastic lymphoma kinase (ALK), with IC_{50} values of 3.4 and 3.9 nM, respectively. **77** exhibited an encouraging PK profile in rats with a high volume of distribution and oral bioavailability (67.1%) (Fig. **20**). Finally, it significantly inhibited ALK driven growth of cancer cells and KARPAS-299 xenograft model, with a better performance compared to Crizotinib, an approved first-generation ALK inhibitor.

CONCLUSION

Today, a lot of resources are being invested in the prevention, diagnosis and treatment of cancer that represent the second leading cause of deaths, causing more than 9.6 million deaths in 2018, worldwide [115]. For these reasons, the discovery and development of novel and effective anticancer agents are a key focus of several studies. Conventional anticancer drugs are cytotoxic agents, that present significant cytostatic or cytotoxic activity on tumor cell lines and induce tumor regression *in vivo*. Although this strategy has achieved significant successes, recent developments in molecular biology and detailed studies of the molecular pathways, involved in tumorigenesis, have challenged researchers to design target-based drugs, able to inhibit or modify selected molecular markers involved in cancer prognosis, growth and metastasis. Several target-based compounds have emerged in recent years and among them, kinase inhibitors play a crucial role in cancer treatment [116]. PKs are a family of enzymes able to modulate phosphorylation signaling networks that are critically involved in several aspects of cellular functions as well as in cancer emergence and development. The abnormal activation of protein phosphorylation is frequently associated with cancer development and progression [117]. In several types of cancer, these enzymes are over-expressed/over-activated and involved in various cell functions (signaling, growth and division), thus their inhibition should prevent the proliferation of cancer cells [118]. Currently, more than 25 PKs inhibitors have been approved as anticancer drugs and several other compounds are in clinical evaluation [34].

In this survey, we reported an overview of small molecules, either of natural or synthetic origin, based on a carbazole scaffold and claimed as PKs inhibitors [87, 92, 114, 119-121]. PKs are a heterogeneous family of enzymes that although, differing in terms of structure, cellular localization and activation modalities, share a highly conserved catalytic site. Due to their several functions, numerous studies have focused on the identification of compounds capable of modulating their activity and, consequently, there are plenty of compounds described in the literature as PKs ligands. For this reason, although there is not a fixed distinction, in this work, we have tried to classify them according to their specific targeted kinase, focusing our attention on carbazole based compounds. These latter have been grouped into three main classes: inhibitors of Pim kinases (a sub-family of calmodulin-dependent PKs), Checkpoint kinase 1, and Tyrosine kinases. The activity of the described compounds, evaluated in dedicated biological assays, was mostly confirmed by computational studies that demonstrated the ability of the various molecules to interact, in a more or less selective manner, with the key amino acid residues for the biological activity of the specific PKs.

Pim kinases inhibitors, are in general, tetracyclic compounds obtained by the addition of a further pentacyclic on the carbazole scaffold and some of them even showed selectivity towards the three subclasses of Pim proteins. Most of the Checkpoint kinase 1 inibitors are instead, derivative of the natural compound Ganulatimide, containing a bis**-**imides moiety. More heterogeneous is the class of tyrosine kinase inhibitors, which include variously substituted tetra or pentacyclic compounds. Most of the compounds described showed, besides a PKs inhibitory activity at doses in the low micromolar range, a significant anti-proliferative activity in several *in vitro* and *in vivo* human cancer models, thus representing attractive lead compounds for the development of novel antineoplastic drugs. Furthermore, the favorable pharmacokinetic and the good oral bioavailability of several of the described carbazoles confirm their potential for future clinical use.

Finally, since one of the main objectives of pharmaceutical chemists remains the identification and development of drugs without long-term toxicity and off-target effects, taking into consideration the whole data so far obtained, carbazole based PKs inhibitors could represent interesting tools for a rational design of alternative, safe and efficient anticancer agents.

LIST OF ABBREVIATIONS

% F	=	Oral Exposure
A375	=	Human Malignant Melanoma
A549	=	Adenocarcinomic Human Alveolar Basal Epithelial Cells
AAL993	=	2-((4-Pyridyl)methyl)amino-N-(3-(trifluoromethyl)phenyl)benzamide
AGC	=	Protein Kinase K A, G and C
ALK	=	Anaplastic Lymphoma Kinase
ATP	=	Adenosine Triphosphate
Bcr-Abl	=	Breakpoint Cluster Region Protein-Abelson Murine Leukemia Viral Oncogene Homolog
BIRB796	=	Doramapimod
B-raf	=	Serine/Threonine-Protein Kinase B-Raf
BRAF	=	v-Raf Murine Sarcoma Viral Oncogene Homolog B
CAMK	=	Calmodulin/Calcium Regulated Kinases
CDK	=	Cyclin-Dependent Kinase
CEP-5214	=	3-(5,6,7,13-Tetrahydro-9-((1-methylethoxy)methyl)-5-oxo-12H-

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		indeno(2,1-a)pyrrolo(3,4-c)carbazol-12-yl)propanol
CEP-7055	=	N,N-dimethyl-, 3-[5,6,7,13-tetrahydro-9-[(1-methylethoxy)methyl]-5-oxo-12H-indeno[2,1-a]pyrrolo[3,4-c]carbazol-12-yl]propyl ester
Chk1	=	Checkpoint Kinase 1
CI-1040	=	2-[(2-Chloro-4-iodophenyl)amino]-N-(cyclopropylmethoxy)-3,4-difluorobenzene-1-carboximidic acid
CK1	=	Casein Kinase 1
c-Kit	=	Tyrosine-Protein Kinase Kit
CL	=	Clearance
CL-387785	=	N-[4-[(3-Bromophenyl)amino]-6-quinazolinyl]-2-butynamide
CLK	=	Cdc2-like Kinase
CMGC	=	Including cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAP kinases), glycogen synthase kinases (GSK) and CDK-like kinases
D835Y	=	Mutation of FLT in which Aspartic Acid (D) is Substituted by Tyrosine (Y) at Position 838
DHI-carbazole	=	Dihydroindazolocarbazole
DNA	=	Deoxyribonucleic Acid
DU145	=	Human Prostate Cancer Cell Line
EGFR	=	Epidermal Growth Factor Receptor
ErbB	=	Erythroblastosis Oncogene B
FDA	=	Food and Drug Administration
FLT	=	FMS-like Tyrosine Kinase
G2	=	Gap 2
GIST	=	Gastrointestinal Stromal Tumor
Glu121	=	Glutamic Acid 121
GNF-2	=	3-[6-[[4-(Trifluoromethoxy)phenyl] amino]-4-pyrimidinyl]benzamide
GSK3	=	Glycogen Synthase Kinase 3
HCT116	=	Human Colon Cancer Cell Line
Her-2/Neu	=	Human Epidermal Growth Factor Receptor 2
HER-2	=	Herceptin-2
HKI-272	=	Neratinib
HT29	=	Human Colon Adenocarcinoma Cell Line
i-Bu	=	Isobutyl
IC_{50}	=	Half Maximal Inhibitory Concentration
IGF1R	=	Insulin-like Growth Factor Receptor 1
IRK	=	Insulin Receptor Kinase
ITD	=	Internal Tandem Duplications
JAK	=	Janus Kinase
JNK	=	c-Jun N-terminal Kinase
KARPAS-299	=	Human Non-Hodgkin's Ki-positive Large Cell Lymphoma cell line
KDR	=	Kinase Insert Domain Receptor
L1210	=	Mouse Lymphocytic Leukemia Cell Line
LNCaP	=	Androgen-Sensitive Human Prostate Adenocarcinoma Cells
M	=	Mitosis
MAPK	=	Mitogen-Activated Protein Kinase
MEK	=	Mitogen-Activated Protein Kinase Kinase
NSCLC	=	Non-small-Cell Lung Carcinoma
PA1	=	Human Ovarian Teratocarcinoma Cell Line
PC3	=	Human Prostate Cancer Cell Line
PDB	=	Protein Data Bank
PDGFR	=	Platelet-Derived Growth Factor Receptors
Phe51	=	Phenylalanine 51
Pim	=	Proto-Oncogene Serine/Threonine-Protein Kinase Pim
PKC	=	Protein-Kinase C
PKs	=	Protein KINASES
Pr	=	Propyl
PTKs	=	Protein-Tyrosine Kinases
RCC	=	Renal Cell Carcinoma
RO0281675	=	(2R)-3-Cyclopentyl-2-(4-methanesulfonylphenyl)-N-thiazol-2-yl-propionamide
RTK	=	Receptor Tyrosine Kinase
SAR	=	Structure Activity Relationship
STE	=	Homologues of Yeast Sterile 7, 11, 20 Kinases
T790M	=	Mutation of EGFR in which Threonine (T) is Substituted by Methionine (M) at Aminoacid 790
TAK-733	=	3-[(2R)-2,3-Dihydroxypropyl]-6-fluoro-5-(2-fluoro-4-iodoanilino)-8-methylpyrido[2,3-d]pyrimidine-4,7-dione

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Tie-2 = Tyrosine Kinase with Immunoglobulin and Epidermal Growth Factor Homology Domains

TKL = Tyrosine Kinase-Like PKs

TKs = Tyrosine Kinases

TrkA = Tropomyosin Receptor Kinase A

TTT-3002 = (5R,7R,8S)-7-amino-N,8-dimethyl-15-oxo-5,6,7,8,14,15-hexahydro-13H-16-oxa-4b,8a,14-triaza-5,8-methanodi-benzo[b,h]cycloocta[jkl]cyclopenta[e]-as-indacene-7-carboxamide

V600E = Mutation of BRAF in which Valine (V) is Substituted by Glutamic acid (E) at Aminoacid 600.

V600K = Mutation of BRAF in which Valine (V) is Substituted by Lysin (K) at Aminoacid 600.

V_d = Volume of Distribution

VEGF-R = Vascular Endothelial Growth Factor

Wee1 = Nuclear Kinase Belonging to the Serine/ Threonine Family

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

REFERENCES

[1] Gatzka, M.V. Targeted Tumor Therapy Remixed-An Update on the Use of Small-Molecule Drugs in Combination Therapies. *Cancers (Basel),* **2018**, *10*(6), E155. [http://dx.doi.org/10.3390/cancers10060155] [PMID: 29794999]

[2] Dervisis, N.; Klahn, S. Therapeutic Innovations: Tyrosine Kinase Inhibitors in Cancer. *Vet. Sci.,* **2016**, *3*(1), E4. [http://dx.doi.org/10.3390/vetsci3010004] [PMID: 29056714]

[3] Arora, A.; Scholar, E.M. Role of tyrosine kinase inhibitors in cancer therapy. *J. Pharmacol. Exp. Ther.,* **2005**, *315*(3), 971-979. [http://dx.doi.org/10.1124/jpet.105.084145] [PMID: 16002463]

[4] Pearson, M.A.; Fabbro, D. Targeting protein kinases in cancer therapy: A success? *Expert Rev. Anticancer Ther.,* **2004**, *4*(6), 1113-1124. [http://dx.doi.org/10.1586/14737140.4.6.1113] [PMID: 15606337]

[5] Blume-Jensen, P.; Hunter, T. Oncogenic kinase signalling. *Nature,* **2001**, *411*(6835), 355-365. [http://dx.doi.org/10.1038/35077225] [PMID: 11357143]

[6] García-Aranda, M.; Redondo, M. Protein kinase targets in breast cancer. *Int. J. Mol. Sci.,* **2017**, *18*(12), 1-31. [http://dx.doi.org/10.3390/ijms18122543] [PMID: 29186886]

[7] Kissau, L.; Stahl, P.; Mazitschek, R.; Giannis, A.; Waldmann, H. Development of natural product-derived receptor tyrosine kinase inhibitors based on conservation of protein domain fold. *J. Med. Chem.,* **2003**, *46*(14), 2917-2931. [http://dx.doi.org/10.1021/jm0307943] [PMID: 12825933]

[8] Bari, S.B.; Adhikari, S.; Surana, S.J. Tyrosine Kinase Receptor Inhibitors: A new target for anticancer drug development. *J. Pharm. Sci. Technol.,* **2012**, *1*(2), 36-45.

[9] Sinicropi, M.S.; Caruso, A.; Conforti, F.; Marrelli, M.; El Kashef, H.; Lancelot, J.C.; Rault, S.; Statti, G.A.; Menichini, F. Synthesis, inhibition of NO production and antiproliferative activities of some indole derivatives. *J. Enzyme Inhib. Med. Chem.,* **2009**, *24*(5), 1148-1153. [http://dx.doi.org/10.1080/14756360802693890] [PMID: 19555184]

[10] Lappano, R.; Santolla, M.F.; Pupo, M.; Sinicropi, M.S.; Caruso, A.; Rosano, C.; Maggiolini, M. MIBE acts as antagonist ligand of both estrogen receptor α and GPER in breast cancer cells. *Breast Cancer Res.,* **2012**, *14*(1), R12. [http://dx.doi.org/10.1186/bcr3096] [PMID: 22251451]

[11] Caruso, A.; Chimento, A.; El-Kashef, H.; Lancelot, J.C.; Panno, A.; Pezzi, V.; Saturnino, C.; Sinicropi, M.S.; Sirianni, R.; Rault, S. Antiproliferative activity of some 1,4-dimethylcarbazoles on cells that express estrogen receptors: Part I. *J. Enzyme Inhib. Med. Chem.,* **2012**, *27*(4), 609-613. [http://dx.doi.org/10.3109/14756366.2011.603132] [PMID: 21883039]

[12] Caruso, A.; Sinicropi, M.S.; Lancelot, J.C.; El-Kashef, H.; Saturnino, C.; Aubert, G.; Ballandonne, C.; Lesnard, A.; Cresteil, T.; Dallemagne, P.; Rault, S. Synthesis and evaluation of cytotoxic activities of new guanidines derived from carbazoles. *Bioorg. Med. Chem. Lett.,* **2014**, *24*(2), 467-472. [http://dx.doi.org/10.1016/j.bmcl.2013.12.047] [PMID: 24374274]

[13] Rizza, P.; Pellegrino, M.; Caruso, A.; Iacopetta, D.; Sinicropi, M.S.; Rault, S.; Lancelot, J.C.; El-Kashef, H.; Lesnard, A.; Rochais, C.; Dallemagne, P.; Saturnino, C.; Giordano, F.; Catalano, S.; Andò, S. 3-(Dipropylamino)-5-hydroxybenzofuro[2,3-f]quinazolin-1(2H)-one (DPA-HBFQ-1) plays an inhibitory role on breast cancer cell growth and progression. *Eur. J. Med. Chem.,* **2016**, *107*, 275-287. [http://dx.doi.org/10.1016/j.ejmech.2015.11.004] [PMID: 26599533]

[14] Caruso, A.; Lancelot, J.; El-Kashef, H.; Sinicropi, M.S.; Legay, R.; Lesnard, A.; Rault, S. A rapid and versatile synthesis of novel pyrimido[5,4-b]carbazoles. *Tetrahedron,* **2009**, *65*, 10400-10405. [http://dx.doi.org/10.1016/j.tet.2009.10.025]

[15] Caruso, A.; Lancelot, J.C.; El-Kashef, H.; Panno, A.; Sinicropi, M.S.; Legay, R.; Lesnard, A.; Lepailleur, A.; Rault, S. Four Partners, Three-Step, One-Pot Reaction for a Library of New 2-Alkyl(dialkyl)aminoquinazolin-4(3H)-ones. *J. Heterocycl. Chem.,* **2014**, *51*, 282-293. [http://dx.doi.org/10.1002/jhet.1942]

[16] Panno, A.; Sinicropi, M.S.; Caruso, A.; El-Kashef, H.; Lancelot, J.C.; Aubert, G.; Lesnard, A.; Cresteil, T.; Rault, S. New Trimethoxybenzamides and Trimethoxyphenylureas derived from dimethylcarbazole as cytotoxic agents. Part I. *J. Heterocycl. Chem.,* **2014**, *51*, 294-302. [http://dx.doi.org/10.1002/jhet.1951]

[17] Saturnino, C.; Caruso, A.; Iacopetta, D.; Rosano, C.; Ceramella, J.; Muià, N.; Mariconda, A.; Bonomo, M.G.; Ponassi, M.; Rosace, G.; Sinicropi, M.S.; Longo, P. Inhibition of human Topoisomerase II by N,N,N-Trimethylethanammonium Iodide Alkylcarbazole derivatives. *Chem. Med. Chem.,* **2018**, *13*(24), 2635-2643. [http://dx.doi.org/10.1002/cmdc.201800546] [PMID: 30347518]

[18] Sinicropi, M.S.; Lappano, R.; Caruso, A.; Santolla, M.F.; Pisano, A.; Rosano, C.; Capasso, A.; Panno, A.; Lancelot, J.C.; Rault, S.; Saturnino, C.; Maggiolini, M. (6-bromo-1,4-dimethyl-9H-carbazol-3-yl-methylene)-hydrazine (carbhydraz) acts as a GPER agonist in

Personal Use Only Not For Distribution

breast cancer cells. *Curr. Top. Med. Chem.,* **2015**, *15*(11), 1035-1042.
[http://dx.doi.org/10.2174/1568026615666150317221549] [PMID: 25786510]

[19] Saturnino, C.; Caruso, A.; Longo, P.; Capasso, A.; Pingitore, A.; Caroleo, M.C.; Cione, E.; Perri, M.; Nicolo, F.; Nardo, V.M.; Scolaro, L.M.; Sinicropi, M.S.; Plutino, M.R.; El-Kashef, H. Crystallographic study and biological evaluation of 1,4-dimethyl-N-alkylcarbazoles. *Curr. Top. Med. Chem.,* **2015**, *15*(11), 973-979.
[http://dx.doi.org/10.2174/1568026615666150317222444] [PMID: 25786507]

[20] Saturnino, C.; Iacopetta, D.; Sinicropi, M.S.; Rosano, C.; Caruso, A.; Caporale, A.; Marra, N.; Marengo, B.; Pronzato, M.A.; Parisi, O.I.; Longo, P.; Ricciarelli, R. N-alkyl carbazole derivatives as new tools for Alzheimer's disease: Preliminary studies. *Molecules,* **2014**, *19*(7), 9307-9317.
[http://dx.doi.org/10.3390/molecules19079307] [PMID: 24991761]

[21] Caruso, A.; Iacopetta, D.; Puoci, F.; Cappello, A.R.; Saturnino, C.; Sinicropi, M.S. Carbazole derivatives: A promising scenario for breast cancer treatment. *Mini-Rev. Med. Chem.,* **2016**, *16*(8), 630-643.
[http://dx.doi.org/10.2174/1389557515666150709111342] [PMID: 26156543]

[22] Iacopetta, D.; Rosano, C.; Puoci, F.; Parisi, O.I.; Saturnino, C.; Caruso, A.; Longo, P.; Ceramella, J.; Malzert-Fréon, A.; Dallemagne, P.; Rault, S.; Sinicropi, M.S. Multifaceted properties of 1,4-dimethylcarbazoles: Focus on trimethoxybenzamide and trimethoxyphenylurea derivatives as novel human topoisomerase II inhibitors. *Eur. J. Pharm. Sci.,* **2017**, *96*, 263-272.
[http://dx.doi.org/10.1016/j.ejps.2016.09.039] [PMID: 27702608]

[23] Maji, B.; Kumar, K.; Muniyappa, K.; Bhattacharya, S. New dimeric carbazole-benzimidazole mixed ligands for the stabilization of human telomeric G-quadruplex DNA and as telomerase inhibitors. A remarkable influence of the spacer. *Org. Biomol. Chem.,* **2015**, *13*(30), 8335-8348.
[http://dx.doi.org/10.1039/C5OB00675A] [PMID: 26149178]

[24] Sinicropi, M.S.; Iacopetta, D.; Rosano, C.; Randino, R.; Caruso, A.; Saturnino, C.; Muià, N.; Ceramella, J.; Puoci, F.; Rodriquez, M.; Longo, P.; Plutino, M.R. N-thioalkylcarbazoles derivatives as new anti-proliferative agents: synthesis, characterisation and molecular mechanism evaluation. *J. Enzyme Inhib. Med. Chem.,* **2018**, *33*(1), 434-444.
[http://dx.doi.org/10.1080/14756366.2017.1419216] [PMID: 29383954]

[25] Saturnino, C.; Palladino, C.; Napoli, M.; Sinicropi, M.S.; Botta, A.; Sala, M.; Carcereri de Prati, A.; Novellino, E.; Suzuki, H. Synthesis and biological evaluation of new N-alkylcarbazole derivatives as STAT3 inhibitors: Preliminary study. *Eur. J. Med. Chem.,* **2013**, *60*, 112-119.
[http://dx.doi.org/10.1016/j.ejmech.2012.11.004] [PMID: 23287056]

[26] Parisi, O.I.; Morelli, C.; Puoci, F.; Saturnino, C.; Caruso, A.; Sisci, D.; Trombino, G.E.; Picci, N.; Sinicropi, M.S. Magnetic Molecularly Imprinted Polymers (MMIPs) for carbazole derivative release in targeted cancer therapy. *J. Mater. Chem. B Mater. Biol. Med.,* **2014**, *2*(38), 6619-6625.
[http://dx.doi.org/10.1039/C4TB00607K]

[27] Grande, F.; Rizzuti, B.; Occhiuzzi, M.A.; Ioele, G.; Casacchia, T.; Gelmini, F.; Guzzi, R.; Garofalo, A.; Statti, G. Identification by Molecular Docking of Homoisoflavones from Leopoldia comosa as Ligands of Estrogen Receptors. *Molecules,* **2018**, *23*(4), E894.
[http://dx.doi.org/10.3390/molecules23040894] [PMID: 29649162]

[28] Casacchia, T.; Occhiuzzi, M.A.; Grande, F.; Rizzuti, B.; Granieri, M.C.; Rocca, C.; Gattuso, A.; Garofalo, A.; Angelone, T.; Statti, G. A pilot study on the nutraceutical properties of the Citrus hybrid Tacle (R) as a dietary source of polyphenols for supplementation in metabolic disorders. *J. Funct. Foods,* **2019**, *52*, 370-381.
[http://dx.doi.org/10.1016/j.jff.2018.11.030]

[29] Bossemeyer, D. Protein kinases--structure and function. *FEBS Lett.,* **1995**, *369*(1), 57-61.
[http://dx.doi.org/10.1016/0014-5793(95)00580-3] [PMID: 7641885]

[30] Knighton, D.R.; Zheng, J.H.; Ten Eyck, L.F.; Ashford, V.A.; Xuong, N.H.; Taylor, S.S.; Sowadski, J.M. Crystal structure of the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase. *Science,* **1991**, *253*(5018), 407-414.
[http://dx.doi.org/10.1126/science.1862342] [PMID: 1862342]

[31] Krause, D.S.; Van Etten, R.A. Tyrosine kinases as targets for cancer therapy. *N. Engl. J. Med.,* **2005**, *353*(2), 172-187.
[http://dx.doi.org/10.1056/NEJMra044389] [PMID: 16014887]

[32] Manning, G.; Whyte, D.B.; Martinez, R.; Hunter, T.; Sudarsanam, S. The protein kinase complement of the human genome. *Science,* **2002**, *298*(5600), 1912-1934.
[http://dx.doi.org/10.1126/science.1075762] [PMID: 12471243]

[33] Fabbro, D.; Cowan-Jacob, S.W.; Moebitz, H. Ten things you should know about protein kinases: IUPHAR Review 14. *Br. J. Pharmacol.,* **2015**, *172*(11), 2675-2700.
[http://dx.doi.org/10.1111/bph.13096] [PMID: 25630872]

[34] Bhullar, K.S.; Lagarón, N.O.; McGowan, E.M.; Parmar, I.; Jha, A.; Hubbard, B.P.; Rupasinghe, H.P.V. Kinase-targeted cancer therapies: progress, challenges and future directions. *Mol. Cancer,* **2018**, *17*(1), 48.
[http://dx.doi.org/10.1186/s12943-018-0804-2] [PMID: 29455673]

[35] Blagden, S.; de Bono, J. Drugging cell cycle kinases in cancer therapy. *Curr. Drug Targets,* **2005**, *6*(3), 325-335.
[http://dx.doi.org/10.2174/1389450053765824] [PMID: 15857291]

[36] Paul, M.K.; Mukhopadhyay, A.K. Tyrosine kinase - Role and significance in Cancer. *Int. J. Med. Sci.,* **2004**, *1*(2), 101-115.
[http://dx.doi.org/10.7150/ijms.1.101] [PMID: 15912202]

[37] Fehm, T.; Jäger, W.; Krämer, S.; Sohn, C.; Solomayer, E.; Wallwiener, D.; Gebauer, G. Prognostic significance of serum HER2 and CA 15-3 at the time of diagnosis of metastatic breast cancer. *Anticancer Res.,* **2004**, *24*(3b), 1987-1992.
[PMID: 15274389]

[38] Cheng, H.C.; Qi, R.Z.; Paudel, H.; Zhu, H.J. Regulation and function of protein kinases and phosphatases. *Enzyme Res.,* **2011**, *2011*794089
[http://dx.doi.org/10.4061/2011/794089] [PMID: 22195276]

[39] Cheetham, G.M. Novel protein kinases and molecular mechanisms of autoinhibition. *Curr. Opin. Struct. Biol.,* **2004**, *14*(6), 700-705.
[http://dx.doi.org/10.1016/j.sbi.2004.10.011] [PMID: 15582394]

[40] Kondapalli, L.; Soltani, K.; Lacouture, M.E. The promise of molecular targeted therapies: Protein kinase inhibitors in the treatment of cutaneous malignancies. *J. Am. Acad. Dermatol.,* **2005**, *53*(2), 291-302.
[http://dx.doi.org/10.1016/j.jaad.2005.02.011] [PMID: 16021125]

[41] Cohen, P. Protein kinases--the major drug targets of the twenty-first century? *Nat. Rev. Drug Discov.,* **2002**, *1*(4), 309-315.
[http://dx.doi.org/10.1038/nrd773] [PMID: 12120282]

[42] Melnikova, I.; Golden, J. Targeting protein kinases. *Nat. Rev. Drug Discov.,* **2004**, *3*(12), 993-994.
[http://dx.doi.org/10.1038/nrd1600] [PMID: 15645605]

[43] Vlahovic, G.; Crawford, J. Activation of tyrosine kinases in cancer. *Oncologist,* **2003**, *8*(6), 531-538.
[http://dx.doi.org/10.1634/theoncologist.8-6-531] [PMID: 14657531]

[44] Segovia-Mendoza, M.; González-González, M.E.; Barrera, D.; Díaz, L.; García-Becerra, R. Efficacy and mechanism of action of the tyrosine kinase inhibitors gefitinib, lapatinib and neratinib in the treatment of HER2-positive breast cancer: Preclinical and clinical evidence. *Am. J. Cancer Res.,* **2015**, *5*(9), 2531-2561.
[PMID: 26609467]

[45] Zhang, J.; Yang, P.L.; Gray, N.S. Targeting cancer with small molecule kinase inhibitors. *Nat. Rev. Cancer,* **2009**, *9*(1), 28-39.
[http://dx.doi.org/10.1038/nrc2559] [PMID: 19104514]

[46] Midland, A.A.; Whittle, M.C.; Duncan, J.S.; Abell, A.N.; Nakamura, K.; Zawistowski, J.S.; Carey, L.A.; Earp, H.S., III; Graves, L.M.; Gomez, S.M.; Johnson, G.L. Defining the expressed breast cancer kinome. *Cell Res.,* **2012**, *22*(4), 620-623.
[http://dx.doi.org/10.1038/cr.2012.25] [PMID: 22310242]

[47] Davis, M.I.; Hunt, J.P.; Herrgard, S.; Ciceri, P.; Wodicka, L.M.; Pallares, G.; Hocker, M.; Treiber, D.K.; Zarrinkar, P.P. Comprehensive analysis of kinase inhibitor selectivity. *Nat. Biotechnol.,* **2011**, *29*(11), 1046-1051.
[http://dx.doi.org/10.1038/nbt.1990] [PMID: 22037378]

Personal Use Only
Not For Distribution

[48] Knight, Z.A.; Shokat, K.M. Features of selective kinase inhibitors. *Chem. Biol.,* **2005**, *12*(6), 621-637. [http://dx.doi.org/10.1016/j.chembiol.2005.04.011] [PMID: 15975507]

[49] Force, T.; Kolaja, K.L. Cardiotoxicity of kinase inhibitors: the prediction and translation of preclinical models to clinical outcomes. *Nat. Rev. Drug Discov.,* **2011**, *10*(2), 111-126. [http://dx.doi.org/10.1038/nrd3252] [PMID: 21283106]

[50] Hasinoff, B.B. The cardiotoxicity and myocyte damage caused by small molecule anticancer tyrosine kinase inhibitors is correlated with lack of target specificity. *Toxicol. Appl. Pharmacol.,* **2010**, *244*(2), 190-195. [http://dx.doi.org/10.1016/j.taap.2009.12.032] [PMID: 20045709]

[51] Cutolo, M. The kinase inhibitor tofacitinib in patients with rheumatoid arthritis: Latest findings and clinical potential. *Ther. Adv. Musculoskelet. Dis.,* **2013**, *5*(1), 3-11. [http://dx.doi.org/10.1177/1759720X12470753] [PMID: 23515130]

[52] Zuccotto, F.; Ardini, E.; Casale, E.; Angiolini, M. Through the "gatekeeper door": Exploiting the active kinase conformation. *J. Med. Chem.,* **2010**, *53*(7), 2681-2694. [http://dx.doi.org/10.1021/jm901443h] [PMID: 20000735]

[53] Kufareva, I.; Abagyan, R. Type-II kinase inhibitor docking, screening, and profiling using modified structures of active kinase states. *J. Med. Chem.,* **2008**, *51*(24), 7921-7932. [http://dx.doi.org/10.1021/jm8010299] [PMID: 19053777]

[54] Davies, S.P.; Reddy, H.; Caivano, M.; Cohen, P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem. J.,* **2000**, *351*(Pt 1), 95-105. [http://dx.doi.org/10.1042/bj3510095] [PMID: 10998351]

[55] Liu, Y.; Gray, N.S. Rational design of inhibitors that bind to inactive kinase conformations. *Nat. Chem. Biol.,* **2006**, *2*(7), 358-364. [http://dx.doi.org/10.1038/nchembio799] [PMID: 16783341]

[56] Wise, D.R.; Thompson, C.B. Glutamine addiction: A new therapeutic target in cancer. *Trends Biochem. Sci.,* **2010**, *35*(8), 427-433. [http://dx.doi.org/10.1016/j.tibs.2010.05.003] [PMID: 20570523]

[57] Nagar, B.; Bornmann, W.G.; Pellicena, P.; Schindler, T.; Veach, D.R.; Miller, W.T.; Clarkson, B.; Kuriyan, J. Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res.,* **2002**, *62*(15), 4236-4243. [PMID: 12154025]

[58] Schindler, T.; Bornmann, W.; Pellicena, P.; Miller, W.T.; Clarkson, B.; Kuriyan, J. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science,* **2000**, *289*(5486), 1938-1942. [http://dx.doi.org/10.1126/science.289.5486.1938] [PMID: 10988075]

[59] Pargellis, C.; Tong, L.; Churchill, L.; Cirillo, P.F.; Gilmore, T.; Graham, A.G.; Grob, P.M.; Hickey, E.R.; Moss, N.; Pav, S.; Regan, J. Inhibition of p38 MAP kinase by utilizing a novel allosteric binding site. *Nat. Struct. Biol.,* **2002**, *9*(4), 268-272. [http://dx.doi.org/10.1038/nsb770] [PMID: 11896401]

[60] Wan, P.T.; Garnett, M.J.; Roe, S.M.; Lee, S.; Niculescu-Duvaz, D.; Good, V.M.; Jones, C.M.; Marshall, C.J.; Springer, C.J.; Barford, D.; Marais, R.; Cancer Genome, P. Cancer Genome Project. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell,* **2004**, *116*(6), 855-867. [http://dx.doi.org/10.1016/S0092-8674(04)00215-6] [PMID: 15035987]

[61] Manley, P.W.; Bold, G.; Brüggen, J.; Fendrich, G.; Furet, P.; Mestan, J.; Schnell, C.; Stolz, B.; Meyer, T.; Meyhack, B.; Stark, W.; Strauss, A.; Wood, J. Advances in the structural biology, design and clinical development of VEGF-R kinase inhibitors for the treatment of angiogenesis. *Biochim. Biophys. Acta,* **2004**, *1697*(1-2), 17-27. [http://dx.doi.org/10.1016/j.bbapap.2003.11.010] [PMID: 15023347]

[62] Ohren, J.F.; Chen, H.; Pavlovsky, A.; Whitehead, C.; Zhang, E.; Kuffa, P.; Yan, C.; McConnell, P.; Spessard, C.; Banotai, C.; Mueller, W.T.; Delaney, A.; Omer, C.; Sebolt-Leopold, J.; Dudley, D.T.; Leung, I.K.; Flamme, C.; Warmus, J.; Kaufman, M.; Barrett, S.; Tecle, H.; Hasemann, C.A. Structures of human MAP kinase kinase 1 (MEK1) and MEK2 describe novel noncompetitive kinase inhibition. *Nat. Struct. Mol. Biol.,* **2004**, *11*(12), 1192-1197. [http://dx.doi.org/10.1038/nsmb859] [PMID: 15543157]

[63] Allen, L.F.; Sebolt-Leopold, J.; Meyer, M.B. CI-1040 (PD184352), a targeted signal transduction inhibitor of MEK (MAPKK). *Semin. Oncol.,* **2003**, *30*(5)(Suppl. 16), 105-116. [http://dx.doi.org/10.1053/j.seminoncol.2003.08.012] [PMID: 14613031]

[64] Eglen, R.; Reisine, T. Drug discovery and the human kinome: recent trends. *Pharmacol. Ther.,* **2011**, *130*(2), 144-156. [http://dx.doi.org/10.1016/j.pharmthera.2011.01.007] [PMID: 21256157]

[65] Adrián, F.J.; Ding, Q.; Sim, T.; Velentza, A.; Sloan, C.; Liu, Y.; Zhang, G.; Hur, W.; Ding, S.; Manley, P.; Mestan, J.; Fabbro, D.; Gray, N.S. Allosteric inhibitors of Bcr-abl-dependent cell proliferation. *Nat. Chem. Biol.,* **2006**, *2*(2), 95-102. [http://dx.doi.org/10.1038/nchembio760] [PMID: 16415863]

[66] Dong, Q.; Dougan, D.R.; Gong, X.; Halkowycz, P.; Jin, B.; Kanouni, T.; O'Connell, S.M.; Scorah, N.; Shi, L.; Wallace, M.B.; Zhou, F. Discovery of TAK-733, a potent and selective MEK allosteric site inhibitor for the treatment of cancer. *Bioorg. Med. Chem. Lett.,* **2011**, *21*(5), 1315-1319. [http://dx.doi.org/10.1016/j.bmcl.2011.01.071] [PMID: 21310613]

[67] Schadendorf, D.; Amonkar, M.M.; Milhem, M.; Grotzinger, K.; Demidov, L.V.; Rutkowski, P.; Garbe, C.; Dummer, R.; Hassel, J.C.; Wolter, P.; Mohr, P.; Trefzer, U.; Lefeuvre-Plesse, C.; Rutten, A.; Steven, N.; Ullenhag, G.; Sherman, L.; Wu, F.S.; Patel, K.; Casey, M.; Robert, C. Functional and symptom impact of trametinib versus chemotherapy in BRAF V600E advanced or metastatic melanoma: quality-of-life analyses of the METRIC study. *Ann. Oncol.,* **2014**, *25*(3), 700-706. [http://dx.doi.org/10.1093/annonc/mdt580] [PMID: 24504441]

[68] Grimsby, J.; Sarabu, R.; Corbett, W.L.; Haynes, N.E.; Bizzarro, F.T.; Coffey, J.W.; Guertin, K.R.; Hilliard, D.W.; Kester, R.F.; Mahaney, P.E.; Marcus, L.; Qi, L.; Spence, C.L.; Tengi, J.; Magnuson, M.A.; Chu, C.A.; Dvorozniak, M.T.; Matschinsky, F.M.; Grippo, J.F. Allosteric activators of glucokinase: Potential role in diabetes therapy. *Science,* **2003**, *301*(5631), 370-373. [http://dx.doi.org/10.1126/science.1084073] [PMID: 12869762]

[69] Guertin, K.R.; Grimsby, J. Small molecule glucokinase activators as glucose lowering agents: A new paradigm for diabetes therapy. *Curr. Med. Chem.,* **2006**, *13*(15), 1839-1843. [http://dx.doi.org/10.2174/092986706777452551] [PMID: 16787225]

[70] Cohen, M.S.; Zhang, C.; Shokat, K.M.; Taunton, J. Structural bioinformatics-based design of selective, irreversible kinase inhibitors. *Science,* **2005**, *308*(5726), 1318-1321. [http://dx.doi.org/10.1126/science1108367] [PMID: 15919995]

[71] Kwak, E.L.; Sordella, R.; Bell, D.W.; Godin-Heymann, N.; Okimoto, R.A.; Brannigan, B.W.; Harris, P.L.; Driscoll, D.R.; Fidias, P.; Lynch, T.J.; Rabindran, S.K.; McGinnis, J.P.; Wissner, A.; Sharma, S.V.; Isselbacher, K.J.; Settleman, J.; Haber, D.A. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. *Proc. Natl. Acad. Sci. USA,* **2005**, *102*(21), 7665-7670. [http://dx.doi.org/10.1073/pnas.0502860102] [PMID: 15897464]

[72] Blanc, J.; Geney, R.; Menet, C. Type II kinase inhibitors: An opportunity in cancer for rational design. *Anticancer. Agents Med. Chem.,* **2013**, *13*(5), 731-747. [http://dx.doi.org/10.2174/18715206113130500008] [PMID: 23094911]

[73] Leproult, E.; Barluenga, S.; Moras, D.; Wurtz, J.M.; Winssinger, N. Cysteine mapping in conformationally distinct kinase nucleotide binding sites: Application to the design of selective covalent inhibitors. *J. Med. Chem.,* **2011**, *54*(5), 1347-1355. [http://dx.doi.org/10.1021/jm101396q] [PMID: 21322567]

[74] Liu, Q.; Sabnis, Y.; Zhao, Z.; Zhang, T.; Buhrlage, S.J.; Jones, L.H.; Gray, N.S. Developing irreversible inhibitors of the protein kinase cysteinome. *Chem. Biol.,* **2013**, *20*(2), 146-159. [http://dx.doi.org/10.1016/j.chembiol.2012.12.006] [PMID: 23438744]

[75] Singh, J.; Petter, R.C.; Baillie, T.A.; Whitty, A. The resurgence of covalent drugs. *Nat. Rev. Drug Discov.,* **2011**, *10*(4), 307-317. [http://dx.doi.org/10.1038/nrd3410] [PMID: 21455239]

Personal Use Only
Not For Distribution

[76] Barf, T.; Kaptein, A. Irreversible protein kinase inhibitors: Balancing the benefits and risks. *J. Med. Chem.,* **2012**, *55*(14), 6243-6262.
[http://dx.doi.org/10.1021/jm3003203] [PMID: 22621397]

[77] Zhao, Z.; Liu, Q.; Bliven, S.; Xie, L.; Bourne, P.E. Determining cysteines available for covalent inhibition across the human kinome. *J. Med. Chem.,* **2017**, *60*(7), 2879-2889.
[http://dx.doi.org/10.1021/acs.jmedchem.6b01815]
[PMID: 28326775]

[78] Rabindran, S.K.; Discafani, C.M.; Rosfjord, E.C.; Baxter, M.; Floyd, M.B.; Golas, J.; Hallett, W.A.; Johnson, B.D.; Nilakantan, R.; Overbeek, E.; Reich, M.F.; Shen, R.; Shi, X.; Tsou, H.R.; Wang, Y.F.; Wissner, A. Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. *Cancer Res.,* **2004**, *64*(11), 3958-3965.
[http://dx.doi.org/10.1158/0008-5472.CAN-03-2868]
[PMID: 15173008]

[79] Kobayashi, S.; Ji, H.; Yuza, Y.; Meyerson, M.; Wong, K.K.; Tenen, D.G.; Halmos, B. An alternative inhibitor overcomes resistance caused by a mutation of the epidermal growth factor receptor. *Cancer Res.,* **2005**, *65*(16), 7096-7101.
[http://dx.doi.org/10.1158/0008-5472.CAN-05-1346]
[PMID: 16103058]

[80] Liao, B.C.; Lin, C.C.; Yang, J.C. Second and third-generation epidermal growth factor receptor tyrosine kinase inhibitors in advanced nonsmall cell lung cancer. *Curr. Opin. Oncol.,* **2015**, *27*(2), 94-101.
[http://dx.doi.org/10.1097/CCO.0000000000000164]
[PMID: 25611025]

[81] Wang, A.; Yan, X.E.; Wu, H.; Wang, W.; Hu, C.; Chen, C.; Zhao, Z.; Zhao, P.; Li, X.; Wang, L.; Wang, B.; Ye, Z.; Wang, J.; Wang, C.; Zhang, W.; Gray, N.S.; Weisberg, E.L.; Chen, L.; Liu, J.; Yun, C.H.; Liu, Q. Ibrutinib targets mutant-EGFR kinase with a distinct binding conformation. *Oncotarget,* **2016**, *7*(43), 69760-69769.
[http://dx.doi.org/10.18632/oncotarget.11951] [PMID: 27626175]

[82] Akué-Gédu, R.; Rossignol, E.; Azzaro, S.; Knapp, S.; Filippakopoulos, P.; Bullock, A.N.; Bain, J.; Cohen, P.; Prudhomme, M.; Anizon, F.; Moreau, P. Synthesis, kinase inhibitory potencies, and *in vitro* antiproliferative evaluation of new Pim kinase inhibitors. *J. Med. Chem.,* **2009**, *52*(20), 6369-6381.
[http://dx.doi.org/10.1021/jm901018f] [PMID: 19788246]

[83] Akué-Gédu, R.; Nauton, L.; Théry, V.; Bain, J.; Cohen, P.; Anizon, F.; Moreau, P. Synthesis, Pim kinase inhibitory potencies and *in vitro* antiproliferative activities of diversely substituted pyrrolo[2,3-a]carbazoles. *Bioorg. Med. Chem.,* **2010**, *18*(18), 6865-6873.
[http://dx.doi.org/10.1016/j.bmc.2010.07.036] [PMID: 20728368]

[84] Giraud, F.; Akué-Gédu, R.; Nauton, L.; Candelon, N.; Debiton, E.; Théry, V.; Anizon, F.; Moreau, P. Synthesis and biological activities of 4-substituted pyrrolo[2,3-a]carbazole Pim kinase inhibitors. *Eur. J. Med. Chem.,* **2012**, *56*, 225-236.
[http://dx.doi.org/10.1016/j.ejmech.2012.08.029]
[PMID: 22982527]

[85] Akué-Gédu, R.; Letribot, B.; Saugues, E.; Debiton, E.; Anizon, F.; Moreau, P. Kinase inhibitory potencies and *in vitro* antiproliferative activities of N-10 substituted pyrrolo[2,3-a]carbazole derivatives. *Bioorg. Med. Chem. Lett.,* **2012**, *22*(11), 3807-3809.
[http://dx.doi.org/10.1016/j.bmcl.2012.03.098] [PMID: 22543026]

[86] Letribot, B.; Akué-Gédu, R.; Santio, N.M.; El-Ghozzi, M.; Avignant, D.; Cisnetti, F.; Koskinen, P.J.; Gautier, A.; Anizon, F.; Moreau, P. Use of copper(I) catalyzed azide alkyne cycloaddition (CuAAC) for the preparation of conjugated pyrrolo[2,3-a]carbazole Pim kinase inhibitors. *Eur. J. Med. Chem.,* **2012**, *50*, 304-310.
[http://dx.doi.org/10.1016/j.ejmech.2012.02.009]
[PMID: 22386260]

[87] Suchaud, V.; Gavara, L.; Saugues, E.; Nauton, L.; Théry, V.; Anizon, F.; Moreau, P. Identification of 1,6-dihydropyrazolo[4,3-c]carbazoles and 3,6-dihydropyrazolo[3,4-c]carbazoles as new Pim kinase inhibitors. *Bioorg. Med. Chem.,* **2013**, *21*(14), 4102-4111.
[http://dx.doi.org/10.1016/j.bmc.2013.05.011] [PMID: 23735828]

[88] Hénon, H.; Anizon, F.; Golsteyn, R.M.; Léonce, S.; Hofmann, R.; Pfeiffer, B.; Prudhomme, M. Synthesis and biological evaluation of new dipyrrolo[3,4-a:3,4-c]carbazole-1,3,4,6-tetraones, substituted with various saturated and unsaturated side chains *via* palladium catalyzed cross-coupling reactions. *Bioorg. Med. Chem.,* **2006**, *14*(11), 3825-3834.
[http://dx.doi.org/10.1016/j.bmc.2006.01.030] [PMID: 16460946]

[89] Hénon, H.; Messaoudi, S.; Anizon, F.; Aboab, B.; Kucharczyk, N.; Léonce, S.; Golsteyn, R.M.; Pfeiffer, B.; Prudhomme, M. Bisimide granulatimide analogues as potent Checkpoint 1 kinase inhibitors. *Eur. J. Pharmacol.,* **2007**, *554*(2-3), 106-112.
[http://dx.doi.org/10.1016/j.ejphar.2006.10.022]
[PMID: 17134696]

[90] Conchon, E.; Anizon, F.; Aboab, B.; Prudhomme, M. Synthesis and biological activities of new checkpoint kinase 1 inhibitors structurally related to granulatimide. *J. Med. Chem.,* **2007**, *50*(19), 4669-4680.
[http://dx.doi.org/10.1021/jm070664k] [PMID: 17722905]

[91] Conchon, E.; Anizon, F.; Aboab, B.; Golsteyn, R.M.; Léonce, S.; Pfeiffer, B.; Prudhomme, M. Synthesis, *in vitro* antiproliferative activities, and Chk1 inhibitory properties of pyrrolo[3,4-a]carbazole-1,3-diones, pyrrolo[3,4-c]carbazole-1,3-diones, and 2-aminopyridazino[3,4-a]pyrrolo[3,4-c]carbazole-1,3,4,7-tetraone. *Eur. J. Med. Chem.,* **2008**, *43*(2), 282-292.
[http://dx.doi.org/10.1016/j.ejmech.2007.03.026]
[PMID: 17502122]

[92] Conchon, E.; Anizon, F.; Aboab, B.; Golsteyn, R.M.; Léonce, S.; Pfeiffer, B.; Prudhomme, M. Synthesis, checkpoint kinase 1 inhibitory properties and *in vitro* antiproliferative activities of new pyrrolocarbazoles. *Bioorg. Med. Chem.,* **2008**, *16*(8), 4419-4430.
[http://dx.doi.org/10.1016/j.bmc.2008.02.061] [PMID: 18321713]

[93] Smaill, J.B.; Lee, H.H.; Palmer, B.D.; Thompson, A.M.; Squire, C.J.; Baker, E.N.; Booth, R.J.; Kraker, A.; Hook, K.; Denny, W.A. Synthesis and structure-activity relationships of soluble 8-substituted 4-(2-chlorophenyl)-9-hydroxypyrrolo[3,4-c]carbazole-1,3(2H,6H)-diones as inhibitors of the Wee1 and Chk1 checkpoint kinases. *Bioorg. Med. Chem. Lett.,* **2008**, *18*(3), 929-933.
[http://dx.doi.org/10.1016/j.bmcl.2007.12.046] [PMID: 18191399]

[94] Lefoix, M.; Coudert, G.; Routier, S.; Pfeiffer, B.; Caignard, D.H.; Hickman, J.; Pierré, A.; Golsteyn, R.M.; Léonce, S.; Bossard, C.; Mérour, J.Y. Novel 5-azaindolocarbazoles as cytotoxic agents and Chk1 inhibitors. *Bioorg. Med. Chem.,* **2008**, *16*(9), 5303-5321.
[http://dx.doi.org/10.1016/j.bmc.2008.02.086] [PMID: 18342518]

[95] Conchon, E.; Anizon, F.; Golsteyn, R.M.; Leonce, S.; Pfeiffer, B.; Prudhomme, M. Synthesis, *in vitro* antiproliferative activities, and Chk1 inhibitory properties of dipyrrolo[3,4-a:3,4-c]carbazole-triones. *Tetrahedron,* **2006**, *62*(48), 11136-11144.
[http://dx.doi.org/10.1016/j.tet.2006.09.027]

[96] Shah, N.; Pang, B.; Yeoh, K.G.; Thorn, S.; Chen, C.S.; Lilly, M.B.; Salto-Tellez, M. Potential roles for the PIM1 kinase in human cancer - a molecular and therapeutic appraisal. *Eur. J. Cancer,* **2008**, *44*(15), 2144-2151.
[http://dx.doi.org/10.1016/j.ejca.2008.06.044] [PMID: 18715779]

[97] Morishita, D.; Katayama, R.; Sekimizu, K.; Tsuruo, T.; Fujita, N. Pim kinases promote cell cycle progression by phosphorylating and down-regulating p27Kip1 at the transcriptional and posttranscriptional levels. *Cancer Res.,* **2008**, *68*(13), 5076-5085.
[http://dx.doi.org/10.1158/0008-5472.CAN-08-0634]
[PMID: 18593906]

[98] Fox, C.J.; Hammerman, P.S.; Cinalli, R.M.; Master, S.R.; Chodosh, L.A.; Thompson, C.B. The serine/threonine kinase Pim-2 is a transcriptionally regulated apoptotic inhibitor. *Genes Dev.,* **2003**, *17*(15), 1841-1854.
[http://dx.doi.org/10.1101/gad.1105003] [PMID: 12869584]

[99] Aho, T.L.; Sandholm, J.; Peltola, K.J.; Mankonen, H.P.; Lilly, M.; Koskinen, P.J. Pim-1 kinase promotes inactivation of the pro-apoptotic Bad protein by phosphorylating it on the Ser112 gatekeeper site. *FEBS Lett.,* **2004**, *571*(1-3), 43-49.
[http://dx.doi.org/10.1016/j.febslet.2004.06.050]
[PMID: 15280015]

[100] Zheng, H.C.; Tsuneyama, K.; Takahashi, H.; Miwa, S.; Sugiyama, T.; Popivanova, B.K.; Fujii, C.; Nomoto, K.; Mukaida, N.; Takano, Y. Aberrant Pim-3 expression is involved in gastric adenoma-adenocarcinoma sequence and cancer progression. *J. Cancer Res. Clin. Oncol.,* **2008**, *134*(4), 481-488.
[http://dx.doi.org/10.1007/s00432-007-0310-1] [PMID: 17876606]

Personal Use Only
Not For Distribution

[101] Li, Y.Y.; Wu, Y.; Tsuneyama, K.; Baba, T.; Mukaida, N. Essential contribution of Ets-1 to constitutive Pim-3 expression in human pancreatic cancer cells. *Cancer Sci.,* **2009**, *100*(3), 396-404. [http://dx.doi.org/10.1111/j.1349-7006.2008.01059.x] [PMID: 19154409]

[102] Popivanova, B.K.; Li, Y.Y.; Zheng, H.; Omura, K.; Fujii, C.; Tsuneyama, K.; Mukaida, N. Proto-oncogene, Pim-3 with serine/threonine kinase activity, is aberrantly expressed in human colon cancer cells and can prevent Bad-mediated apoptosis. *Cancer Sci.,* **2007**, *98*(3), 321-328. [http://dx.doi.org/10.1111/j.1349-7006.2007.00390.x] [PMID: 17270021]

[103] Bartek, J.; Lukas, J. Chk1 and Chk2 kinases in checkpoint control and cancer. *Cancer Cell,* **2003**, *3*(5), 421-429. [http://dx.doi.org/10.1016/S1535-6108(03)00110-7] [PMID: 12781359]

[104] Zhang, Y.; Hunter, T. Roles of Chk1 in cell biology and cancer therapy. *Int. J. Cancer,* **2014**, *134*(5), 1013-1023. [http://dx.doi.org/10.1002/ijc.28226] [PMID: 23613359]

[105] Merry, C.; Fu, K.; Wang, J.; Yeh, I.J.; Zhang, Y. Targeting the checkpoint kinase Chk1 in cancer therapy. *Cell Cycle,* **2010**, *9*(2), 279-283. [http://dx.doi.org/10.4161/cc.9.2.10445] [PMID: 20023404]

[106] Jiang, X.; Zhao, B.; Britton, R.; Lim, L.Y.; Leong, D.; Sanghera, J.S.; Zhou, B.B.; Piers, E.; Andersen, R.J.; Roberge, M. Inhibition of Chk1 by the G2 DNA damage checkpoint inhibitor isogranulatimide. *Mol. Cancer Ther.,* **2004**, *3*(10), 1221-1227. [PMID: 15486189]

[107] Zhao, B.; Bower, M.J.; McDevitt, P.J.; Zhao, H.; Davis, S.T.; Johanson, K.O.; Green, S.M.; Concha, N.O.; Zhou, B.B. Structural basis for Chk1 inhibition by UCN-01. *J. Biol. Chem.,* **2002**, *277*(48), 46609-46615. [http://dx.doi.org/10.1074/jbc.M201233200] [PMID: 12244092]

[108] Smaill, J.B.; Baker, E.N.; Booth, R.J.; Bridges, A.J.; Dickson, J.M.; Dobrusin, E.M.; Ivanovic, I.; Kraker, A.J.; Lee, H.H.; Lunney, E.A.; Ortwine, D.F.; Palmer, B.D.; Quin, J., III; Squire, C.J.; Thompson, A.M.; Denny, W.A. Synthesis and structure-activity relationships of N-6 substituted analogues of 9-hydroxy-4-phenylpyrrolo[3,4-c]carbazole-1,3(2H,6H)-diones as inhibitors of Wee1 and Chk1 checkpoint kinases. *Eur. J. Med. Chem.,* **2008**, *43*(6), 1276-1296. [http://dx.doi.org/10.1016/j.ejmech.2007.07.016] [PMID: 17869387]

[109] Gingrich, D.E.; Reddy, D.R.; Iqbal, M.A.; Singh, J.; Aimone, L.D.; Angeles, T.S.; Albom, M.; Yang, S.; Ator, M.A.; Meyer, S.L.; Robinson, C.; Ruggeri, B.A.; Dionne, C.A.; Vaught, J.L.; Mallamo, J.P.; Hudkins, R.L. A new class of potent vascular endothelial growth factor receptor tyrosine kinase inhibitors: Structure-activity relationships for a series of 9-alkoxymethyl-12-(3-hydroxypropyl)indeno[2,1-a]pyrrolo[3,4-c]carbazole-5-ones and the identification of CEP-5214 and its dimethylglycine ester prodrug clinical candidate CEP-7055. *J. Med. Chem.,* **2003**, *46*(25), 5375-5388. [http://dx.doi.org/10.1021/jm0301641] [PMID: 14640546]

[110] Becknell, N.C.; Zulli, A.L.; Angeles, T.S.; Yang, S.; Albom, M.S.; Aimone, L.D.; Robinson, C.; Chang, H.; Hudkins, R.L. Novel C-3 N-urea, amide, and carbamate dihydroindazolo[5,4-a]pyrrolo[3,4-c]carbazole analogs as potent TIE-2 and VEGF-R2 dual inhibitors. *Bioorg. Med. Chem. Lett.,* **2006**, *16*(20), 5368-5372. [http://dx.doi.org/10.1016/j.bmcl.2006.07.066] [PMID: 16890434]

[111] Underiner, T.L.; Ruggeri, B.; Aimone, L.; Albom, M.; Angeles, T.; Chang, H.; Hudkins, R.L.; Hunter, K.; Josef, K.; Robinson, C.; Weinberg, L.; Yang, S.; Zulli, A. TIE-2/VEGF-R2 SAR and *in vitro* activity of C3-acyl dihydroindazolo[5,4-a]pyrrolo[3,4-c]carbazole analogs. *Bioorg. Med. Chem. Lett.,* **2008**, *18*(7), 2368-2372. [http://dx.doi.org/10.1016/j.bmcl.2008.02.069] [PMID: 18343109]

[112] Dandu, R.; Zulli, A.L.; Bacon, E.R.; Underiner, T.; Robinson, C.; Chang, H.; Miknyoczki, S.; Grobelny, J.; Ruggeri, B.A.; Yang, S.; Albom, M.S.; Angeles, T.S.; Aimone, L.D.; Hudkins, R.L. Design and synthesis of dihydroindazolo[5,4-a]pyrrolo[3,4-c]carbazole oximes as potent dual inhibitors of TIE-2 and VEGF-R2 receptor tyrosine kinases. *Bioorg. Med. Chem. Lett.,* **2008**, *18*(6), 1916-1921. [http://dx.doi.org/10.1016/j.bmcl.2008.02.001] [PMID: 18308565]

[113] Ma, H.; Nguyen, B.; Li, L.; Greenblatt, S.; Williams, A.; Zhao, M.; Levis, M.; Rudek, M.; Duffield, A.; Small, D. TTT-3002 is a novel FLT3 tyrosine kinase inhibitor with activity against FLT3-associated leukemias *in vitro* and *in vivo. Blood,* **2014**, *123*(10), 1525-1534. [http://dx.doi.org/10.1182/blood-2013-08-523035] [PMID: 24408321]

[114] Jiang, X.; Zhou, J.; Ai, J.; Song, Z.; Peng, X.; Xing, L.; Xi, Y.; Guo, J.; Yao, Q.; Ding, J.; Geng, M.; Zhang, A. Novel tetracyclic benzo[b]carbazolones as highly potent and orally bioavailable ALK inhibitors: Design, synthesis, and structure-activity relationship study. *Eur. J. Med. Chem.,* **2015**, *105*, 39-56. [http://dx.doi.org/10.1016/j.ejmech.2015.10.005] [PMID: 26476749]

[115] Organization, W.H. *Global Health Observatory.,* www.who.int/gho/database/en/

[116] Gross, S.; Rahal, R.; Stransky, N.; Lengauer, C.; Hoeflich, K.P. Targeting cancer with kinase inhibitors. *J. Clin. Invest.,* **2015**, *125*(5), 1780-1789. [http://dx.doi.org/10.1172/JCI76094] [PMID: 25932675]

[117] Shchemelinin, I.; Sefc, L.; Necas, E. Protein kinases, their function and implication in cancer and other diseases. *Folia Biol. (Praha),* **2006**, *52*(3), 81-100. [PMID: 17089919]

[118] Chen, X.W.; Sun, J.; Zhou, S.F. Protein Kinase Inhibitors for Clinical Targeted Cancer Treatment. *Clin. Pharmacol. Biopharm.,* **2013**, *2*(1), 1000-1112. [http://dx.doi.org/10.4172/2167-065X.1000e112]

[119] Sinha, S.; Pal, B.C.; Jagadeesh, S.; Banerjee, P.P.; Bandyopadhaya, A.; Bhattacharya, S. Mahanine inhibits growth and induces apoptosis in prostate cancer cells through the deactivation of Akt and activation of caspases. *Prostate,* **2006**, *66*(12), 1257-1265. [http://dx.doi.org/10.1002/pros.20415] [PMID: 16683271]

[120] Chatterjee, P.; Seal, S.; Mukherjee, S.; Kundu, R.; Bhuyan, M.; Barua, N.C.; Baruah, P.K.; Babu, S.P.; Bhattacharya, S. A carbazole alkaloid deactivates mTOR through the suppression of rictor and that induces apoptosis in lung cancer cells. *Mol. Cell. Biochem.,* **2015**, *405*(1-2), 149-158. [http://dx.doi.org/10.1007/s11010-015-2406-2] [PMID: 25893736]

[121] Liu, Q.; Batt, D.G.; Lippy, J.S.; Surti, N.; Tebben, A.J.; Muckelbauer, J.K.; Chen, L.; An, Y.; Chang, C.; Pokross, M.; Yang, Z.; Wang, H.; Burke, J.R.; Carter, P.H.; Tino, J.A. Design and synthesis of carbazole carboxamides as promising inhibitors of Bruton's tyrosine kinase (BTK) and Janus kinase 2 (JAK2). *Bioorg. Med. Chem. Lett.,* **2015**, *25*(19), 4265-4269. [http://dx.doi.org/10.1016/j.bmcl.2015.07.102] [PMID: 26320619]

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