ROLE OF PYRROLOPYRIMIDINE DERIVATIVES AS ANTICANCER AGENT: MINIREVIEW

Sandip P. Dholakia*, Dr. Madhabhai M. Patel, Dr. Jitendra S. Patel
Shankersinh Vaghela Bapu Institute of Pharmacy, Gandhinagar, Gujarat, India.

ABSTRACT

Cancer is an important area of interest in the life sciences because it has been a major killer disease throughout human history. Pyrrolopyrimidines are well known to play a critical role in health care and pharmaceutical drug design. Currently a number of Pyrrolopyrimidines are available commercially as anticancer drugs and great efforts have been put to the identification of novel anticancer targets for novel anticancer drug discovery. The focus of this review is to provide a comprehensive and up-to-date account on the most recent development in the medicinal chemistry of pyrrolo[2,3-d]pyrimidine derivatives with significant anticancer activity starting from the last exhaustive publication in this field.

Please cite this article in press as Sandip P. Dholakia et al. Role of Pyrrolopyrimidine Derivatives As Anticancer Agent: Minireview. Indo American Journal of Pharm Research. 2015:5(02).

Copy right © 2015 This is an Open Access article distributed under the terms of the Indo American journal of Pharmaceutical Research, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
INTRODUCTION

Cancer is an important area of interest in the life sciences because it has been a major killer disease throughout human history. Pyrrolopyrimidines are well known to play a critical role in health care and pharmaceutical drug design. Currently a number of Pyrrolopyrimidines are available commercially as anticancer drugs and great efforts have been put to the identification of novel anticancer targets for novel anticancer drug discovery. The focus of this review is to provide a comprehensive and up-to-date account on the most recent development in the medicinal chemistry of pyrrolo[2,3-d]pyrimidine derivatives with significant anticancer activity starting from the last exhaustive publication in this field.

ANTICANCER ACTIVITY OF PYRROLOPYRIMIDINES

Stanley D. et al., synthesized a series of 4,6-bis-anilino-1H-pyrrolo[2,3-d]pyrimidines and screened for inhibition of IGF-1R which is attractive target for oncology. In summary, a key developability issue was observed for potent IGF-1R inhibitor (1) with IC$_{50}$-2.0 Nm wherein an acid-mediated cyclization of the pyrimidine moiety onto the pendant carboxamide led to facile hydrolysis in vitro and in vivo. Remarkable improvements in both inhibitor stability and potency were realized via substitution at C(4) with carboxamide-containing 5-membered heteroaryl amines (2) with IC$_{50}$-1.6 Nm, a constrained lactam (3) with IC$_{50}$-0.8 Nm, or indolines (4) with IC$_{50}$-0.5 Nm Further biological characterization and in vivo pharmacokinetics of this subclass of exceptionally potent and acid-stable inhibitors of IGF-1R will be reported in due course.

Taylor E. C. et al., synthesized Thymidylate synthase(TS) inhibitor 2-amino 4-oxo 5,6 disubstituted pyrrolo[2,3-d]pyrimidine[LY231514-Alimta, Pemetrexed] (5) and [TNP351] (6) exhibited excellent antitumor activity with IC$_{50}$- 9.5 X 10$^{-6}$ M and 15.5 X 10$^{-6}$ M approved in Europe and US.

Aleem G. et al., synthesized novel 2-amino-4-oxo-5-[(substituted phenyl)thio]pyrrolo[2,3-d]pyrimidines in which (7) act as potential inhibitor of thymidylate synthase (TS) and as anti tumor agent with IC$_{50}$- 0.21 uM which is more potent than LY231514.

Trexler P. et al., synthesized 4(3-chloro phenyl)amino 5,6-dimethyl pyrrolopyrimidine [CGP-59326] (8) act as potent tyrosine kinase (RTK) inhibitor approved in market by Novartis group.

Aleem G. et al., synthesized novel 2-amino-4-anilino 6-methylphenyl substituted purrolo[2,3-d]pyrimidines in which four compounds (9,10,11,12) were much more potent inhibitory activity against Receptor tyrosine kinase(IC$_{50}$-0.25 uM, 0.62 uM, 28.11 uM, 50 uM respectively) as compare to standard compounds. Two analogues (9 and 11) shows potent cytotoxic effect against A143 cells culture and compound (12) demonstrated high antiangiogenic activity in the Chorioallantonic membrane (CAM) assay.

Erika Pudziuvelite et al., synthesized series of 2,4-disubstituted 6-aryl-1H-pyrrolo[2,3-d]pyrimidine-7-one-5-oxides and examined in vitro antiproliferative activity in the human solid cell lines A2780, HLB-100, HeLa, SW1573 etc.in which compound (13,14,15) Exhibited excellent activity with IC$_{50}$ in range of 0.35-2.0 uM.

Leo Widler et al., synthesized 7-Substituted-5-aryl-pyrrolo[2,3-d]pyrimidines in which (16, 17) present novel class of potent inhibitors of the tyrosine kinase pp60c-Src (IC$_{50}$-0.042 uM, 0.002 uM) with good specificity towards other tyrosine kinases (EGF-R, v-Abl) with IC$_{50}$- 0.34 uM, 0.17 uM respectively.
Eva Altmann et al., synthesized 4-amino 5-phenyl 7-heterocucyl pyrrolo [2,3-d]pyrimidines in which compound (18) represent a new class of highly potent and selective inhibitors of the tyrosine kinase pp60c-Src with IC$_{50}$ – < 0.001 uM.

Ha-soon Choi et al., design and synthesized series of 2-amino-9-aryl-7H-pyrrolo[2,3-d]pyrimidines in which compound (19) exhibited low micromolar inhibitory activities against focal adhesion kinase with IC$_{50}$ – 0.1 uM.

Kevin J. M. et al., developed a series of 2-amino-pyrrolo[2,3-d]pyrimidine (20) derivatives as novel Aurora-A kinase inhibitors. An optimized analog possessed potent in vitro activity with IC$_{50}$–0.0008 uM, good kinase selectivity, and appropriate metabolic and pharmacokinetic properties. The analog was able to induce polyploidy and apoptosis.

Antanino Lauria et al., synthesized Annelated pyrrolo-pyrimidines and evaluate for antiproliferative activity in which compound (21) give moderate activity (IC$_{50}$–10.5 uM) due to stearic parameter.

Sangivamycin (22) is an antitumor substance produced by an unidentified species of Streptomyces. It is acid stable shows significant activity against leukemia L1210 in mice and is strongly cytotoxic toward HeLa cells grown in cell culture. It has very slight antibacterial or antifungal activity. The compound is currently under clinical trials.

The antibiotic nucleoside, tubercidin, was isolated from Streptomyces tubercidicus and has been assigned the 4-amino-7-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine structure. This is the first example of the natural occurrence of a pyrrolo-[2,3-d]pyrimidine ring system. Because of its unique structure, tubercidin (23) as well as toyocamycin (24) (5-cyanothubercidin) has attracted interest due exhibiting excellent antimetabolite activity.

Thymidylate synthase (TS) is a crucial enzyme that catalyzes the reductive methylation of 2α-deoxyuridine-5α-monophosphate (dUMP) to 2α-deoxothymidine-5α-monophosphate (dTMP) utilizing 5,10-methylenetetrahydrofolate, a cofactor which acts as the source of the methyl group as well as the reductant. This is the exclusive de novo source of dTMP; hence inhibition of TS activity, in the absence of salvage, leads to “thymineless death”. Thus inhibition of TS has long been an attractive goal for the development of antitumor agents.
Taylor et al.,\textsuperscript{25} have reported the synthesis of $N$-[4-[(2-amino-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid, a classical antifolate, (LY231514, 25), as an inhibitor of TS ($K_i$) 340 nM against mouse recombinant TS and as an effective antitumor agent. A potential drawback of classical antifolates, such as (25) is their requirement for the reduced folate carrier system for transport into cells,\textsuperscript{26} the impairment of which can lead to drug resistance.

The potent nonclassical TS inhibitors in the literature possess different electron-withdrawing groups in place of the CO-glutamate of classical antifolates. Aleem Gangjee et al.,\textsuperscript{27} synthesized novel 2-amino-4-oxo-5-[(substituted phenyl)sulfanyl]pyrrolo[2,3-d]pyrimidines were synthesized in which (26) is potential inhibitors of thymidylate synthase (TS) and as antitumor agent.

Aleem Gangjee et al.,\textsuperscript{28} synthesized Classical and nonclassical isosteric C$_8$-N$_9$ bridged analogues of the multitargeted antifolate, LY231514 was synthesized as inhibitors of thymidylate synthase (TS), dihydrofolate reductase (DHFR), and as antitumor and antioopportunistic infection agents. The classical analogue (27) was a marginal inhibitor of isolated human TS (IC$_{50}$ 46 µM) and of human DHFR (IC$_{50}$ 10 µM), however, it was a potent inhibitor of the growth of two human head and neck squamous cell carcinoma cell lines and of CCRF-CEM human lymphoblastic leukemia cells in culture and was similar to LY231514 against ZR-75-1 human breast carcinoma cell line.

Aleem Gangjee et al.,\textsuperscript{29,30} reported the synthesis of 2-amino-4-oxo-5-thiopyridyl-6-methyl pyrrolo(2,3-d)pyrimidine (28) as a potent TS inhibitor (IC$_{50}$ 0.34 µM).
Saritha Jyostna et al. synthesized New pyrrolo[2,3-d]Pyrimidines with heteroaryl substitution at 5th position through sulfur linker incorporating putative pharmacophoric moieties like heteroaryl groups. Cytotoxic effect of all the compounds was carried out on HCT116 colon cancer cell lines. Compounds (29) and (30) with nitrobenzimidazole and pyrimidyl heterocycles attached at 5th position via sulfur were the most potent of all with IC50 -17.6 µM. Among the four compounds tested for apoptosis induction activity, (29) induced apoptosis in a dose dependent manner.

Aleem Gangjee et al. synthesized Nine pyrrolo[2,3-d]pyrimidine analogues and reported as antitumor antimitotic agents. All the compounds were found to be one to two digit micromolar inhibitors of the proliferation of MCF-7 cells in culture. Interestingly, all of these compounds were cytotoxic against sensitive and resistant MCF-7/VP (which overexpresses MRP1) and NCI/ADR (which overexpresses pgp) cell lines. Compound (31) out of the nine compounds were found to restore sensitivity of vincristine or vinblastine to either Pgp or MRP1 activity, respectively. It inhibited the growth of most of the cell lines at GI50 values that ranged. Compound (31) had a GI50-0.34 µM against MDA-MD-435 breast cancer cell line. Additionally it exhibited single digit micromolar GI50 values against almost 53 cell lines.

Dalal A. A. E. et al. & Mostafa M. G. et al. performed molecular modeling study and design, synthesized various novel pyrrolo[2,3-d]pyrimidine derivatives and checked for antitumor activity in which compound (32) exhibited excellent antitumor activity.
Myung Ho Jung et al., synthesized Synthesis of a new series of diarylureas and amides having pyrrolo[2,3-d]pyrimidine scaffold. Among all of these derivatives, compounds (33) having imidazole and morpholine moieties, respectively, showed the most potent antiproliferative activity against A375.

Erika Pudziuvelyta et al., synthesized series of 2,4-disubstituted 6-aryl-7H-pyrrolo[3,2-d]pyrimidin-7-one 5-oxides were synthesized and in vitro antiproliferative activities were examined in the human solid tumor cell lines A2780, HBL-100, HeLa, SW1573, T-47D, and WiDr. The most potent analog induced considerably growth inhibition in the range 0.35–2.0 M. The N-alkylaminos or N,N-dialkylaminos substituents at C-2 position induced an enhancement of the biological activity in which compound (34a, 34b, 34c) exhibited excellent activity.

Aleem Gangjee et al., synthesized six novel RTK inhibitors to determine the effect of substitution in the 4-anilino ring along with variations in the 6-substituent of the pyrrolo[2,3-d]pyrimidine scaffold. Compound (35) emerged as the most viable candidate for future evaluation with its remarkably 120-fold and 189-fold increased potency against VEGFR-2 compared to semaxinib and sunitinib, respectively.

Our results indicate that the potency and selectivity of cellular inhibition of different RTKs does indeed vary with different aniline substitutions and that an optimal combination of the substitutions in the 4-anilino ring and the 6-benzyl substituent is essential for RTK inhibition of the N4-aryl-6-substituted phenylmethyl-7H-pyrrolo[2,3-d]pyrimidine-2,4-diamines.

Mostafa M. G. et al., synthesized series of novel 5-phenyl-pyrrolo[2,3-d]pyrimidine(36) derivatives bearing either sulfathiazole or sulfapyridine and evaluated for their in vitro cytotoxicity against liver and breast cancer cell line (HEPG2 - IC_{50}=3.39 μg/ml and MCF7 - IC_{50}=5.1 μg/ml). Most of the screened compounds showed interesting cytotoxic activities compared with the used reference drug (doxorubicin-IC_{50}=5.23 μg/ml and IC_{50}=3.22 μg/ml respectively). The radiosensitizing ability of some of the synthesized compounds was studied and the results showed an increase in the cell killing effect of γ-radiation after combination with the tested compounds.

Myung-Ho Jung et al., Synthesis of a new series of amides having pyrrolo[2,3-d]pyrimidine scaffold is described. Their in vitro antiproliferative activities against A375 human melanoma cell line and HS27 fibroblast cell line were tested and the effect of substituents on pyrrolo[2,3-d]pyrimidine was investigated. Among all of these derivatives, compounds (37) having imidazole and morpholine moieties, respectively, showed the most potent antiproliferative activity against A375 and HS27 fibroblast cell line. IC_{50}=0.8 μg/ml and 1.6 μg/ml respectively.

A series of chiral and non-chiral 4-N-disubstituted 6-aryl-pyrrolopyrimidines have been synthesised, characterised and tested for their in vitro EGFR-TK inhibitor properties. Eight active derivatives were identified as possible drug candidates having IC_{50} values in the range of 2.8-9.0 nM. Four of these contain fluorine atoms at sites potentially susceptible to oxidative metabolism.

Examination of structure reactivity relationships showed that substituents on the 6-aryl group (fragment A-38) could be varied without drastic effects on the EGFR-TK inhibition. In addition to a phenolic group, small substituents at R₂ are all well tolerated. Decreased potency was observed by introducing larger groups such as bromine. For fragment B, the unsubstituted benzylamine, as well as methyl and ethyl derivatives are all potent inhibitors, while limited substitution of the aromatic moiety is tolerated. A study of the effect of the inhibitors on the internalization process of EGFR, confirms that several of the fluorinated derivatives have high activity in HeLa cells. A benefit of the fluoro-containing pyrrolopyrimidines (39) in a therapeutic setting could potentially be higher metabolic stability due to removal of sites for aromatic hydroxylation or O-glucoronidation metabolism.
\[ R = \text{a) } -\text{NHCH}_2\text{CH} = \text{CH}_2 \text{ b) Piperidine} \]
A series of eight N4-phenylsubstituted-6-(2,4-dichloropheny)methyl-7H-pyrrolo[2,3-d]pyrimidine-2,4-diamines were synthesized as vascular endothelial growth factor receptor-2 (VEGFR-2) inhibitors with varied substitutions in the phenyl ring of the 4-anilino moiety. Compounds (40) was potent VEGFR-2 inhibitors and were 100-fold more potent than the standard semaxanib, respectively and it is chosen for further evaluation in a mouse orthotopic model of melanoma and showed significant inhibitory of tumor growth, angiogenesis and metastasis.

Aleem gangjee et al., synthesized series of eight N4-phenylsubstituted-6-(2,4-dichlorophenylmethyl)-7H-pyrrolo[2,3-d]pyrimidine-2,4-diamines as vascular endothelial growth factor inhibitor-2 (VEGFR-2) inhibitors with varied substitutions in the phenyl ring of the 4-anilino moiety in which compounds (41) exhibited excellent VEGF-2 inhibitory activity with IC50-0.1-0.3 µM.

Yuya Oguro et al., synthesized a series of pyrrolo[3,2-d]pyrimidine derivatives and evaluated their application as type-II inhibitors of vascular endothelial growth factor receptor 2 (VEGFR2) kinase. Incorporation of a diphenylurea moiety at the C4-position of the pyrrolo[3,2-d]pyrimidine core via an oxygen linker resulted in compounds that were potent inhibitors of VEGF-2 kinase. Of these derivatives, compound (42) showed the strongest inhibition of VEGF-stimulated proliferation of human umbilical vein endothelial cells (HUVEC) with IC50 – 6.3 µM.

On the basis of the information derived from co-crystal structure analysis of VEGFR2 and (42), a series of pyrrolo[3,2-d]pyrimidine derivatives was designed and synthesized with the goal of obtaining strong inhibitory activity against FGFR kinase and improved solubility. As a result, the urea (43), possessing a piperazine moiety on the terminal benzene ring, strongly inhibited FGFR1 kinase while retaining potent VEGFR2 kinase inhibitory activity. Further evaluation revealed that (43) showed strong inhibitory activities against both VEGF- and FGF-stimulated HUVEC proliferation and improved solubility. Since FGF-FGFR signaling has been shown to not only influence tumor angiogenesis but also directly contribute to tumor growth and survival, simultaneous inhibition of these receptor tyrosine kinases by (43) may enhance antitumor activity.

REFERENCES
1. Rediscovering Biology: Molecular to Global Perspectives. Cell Biology and Cancer. 2012,


