Anaplastic Lymphoma Kinase: Signal Transduction And Therapeutic Voyage of Alk Inhibitors

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Abstract- *The identification of anaplastic lymphoma kinase (ALK) rearrangements in 2-5% of non-small cell lung cancer (NSCLC) patients led to the rapid clinical development of tyrosine kinase inhibitors. Anaplastic lymphoma kinase (ALK), a tyrosine kinase has attracted significant amount of attention because of its oncogenic potential and essential role in the pathogenesis of a wide variety of human cancers such as anaplastic large cell lymphoma (ALCL), non-small cell lung cancer (NSCLC), squamous cell cancer of head and neck (SCCHN), breast cancer, neuro-blastoma and myo-fibroblastic tumor etc. To date nine ALK inhibitors have entered the clinical investigational phase for treatment of cancer. This review presents the role of ALK in certain cancers and signal transduction pathways, recent advances in drug development of ALK inhibitors and their synthetic routes. The review also presents the hit-drug evolution strategies of four ALK inhibitors and describes the current status of the small molecule ALK inhibitors isolated from natural sources and synthesized using variety of scaffolds. It anticipates to provide direction for design and synthesis of new compounds with privileged scaffolds possessing potent ALK inhibitory activity.*

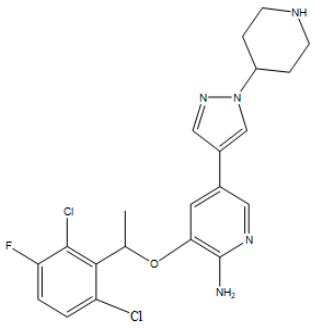
Keywords- ALK, NSCLC, ALCL, ALK inhibitors, crizotinib.

I. INTRODUCTION

Anaplasia is a condition in which cells lose their morphological characteristics and are disoriented with respect to each other as well as endothelial cells. These cells have nuclear pleomorphism, altered nuclear: cytoplasmic ratio, presence of nucleoli and high proliferation index which leads to malignancy.

Anaplastic lymphoma kinase (ALK) is a type of tyrosine kinase receptor which belongs to the insulin receptor superfamily. It is located on the short arm of chromosome 2, it was first identified as oncogene which was activated by chromosomal translocation in anaplastic large cell lymphoma patients. [1,2] Anaplastic lymphoma kinase is generally expressed only in the nervous system regions of thalamus and midbrain which suggests that it has a vital role in development and maintenance of central as well as peripheral nervous system.[3] By far no specific physiological function of ALK has been identified but formation of ALK fusion proteins has been linked to many human cancers which occurs due to over-expression of ALK gene.[4] Nucleophosmin (NPM) –ALK fusion protein was observed in about 75% patients with anaplastic large cell lymphoma patients which had t (2,5) chromosomal translocation. ALK fusion protein has also been observed in pediatricneuroblastoma, [5] breast cancer, ovarian cancer and inflammatory myofibroblastic tumours. [6] Most importantly the fusion protein of ALK with echinoderm micro-tubule associated protein like 4(EMLK-4) was identified in non- small cell lung cancer patients [7] and since then ALK has been an attractive target for anti- tumour activity. Every living organism has the ability to regulate cell cycle progression and maintain the genomic integrity. Activation of ALK gene which is result of variety of chromosomal rearrangements, mutations or its amplification has been associated with tumorigenesis, and progression of certain cancers such as non-small cell lung carcinoma (NSCLC) breast cancer and neuroblastoma. On inhibition of ALK, the formation of fusion protein of a variety of gene that are responsible for ALK-positive cancers is suppressed and hence ALK inhibitors are seen as breakthrough interventions for malignancies arising out of ALK deregulation.

The first generation ALK inhibitor Crizotinib (XALKORI ®) was developed by Pfizer and approved by FDA in 2011 for the treatment of ALK positive non-small cell lung cancer (NSCLC). It inhibits the ALK activity by binding competitively in the ATP binding site thereby inhibiting the activity of the fusion protein formed by ALK.



Crizotinib (PH-02314066)

The structural analogues of crizotinib were thus suggested to be potent ALK inhibitors and in the process of development of novel inhibitors various scaffolds were explored for generation of ALK inhibitors.

II. STRUCTURE OF ANAPLASTIC LYMPHOMA KINASE RECEPTOR

The human anaplastic lymphoma kinase gene encrypts a 176 kDa protein which undergoes post-translational changes such as N-glycosylation.[3,8]The ALK receptor is a single pass trans-membrane protein which comprises of an extracellular region of 1030 amino acids, containing an N-terminal peptide, two meprin, A-5 protein, receptor protein tyrosine phosphatase mu (MAM) domains segregated by a low density lipoprotein class A (LDL-A) domain and a glycine rich region proximal to the transmembrane element that links the extracellular region with the intracellular region. The MAM domain of the receptor consist of about 160 amino acids which are involved in cell interactions. [9] The function of LDL-A domain is still unknown; however, it is thought to be involved in ligand recognition. [10] The intracellular region consists of a juxtamembrane element and a tyrosine kinase domain. The function of juxtamembrane is still unknown in ALK receptor but in other receptors it is found to be involved in modulation of kinase catalytic activity. [11] The kinase domain contains three auto-phosphorylation sites in the tyrosine residues 1278, 1282 and 1283 known as the YXXXYY motif whose phosphorylation controls the kinase activity of anaplastic lymphoma kinase. [12,13]

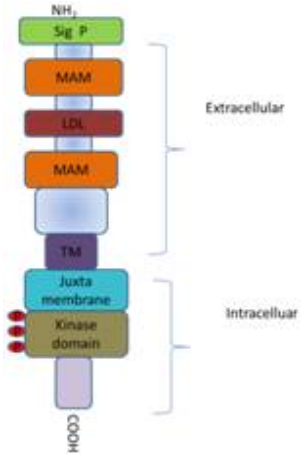


Fig. (1) Structure of ALK protein

III. ROLE OF ANAPLASTIC LYMPHOMA KINASE IN CANCER

Anaplastic lymphoma kinase leads to tumorogenesis because of aberrant signalling. A number of mechanisms for unregulated signalling have been discovered which include:

i. Translocation or structural rearrangements
ii. ALK gene amplification
iii. Mutations
iv. Over-expression

ALK fusion proteins translocation

Translocation is the most common cause of genomic aberration. [14] In physiological ALK signalling, ligand induced homo-dimerization of the extracellular domain is proposed to put forward the tyrosine kinase domain into close proximity to perform trans-phosphorylation and kinase activity. By discrepancy, translocations bring forth pathogenic fusion partners which supply dimerization domains that are ligand independent, leading to unregulated constitutive kinase activity and malignant transformation.[15] Site-targeted mutagenesis of the dimerization domain revoke NPM-ALK-transforming activity suggesting that proximity of the ALK kinase domains is a primary requirement to ease the trans-phosphorylation of the partner kinase.[16,17] The shortened NPM also encodes a nucleolar localization domain that explains why in contrast to other ALK fusions, NPM-ALK can be detected in the cytoplasm, nucleus, and nucleolus whereas most fusion products are largely cytoplasmic.[4] The remaining 20% of translocations fuse ALK to other partners that commonly encrypt coiled-coil oligomerization domains such as Tyrosine Receptor Kinase-fused gene (TFG) Tropomyosin 3 (TPM3), and Tropomyosin 4 (TPM4).

Mutations and genomic amplification

Expression of ALK has been reported in a large fraction of human-derived neuroblastoma cell lines,[18] and ALK was previously identified as a candidate for neuroblastoma oncogene through somatically acquired amplification of the genomic locus.[19,20] Recently both germline and somatic mutations that activate ALK have been discovered in neuroblastoma.[21- 23] Heritable gain of function mutations are been reported in most of the ALK activated human cancers.[24]Heritable mutations have been restricted to the kinase domain of ALK, and it is hypothesised, that the second hit is somatic gain or amplification of the mutant gene, similar to what is seen at the MET locus in hereditary renal papillary cancer. [25] Although the number of families studied remains relatively small, it is evident that the R1275Q mutation is seen most commonly, and it seems that disease penetrance may be credited to mutation type as rarer mutations, such as G1128A seen in a large population, was associated with very low penetrance compared with the near complete penetrance seen with the R1275Q mutation. Both mutations fall within the kinase activation loop in a region

strongly associated with activating mutations seen in other kinases that are capable to cause cancer. [21]

Expression

ALK expression is of uncertain pathogenic importance in several human cancers. The detection of ALK mRNA in diverse human tumour-derived cell lines, as well as the detection of higher ALK expression in tumour compared with neighbouring normal tissue, suggests that physiological expression is more prominent.[26,27] ALK has previously been shown to be upregulated and functionally relevant in glioblastoma, and recent work has shown that ribozyme-mediated knockdown of ALK results in eradication of tumour growth in xenograft model.[28] This offers potentially novel therapeutic options for an otherwise lethal brain tumour. Immuno- histochemical detection of ALK protein overexpression is pathognomonic for ALCL, and recent work suggests that ALK immune-histochemistry may have utility as a screening tool or surrogate marker for EML4-ALK fusion gene-positive NSCLC tumours. Recent studies are focused on developing reliable techniques for phospho-ALK staining in routine clinical specimens, and this may be critically important to properly evaluate future ALK inhibitor clinical trials.[29]

Anaplastic Lymphoma Kinase as Oncogene

ALK was first identified as an oncogene in anaplastic large cell lymphoma and since then aberrant signalling of ALK has been observed and reported in a variety of cancers. This aberrant signalling is linked with chromosomal rearrangements, mutations or amplification of ALK gene leading to tumorigenesis. Because of these chromosomal rearrangements of ALK gene it produces fusion proteins. These fusion proteins retain the intracellular region of the ALK gene which contains the tyrosine kinase domain, and the extracellular region which contains the ligand-binding site is replaced by the dimerization domain of the partner protein which promotes trans-phosphorylation of the kinase domain independently of the ligand. These formation of the fusion protein derived from translocation is involved in ALK generated human malignancies.

IV. ANAPLASTIC LYMPHOMA KINASE SIGNALLING

The ALK signal transduction starts when its ligand binds, which in course stimulates dimerization and trans-phosphosphorylation. Following activation by the ligand, ALK leads to cellular processes that form a part of the oncogenesis in humans. The ALK receptor is responsible for activation of various signal transduction pathways. The important ALK induced pathways are extracellular signal regulated kinase (Ras/ERK), signal transducers and activator of transcription/ Janus kinase (JAK/STAT) and Phosphatidyl inositol 3 (PI3/AKT) which are actively involved in proliferation, migration and cell survival.

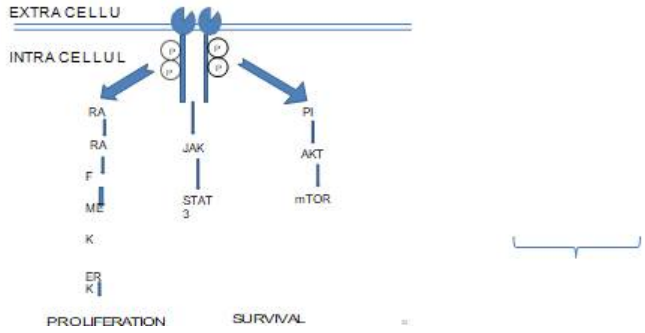


Fig. (2). Overview of ALK signalling pathways.

The Ras/ERK Pathway

The extracellular signal regulated kinase (ERK) is involved in controlling of cellular processes such as differentiation, proliferation and migration. ERK signalling is activated by a number of extracellular signals like growth factors. [30] Stimulation of receptor tyrosine kinase such as ALK promotes the exchange of GDP for GTP in the RasGTPase, which then enrols Raf kinase to the plasma membrane for its activation. [31] This Raf kinase activates MEK by phosphorylation of its two serine residues. Activated MEK then phosphorylates ERK in threonine and tyrosine residues, phosphorylated ERK translocate to the nucleus to modify gene expression through phosphorylation of transcription factors. [32,33]

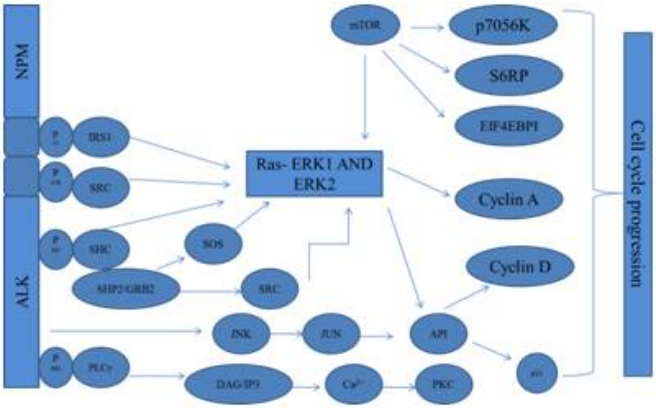


Fig.(3). Ras/ ERK pathway

The JAK/STAT Pathway

Signal transducers and activator of transcription (STAT) are a family of latent cytoplasmic transcription factors

which are activated in most of the cases by growth factor receptors, such as ALK. [34] The STATs regulate various processes leading to oncogenesis, including angiogenesis, proliferation and survival by regulating the expression of a variety of genes. The Janus protein tyrosine kinases (JAKs) are enzymes that intercede deactivation of STATs in response to growth factors. [35] Stimulation of growth factor receptors promotes activation of JAK through trans-phosphorylation mechanisms. Once activated, JAK phosphorylates to
STATs in conserved tyrosine residues, resulting in their dimerization and translocation to the nucleus, where STAT dimers vary expression of their target genes involved in proliferation and survival. [36,37]

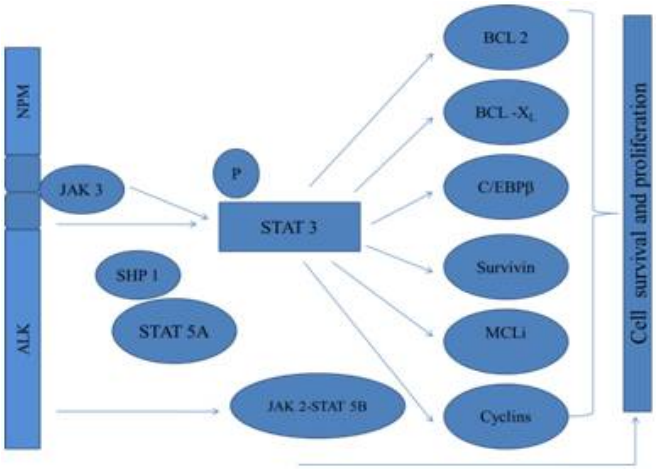


Fig. (4). The JAK/STAT Pathway

The PI3/AKT Pathway

PI3K is a lipid kinase that is activated by RTK like ALK.[38] PI3K generates phosphatidylinositol-3,4,5-trisphosphate second messenger that enrols proteins with the pleckstrin homology domain (PH domain) to the plasma membrane.[39] AKT is a serine/threonine kinase with PH domain that plays an significant role in multiple cellular processes, such as proliferation, migration, and survival.[40] AKT is activated by phosphorylation on two sites, threonine 308 and serine 473, by the phosphoinositide-dependent kinase (PDK1) and the hypothetical PDK2 kinase, respectively. Activated AKT can phosphorylate numerous downstream substrates involved in proliferation and survival, thus showing active involvement in oncogenesis. [41,42]

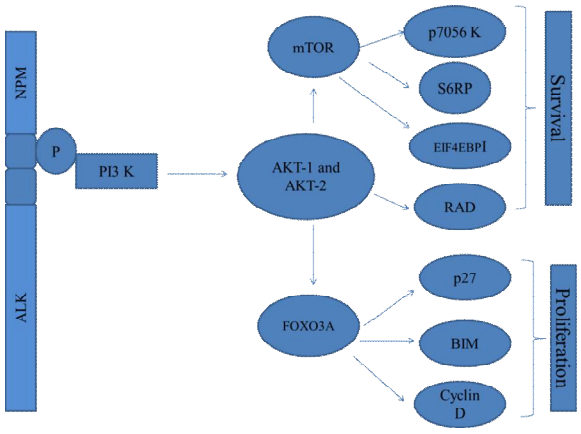


Fig. (5) The PI3/AKT Pathway

Table (1): Alteration of anaplastic lymphoma kinase gene in cancer [43]

Disease	Fusion Protein	Chromosomal abnormality	Gene amplification
ALCL	NPM-ALK TPM3-ALK	t(2;5)(p23;q35) t(1;2)(p25;q35)	
DLBCL	NPM-ALK	t(2;5)(p23;q35)	
NSCLC	EML4-ALK	Inv(2)(p21;p23)	
Breast cancer	EML4-ALK	Inv(2)(p21;p23)	
Neuroblastoma			ALK

ALCL= anaplastic large cell lymphoma; DLBCL=diffuse large B-cell lymphoma; NSCLC= non-small cell lung cancer

Table (2): Current status of ALK Inhibitors

Agent	Chemical series	Company	Disease	Clinical status
Crizotinib	Aminopyridine	Pfizer	NSCLC	Approved
Alectinib	Benzocarbazole	Chugai Pharmaceuticals	NSCLC, ALCL, Neuroblastoma	Approved
Ceritinib	Diaminopyrimidine	Novartis	NSCLC	Approved
Brigatinib	Diaminopyrimidine	Ariad Pharmaceuticals	NSCLC	Phase 2
Lorlatinib	Aminopyridine	Pfizer	NSCLC	Phase 2
X-396	Pyridazinecarboxamide	Xcovery	NSCLC, ALCL, Neuroblastoma	Preclinical
TSR-011	Benzamide	Tesaro	NSCLC, Pancreatic	Phase 2
			cancer, Salivary gland cancer	
CEP-37440	Benzamide	Teva Pharmaceuticals	NSCLC, Prostate cancer, Colorectal cancer, SCCHN	Phase 1
NMS-E628 (Enterictinib)	Benzamide	Nerviano Medical Sciences.	Advanced solid tumours	Phase 2

NSCLC= Non-small cell lung cancer, ALCL= Anaplastic large cell lymphoma, SCCHN= Squamous cell cancer of head and neck.

V. ANAPLASTIC LYMPHOMA KINASE INHIBITORS

These are chemical agents that are involved in the destruction of tumours arising out of ALK aberrant signalling.

First generation ALK Inhibitors

The first generation ALK inhibitors comprise of only one drug crizotinib.

Crizotinib was developed from lead compound SU11274**(1)** which was identified as c-MET inhibitor with IC50 of 10 nM. Optimization of SU11274 lead to PHA665732**(2)** with a significantly improved cellular potency but had poor pharmaceutical properties which limited its development for human clinical studies. Thus smaller and less lipophilic inhibitors with good kinase activity were sought. Based on the co-crystal structure of PHA66572 bound to c-MET kinase domain a series of 2-amino-5-aryl-3-benzyloxypyiridines were designed. In this new chemo type the 2- aminopyridine NH and ring nitrogen would make hydrogen bond to the hinge protein residues. 2– amino pyridines core allowed a 3-benzyloxy group to fit into the same pocket as the 2,6- dichlorophenyl group of PHA665732 which gave better ligand efficiency 5-aryl group of this scaffold would point towards the solvent in the same way as oxindole – pyrrole substituents do providing an handle to modulate lipophilicity. [45] It has been approved for treatment of locally advanced or metastatic NSCLC that are ALK positive. It consists of the aminopyridine chemical motif. It has an IC50 value of 10nM-24Nm. Unfortunately, patients develop invariable resistance during the first year of the initiation of therapy and thus the drug proves to be inactive. There have been case reports of significant adverse effects that were not reported in the initial trials. These included erythema multiform, acute interstitial lung disease, renal polycytosis, contact esophagitis, decrease in GFR, and hypersensitivity. [43]

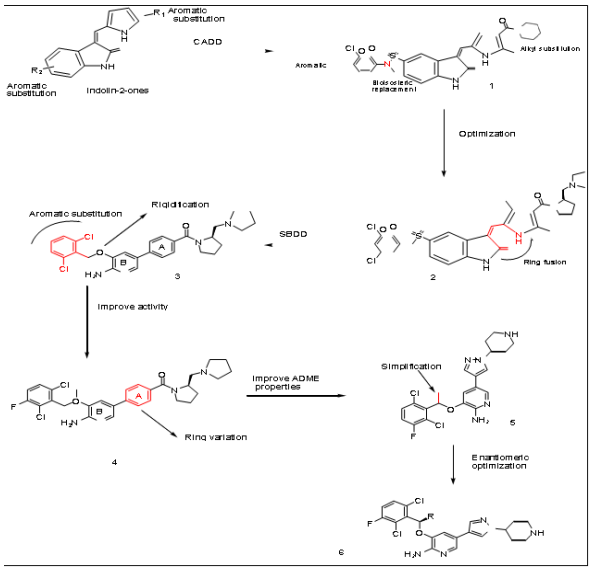


Fig. (6). Discovery of crizotinib

Second-generation ALK inhibitors

Alectinib

Alectinib (CH5424802, alecensa) is a potent and highly selective inhibitor of ALK tyrosine kinase with IC50 of 1.9 nM. Alectinib consist of benzo[b] carbazole scaffold. Significantly, alectinib showed potent antitumor activity by overcoming crizotinib-resistant L1196M and C1156Y mutants, as well as inhibiting EML4–ALKL1196M driven cell growth. Compound **7** was identified as a weak ALK inhibitor (IC50 ¼ 1.3 mM) without c-Met inhibitory activity (IC50> 50 mM) through HTS of an in-house library. From discovery of crizotinib (a dual c- Met/ALK inhibitor) that a highly selective inhibitor may increase the therapeutic window of drug safety, selective ALK inhibitor **7** was further developed. [44] At first, variation of the benzofuran scaffold by bioisosteric replacement with an indole ring in order to increase the H-bonding interaction between the carbonyl group at the 11-position and the hinge region Met- 1199 of ALK was attempted. The replacement of the ethoxy group at the 3-position with a cyano group also showed a favourable interaction with the protein. The cyano group was thought to form a critical interaction with the ALK protein, and played an important role in the development of ALK inhibitors. The above two modifications led to the identification of compound **8** which showed a 100-fold ALK activity increase compared to compound **7** and also possessed good cell growth inhibition against NPM–ALK driven cell line KARPAS-299, with an IC50 value of 60 nM. Unfortunately, compound **8** was found to have high in vitro clearance in both mouse (CL ¼ 51.4 mL min_1 mg_1) and human (CL 54.5 mL/min/mg) liver microsomes, perhaps due to oxidative dealkylation of the diethylamino group. The next strategy used was to modify the 8-position substituent of the 3-cyanobenzo[b] carbazole scaffold in order to block or slow down the metabolism and clearance. It was found that substituents with a cationic nitrogen atom at the 8-position would interact with the solvent- accessible region of the ALK protein, as seen in the N-oxetan-3-yl-piperidin-4-yl derivative**9**, resulting in improved metabolic stability (CL 10.2 mL/min/mg) in human liver microsomes). Derivative 9 showed inhibitory activity against enzymatic ALK and cellular KARPAS-299 with IC50 values of 1.5 nM and 21.4 nM, respectively. Compound **9** further showed significant tumour regression. Even though ALK inhibitor **9** had weak inhibitory activity against c-Met (IC50 7200 nM), it was much more active against KDR (IC50 ¼ 100 nM), which is associated with hypertensive side effects. [45] Docking studies of compound **9** to an ALK homology modelled structure showed that the hydrophobic substituents at the 9-position play an essential role in kinase selectivity. Among several synthesized analogues bearing various alkyl

and alkynyl groups at the 9-position and an N-(oxetan-3-yl) piperazine moiety at the 8-position, compound **10** bearing a 9-ethyl substituent showed excellent selectivity against enzymatic ALK (IC50 2.9 nM). The greatly improved target selectivity of compound **10** compared to that of compound **9**, as well as strong anti-proliferative activity against KARPAS-299 (IC5012.8 nM), suggested further testing of this compound would be worthwhile. In vivo study of compound 10 revealed significant tumour regression at 20 mg kg^{-1} oral dosage without significant body weight loss in NPM–ALK positive ALCL mouse xenograft models. The final design focused on varying the substituents at the 8- position to achieve better ADME properties, since compound 10 only had 28.2% oral bioavailability. Compound **11** (alectinib) bearing the 4-morpholinopiperidine group displayed superior bioavailability (F ¼ 50.4% in monkey) compared to compound **10**, and showed potent activity against enzymatic ALKWT (1.9 nM) as well as the mutated ALK enzymes such as ALKL1196M (Ki 1.56 nM), ALKF1174L (IC501.0 nM)[46]

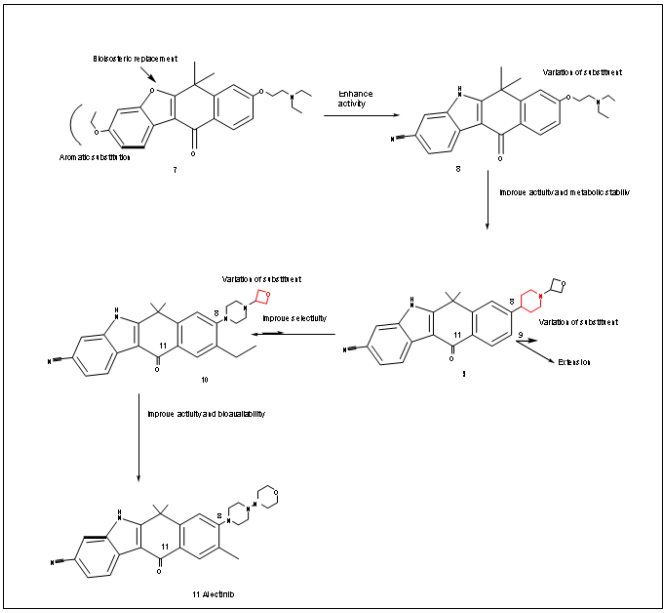


Fig. (7). Discovery of alectinib

Ceritinib

Ceritinib is an orally available second-generation ALK inhibitor developed by Novartis, which received accelerated approval by the FDA in April 2014 as a treatment for patients with ALK positive metastatic NSCLC, [44] who were previously treated with crizotinib. Compound **12** with the diaminopyrimidine scaffold is a potent and highly selective ALK inhibitor identified in a cellular screening designed to discover compounds with activities against NPM–ALK activated Ba/F3 cells. Hit 12showed good activity against Ba/F3–NPM–ALK with an IC50 value of 3 nM,and displayed higher potency against the two EML4–ALK positive NSCLC cell lines, NCI-H2228 and NCI-H3122 (IC50 values of16 nM and 44 nM) than crizotinib (871 nM and 1551 nM respectively).Although compound **12** showed potent in vivo efficacy, clinical development was not possible due to the toxicity associated with the generated 1,4- diiminoquinone as a result of oxidative metabolism. Three approaches to slow the metabolism rate were designed: an increase in steric bulk by replacing the methoxy moiety by an isopropoxy group; blockage of the para position of the alkoxy group by adding a methyl group; and reversal of piperidine attachment to avoid the formation of 1,4- diiminoquinone.[47] After synthesizing several analogues using the above design strategy, compound **13** (ceritinib) was found to be the most effective against Ba/F3–NPM–ALK and KARPAS-299 with IC50 values of 26 nM and 22.8 nM, respectively. Ceritinib was approved for treatment of relapsed or refractory NSCLC after crizotinib.[48]

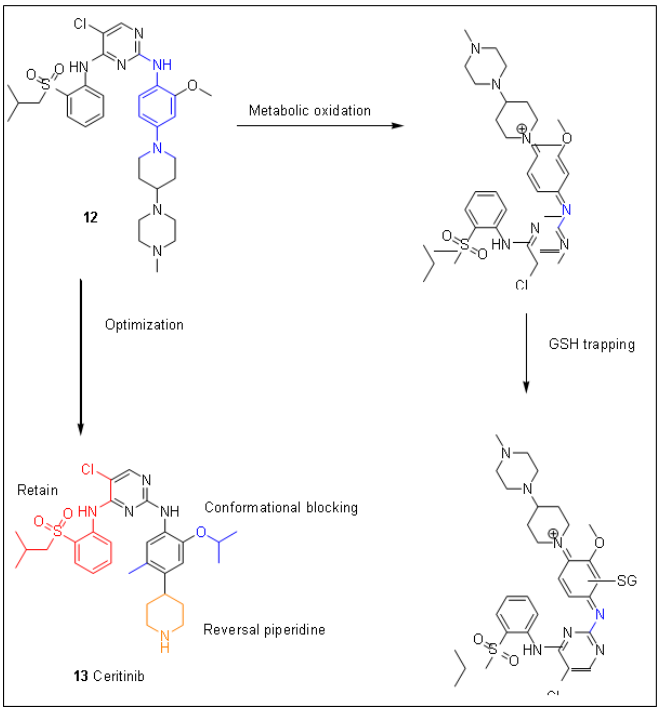
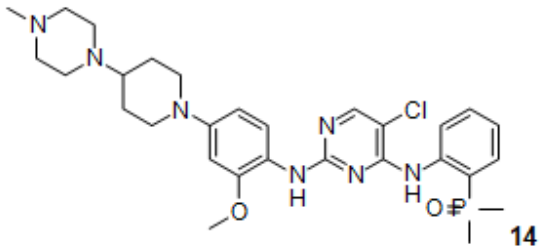


Fig. (8). Discovery of ceritinib

Brigatinib (AP26113)

Brigatinib**14** is another second-generation ALK inhibitor. It is a potent dual inhibitor of ALK and EGFR, including ALK L1196M and EGFR T790M mutants, shown in preclinical and first- in-human studies. [49] Brigatinib was active in crizotinib-resistant NSCLC and showed activity in CNS lesions. A randomized phase2 trial of brigatinib in

crizotinib-resistant ALK+ NSCLC(ALTA) is underway. It consists of the diaminopyrimidine chemical motif. [50]



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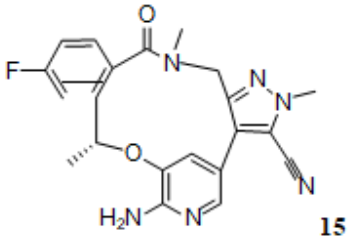
Third-generation ALK inhibitor

Lorlatinib

Lorlatinib (PF-06463922) is a novel, reversible, potent ATP-competitive small molecule inhibitor of ALK andROS1. This third-generation inhibitor is effective against all known resistant mutants. In preclinical studies, lorlatinib was proven to be active in crizotinib-resistant cancers both *in vitro* and in xenograft models.

To overcome ALK mutations and ALK inhibitor resistance, lorlatinib**15** was combined with PI3K pathway inhibitors, such as PF-05212384 (PI3K/mTOR), GDC0941 (pan-PI3K),or GDC0032 (beta-sparing). Such rational combination was reported to lead to more robust activity in vitro and greater duration of efficacy *in vivo* in the ALK inhibitor resistant models.[51-53] Lorlatinib is being studied in a phase I clinical trial in patients who were refractory to crizotinib and ceritinib.[54] One patient enrolled to this trial responded to lorlatinib for 8 months. [55] Interestingly, the patient was resensitized to crizotinib after the patient failed the lorlatinib treatment, indicating that retreatment under molecular guidance can be a clinically important approach. It consists of carbonitrile chemical motif.



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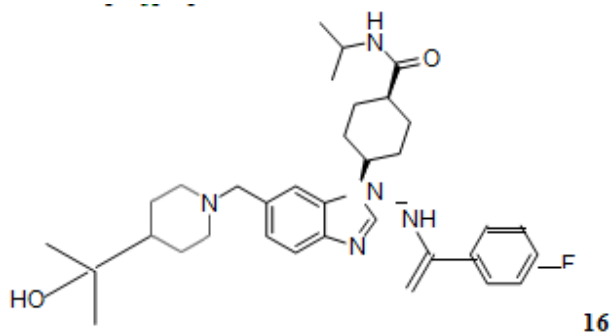
Other ALK Inhibitors in clinical trials

5.4.1TSR-011

TSR-011, **16** is an orally active dual ALK and TRK (tropomyosin-related kinases) inhibitor developed by Tesaro. It consists of the benzamide scaffold. It showed inhibitory activity against ALK, ALKL1196M and ALKR1275Q with IC50 values of 0.7 nM, 0.1 nM and 0.5 nM, respectively. It also showed potent anti-proliferative activity against KARPAS-299 (IC501 nM), Sup-M2 (IC50 4 nM), NCIH3122 (IC501nM), and NB-1 (IC5010 nM). In addition, it exhibited potent inhibition of TRK with IC50values of 0.5–2.4nM, and inhibited the proliferation of either TRKA-rearranged(IC5025nM) or NGF-stimulated TRKA (IC5042 nM) cell lines. TSR-011 further showcased promising activity in mouse models and is currently undergoing phase I/II clinical trials. According to the preliminary clinical results in 17 patients with treatment of TSR-011 for 8 weeks, 11 patients (65%) showed disease control. Three patients with ALK-positive NSCLC were progressed on prior treatment of crizotinib, and two patients showed response and one patient showed stable disease. In addition, one patient with papillary thyroid carcinoma and one patient with pancreatic cancer exhibited stable disease on treatment with TSR-011[44][55]



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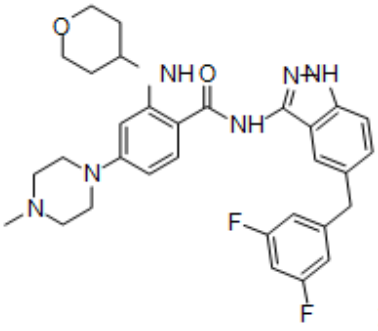
5.4.2 NMS-E628

NMS-E628, **17** is an orally available ALK inhibitor developed by Nerviano Medical Sciences. It consists of the benzamide scaffold. It showed good activity against NPM–ALK with an IC50 value of 55 nM. In vivo study of NMSE-628 showed complete tumour regression in both KARPAS-299 and SR-768 mouse xenograft models. When NMS-E628 was evaluated in Ba/F3–ALKL1196M and Ba/F3–ALKC1156Y cells that were identified in crizotinib resistant patients, it was able to inhibit them at lower dose than crizotinib, in both *in vitro* and *in-vivo* conditions. NMS-E628 also showed ATP competitive inhibition of ROS1 with an IC50 value of 7nM and induced complete tumour regression in Ba/F3-ROS1 mouse xenograft models for 10 days. It is currently being evaluated in phase I/II. [56,57]



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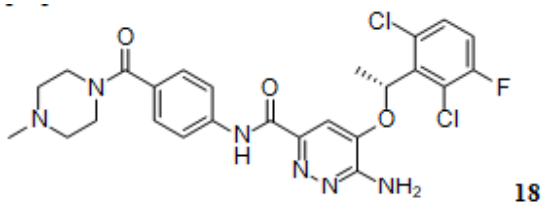
5.4.3 X-396

Xcovery reported X-396, **18** as a novel ALK inhibitor bearing an aminopyrazine scaffold. It showed comparatively superior ALK inhibition (IC50< 0.4 nM) than crizotinib (IC504.5 nM), and displayed inhibitory activity against NCI-H3122 (IC5015 nM), NCI-H2228 (IC5045 nM), SUDHL-1 (IC509 nM), and SY5Yneuroblastoma cells (ALKF1174L, IC5068 nM). In Ba/F3– NPM– ALK cells, X-396 was approximately 10-fold more potent than crizotinib (IC50 values of 22 nM and 250 nM, respectively). In two crizotinib-resistant mutations, EML4– ALKL1196M and EML4–ALKC1156Y, it exhibited IC50 values of 106 nM and 48 nM, respectively. In vivo study of X-396 showed significant tumour inhibition in NCI-H3122 (25 mg kg^{-1}, po, bid) mouse xenograft models without any signs of body weight loss or toxicity.

Based on these promising preclinical results, X-396 entered into phase I clinical trial testing. [58]



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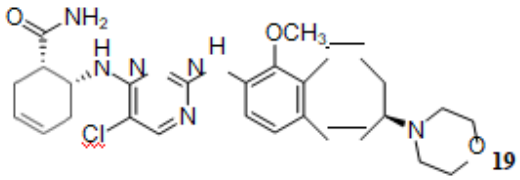
5.4.4 CEP-28122 and CEP-37440

CEP-28122,**19** bearing a diaminopyrimidine scaffold was identified by Cephalon as a highly potent and selective ALK inhibitor (>600-fold selectivity with respect to the insulin receptor), which showed inhibitory activity against enzymatic ALK and KARPAS-299 with IC50values of 1.9 nM and 20 nM, respectively. It also inhibited the growth of neuroblastoma cell lines NB- 1643 and SHSY5Y, harbouring ALK activating mutants F1174L and R1275Q. Moreover, CEP-28112 led to tumour regression in two ALK-positive ALCL mouse tumour xenografts, KARPAS-299 (30 mg kg^{-1}, po, bid) and Sup-M2 (55 or 100 mg kg^{-1}, po, bid for 4 weeks). In EML4–ALK-positive NSCLC mouse xenograft models (NCI-H2228, NCI-3122, and NB-1), CEP-28112 also demonstrated good antitumor activities at 50 mg kg^{-1}, po, bid for 12 days. However, the development of CEP-28122 was terminated due to the occurrence of severe lung toxicity in the 4- and 13-week monkey studies. [59] Cephalon has turned its attention on a new analogue, CEP-37440, **20** which showed potent inhibitory activity against enzymatic ALK and FAK with IC50 values of 3.5 and 2.3 nM, respectively. CEP-37440 possessed more favourable properties than CEP-28112, such as superior solubility and metabolic stability, improved oral bioavailability, as well as lower clearance and toxicity. In vivo study of CEP-37440 in both Sup-M2 (30 mg kg_1, po, bid or 50 mg kg^{-1}, po, qd) and KARPAS-299 (30 mg kg^{-1}, po, bid or 50 mg kg^{-1}, po, qd) mouse xenograft models for 12 days displayed complete tumour regression without overt toxicity and significant body weight loss. Moreover, oral administration of CEP-37440 caused tumour stasis and partial regression in NCI-H3122 (30 mg kg^{-1}, po, bid and 55 mg kg^{-1}, po, bid) mouse xenograft models for 12 days, but it exhibited tumour regression in NCI-H2228 (30 mg kg^{-1}, po, qd and bid or 55 mg kg^{-1}, po, bid) mouse xenograft models with no overt toxicity and body weight loss (except at 30 mg kg_1, bid). Based on these results, CEP-37440 underwent a phase I clinical trial in August 2013. [60-61]



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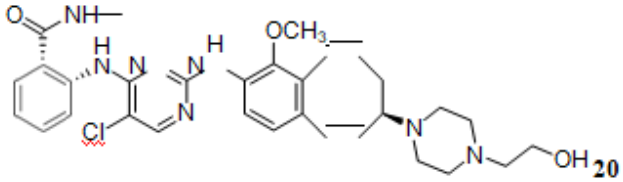

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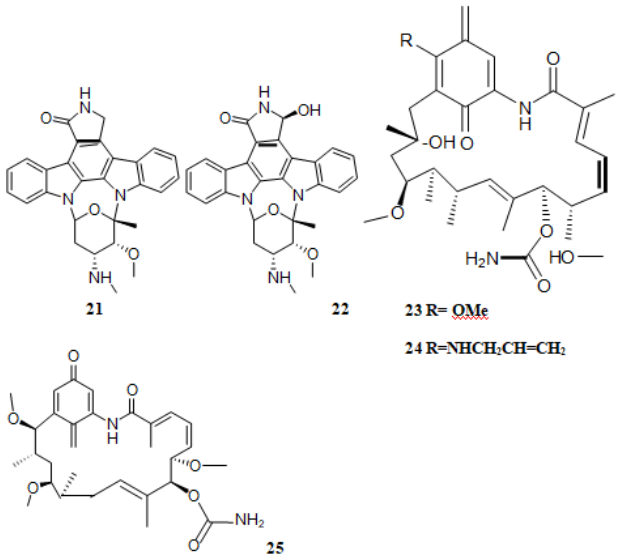
VI. CURRENT STATUS OF ALK SMALL MOLECULE INHIBITOR DEVELOPMENT

Various chemical scaffolds possessing ALK inhibitory activity from natural as well as synthetic sources have been identified and are been studied for their potency against malignant cells arising out of ALK malfunction.

ALK Inhibitors from naturally occurring sources

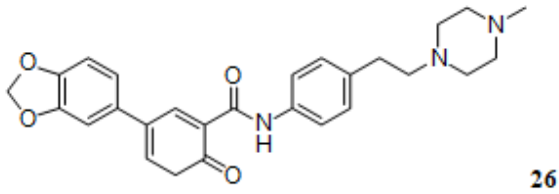
Till date only a few compounds from naturally occurring sources possessing ALK inhibitory activity have

been reported. Staurosporine, **21** was reported as an ALK inhibitor with IC50values of 123 and 150 nM in a*in-vitro* radioactive kinase assay and ALK-ELISA respectively. [62] 7-Hydroxy staurosporine, **22** also known as UCN-01 a derivative of staurosporine was reported to have anti-tumour activity in a patient with ALK-positive ALCL that was refractory to the existing chemotherapy and radiotherapy. But it only showed weak ALK inhibition with an IC50 of 5µM in the presence of 30µM ATP and no inhibition at 300µM ATP in an ALK-ELISA. [62] Another series of compounds showing ALK inhibitory activity are, structurally related benzoquinone ansamycin antibiotics, geldanamycin, **23**[63] and 17- allylamino-17-demethoxy geldanamycin, **24**[64] and herbimycin A, **25**[65] which exert their activity via the heat shock protein pathways, which enhances the proteasome mediated degradation of the ALK protein.



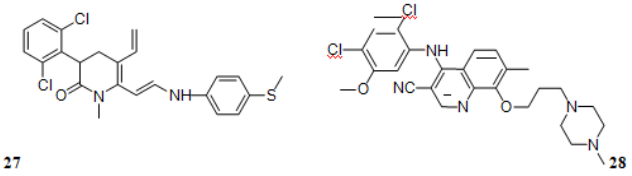
ALK Inhibitors Designed Based on Structural Insights of the Kinase Domain Pyridones

A novel chemo type aryl-pyridonecarboxamide, **26** was reported to possess ALK inhibitory activity. A virtual chemical library around the pyridone scaffold was designed to optimize one of the original hit molecule. After iterative optimization of the pyridone structure, was discovered with an appropriate spacer and basic moiety. This compound has an enzymatic IC50 of 380nM and an ALK kinase inhibition of 200 ± 100nM. It was found that the intact pyridone ring was essential for potent kinase inhibitory activity. A methyl substitution on the amide nitrogen reduced the activity 4-fold, presumably because of loss of hydrogen bond donor from amide -NH. A co-planar geometric arrangement of the pyridone core with aniline aromatic ring seems to be preferred, co-planarity was distributed by ortho-substitution with either methyl or methoxy group. Pyridone carbonyl was found to act as hydrogen bond acceptor since a decrease in activity (20-30 fold) was observed and hence it was substituted by methoxy or chlorine group. [66]



Dialkoxyquinolines and Aminopyridopyrimidinones

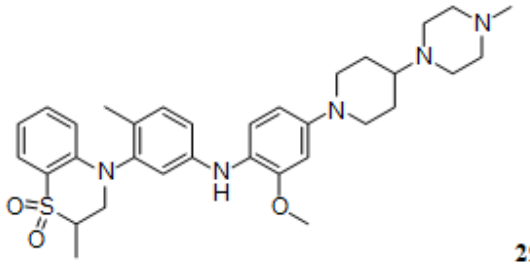
Structural insights into the ATP- binding pocket of ALK, assisted in development of a series of chemical entities possessing ALK inhibitory activity, because majority of the kinase inhibitors developed bind to the ATP pocket of the kinase domain. In an attempt to discover a novel scaffold having ALK inhibitory activity various tyrosine kinase inhibitors were screened by QSAR techniques and in the process two ABL (Abelson murine leukaemia viral oncogene homology 1) inhibitors PD173955 and SKI-606 showed ALK inhibition. In a study conducted by Gunby et al.; PD173955, **27** was able to inhibit NPM-ALK, the fusion kinase protein found in malignancies arising out of ALK deregulation at micromolar concentration (IC50=2.5µM) whereas SKI-606,**28** inhibited NPM-ALK at nano molar concentration(IC50=150nM).[67-68]



Diaminopyrimidines

Galkin*et al.* have reported 5-chloro-2,4-diaminopyrimidine(NVP-TAE684),**29** as a highly potent and specific inhibitor of NPM-ALK. [69] The compound inhibited the proliferation of the murine lymphoid Ba/F3 cells engineered to express NPM-ALK with an IC50 of 3nM without affecting the survival of parental Ba/F3 cells at a concentration of 1µM. A series of diaminopyrimidines were reported in a patent application by Novartis. Compound **30**[70]and its derivatives showed ALK inhibition with an IC50 between 0.01 to 1µM. The structurally closely related compound **31** and its derivatives were also found to have ALK inhibitory activity in the same IC50 range. Compound **32**[71]

and its derivatives also contain the same core as that of NVP-TAE684 but with different head and tail groups, showed ALK inhibitory activity with an IC50 between 0.01 to 1µM. Researchers have reported diaminopyrimidine bearing bicyclic benzazepine compound, **33** as ALK inhibitors with an IC50 value in the nanomolar range. 2,3,4,5-tetrahydro-benzo[d]azepine derivatives of 2,4-diaminopyrimidine were also reported by researchers at Cephalon to have ALK inhibitory activity. SAR studies on this series showed that basicity of the nitrogen of benzazepine group is of utmost importance. N- substituent rendered the activity, amide substitution showed most promising result. Bulky group substituent decrease the activity. [72] Investigators at the Korea Institute of chemical technology reported tetrahydroisoquinoline pyrimidine derivatives as ALK inhibitors, 2 compounds **34,35** of the series showed potent inhibition with an IC50 of 0.001µM.[73]



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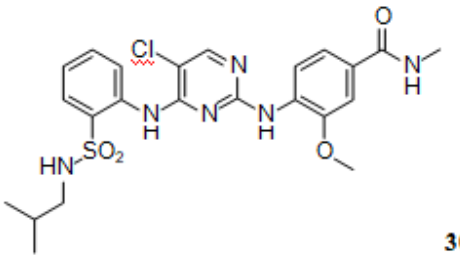

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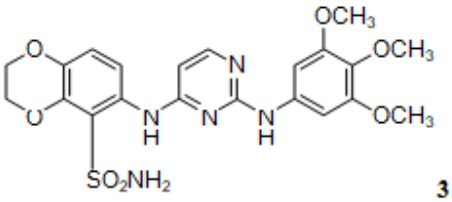

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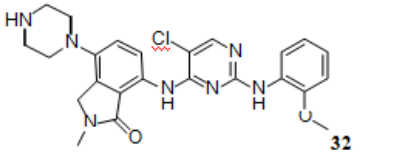

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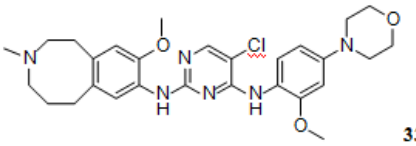

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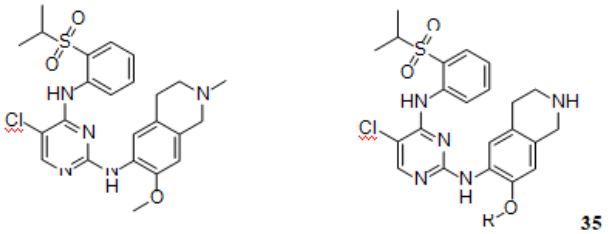
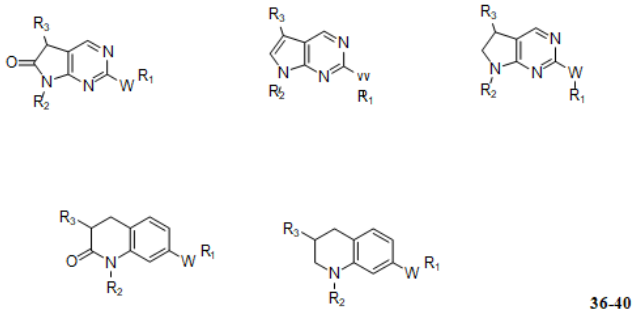


34 **35**

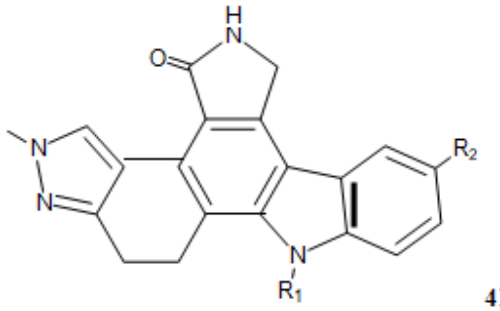
Other ALK Inhibitors

6.3.1. The fused ring version of diaminopyrimidines, pyrrolopyrimidines, **36-40** were claimed by Novartis investigators as novel class of ALK inhibitors in a patent application. The series of more than 200 compounds were reported to inhibit a variety of kinases including ALK. The inhibitory activity of these compounds had an IC50 value between 0.001 to 0.5µM. (W=NR4) [74]



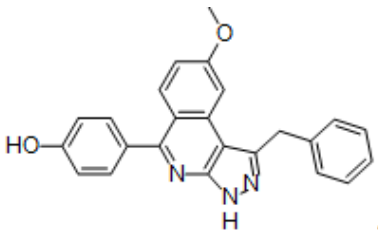
36-40

The fused pyrrolocarbazoles as ALK inhibitors were discovered by investigators at Cephalon via high throughput screening, this staurosporine like chemotype showed very potent inhibitory activity in both in-vitro enzymatic assays (IC50< 5nM), and cell based assays of ALK tyrosine phosphorylation (IC50< 30nM) . Compound **41** which has a smaller alkyl substitution for R1 and R2 on the other hand was at-least 1,000 fold less active in both assays. Unfortunately due to undesirable physical properties of these compounds were withdrawn from further studies. [74]



41

A series of pyrazoloisoquinolines, **42** were reported by investigators at Exelixis to possess ALK inhibitory activity. The most potent compound of this series showed ALK inhibition with an IC50 of 99nM. [76] Thiazole and analogues, **43** a more linear and flexible molecules showed ALK inhibition with an IC50< 50nM in a luciferase coupled chemiluminescent kinase assays. [77]



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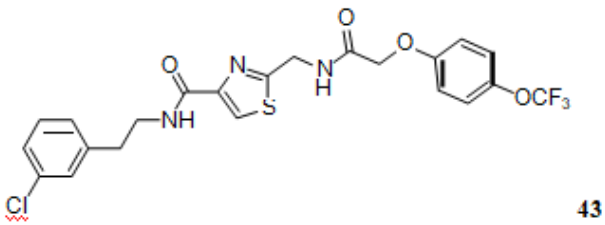

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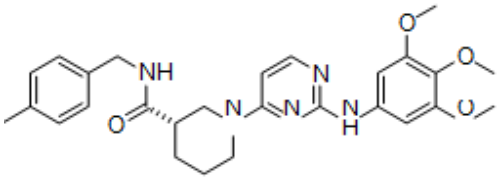
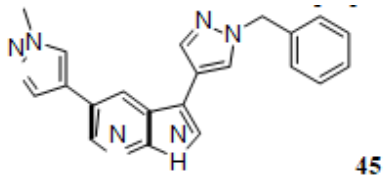
Investigators at Amgen have reported a novel chemo type piperidine-carboxamide to possess ALK inhibitory activity. The most potent compound of the series showed ALK inhibition at an IC50 of 0.174µM.[78]



44

A novel chemo type azaindole was reported to have ALK inhibitory activity. Various analogues were synthesised and most potent compound of the series had an IC50 value of 90nM. [79]



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VII. CONCLUSION

One third of all the current drug discovery programs target the protein kinases. Anaplastic lymphoma kinase has become a promising new target in the development of anti-cancer agents. This review mentions various chemical classes of compounds that have showed ALK inhibitory activity. Since the discovery of crizotinib a lot of research has been directed towards identification and development of novel ALK inhibitors with better activity and tolerability, and in the process various agents were developed which could be used in the patients with crizotinib resistance. A lot of compounds from natural sources and synthetic sources possess ALK inhibitory activity and need to be looked upon as potential inhibitors. Various novel agents are being studied in clinical trials for the discovery of potential ALK inhibitors. In summary an exciting new class of drugs have entered the arena of cancer treatment with wider clinical indications.

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