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Pyrazolo[3,4-d]pyrimidine based scaffold derivatives targeting kinases as anticancer agents

Nasser S.M. Ismail [b, *], Eslam M.H. Ali [d, 1], Diaa A. Ibrahim [c], Rabah A.T. Serya [a], Dalal A. Abou El Ella [a]

[a] Pharmaceutical Chemistry Department, Faculty of Pharmacy Ain Shams University, Abbassia, Cairo 11566, Egypt
[b] Pharmaceutical Chemistry Department, Faculty of Pharmaceutical Sciences and Pharmaceutical Industries, Future University, Cairo 12311, Egypt
[c] National Organization for Drug Control & Research, P.O. Box: 29, Cairo, Egypt
[d] Medical Union Pharmaceuticals Company, Abu Sultan, Al Ismaillia, Egypt

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ABSTRACT

Pyrazolopyrimidines are fused heterocyclic ring systems which structurally can consider as bioisosteres of adenine, which is fundamental for every aspect of cell life. Pyrazolo[3,4-d]pyrimidines derivatives have been explored for their inhibitory activity towards various protein kinase enzymes and their role as anticancer agents. The present review to the best of our knowledge is the first compilation on synthesis and medicinal aspects including structure–activity relationships of pyrazolo[3,4-d]pyrimidines reported to date.

1. Introduction

Several pyrazole derivatives received great attention due to their biological and pharmacological activities, not only as potential inhibitors of HIV-1 [1], pesticides [2], fungicides [3], analgesic drugs [1], antihypertensive agents [4] and anticancer agents [5], but they are also important and useful as starting materials for the synthesis of other fused heterocyclic systems. One of the most important fused pyrazoloes is prazolopyrimidine derivative which possess a wide variety of biological activities. In the current work we will put highlights on the most important aspect of pyrazolopyrimidines, in particular pyrazolo[3,4-*d*]pyrimidines, bioisosteres of purines. Pyrazolo[3,4-d]pyrimidines are reported to encompass pharmacological potential as antiviral [6–9], anticoccidials [10,11], antimicrobial [12–15], antitumor [16–19], herbicidal, antileukemic [20,21], pesticides [22], CNS agents [23], tuberculostatic [24–26], antileishmanial [27–29], radioprotectant [30], antiinflammatory [31] and cardiovascular activities [32,33]. The present review outlines synthetic strategies and anticancer activity of pyrazolo[3,4-d] pyrimidines as protein kinase inhibitors. In addition, the radioactive and active oxygen species generation activities of pyrazolo[3,4-d]pyrimidines were discussed in this article.

Protein kinases are the most important human enzyme classes. These enzymes are responsible for protein phosphorylation by catalyzing the transfer of the ɣ-phosphoryl group from a nucleoside triphosphate, usually ATP, to the side chain of an amino acid residue in the substrate protein [34,35].

Based upon their catalytic specificity, the enzyme classes can be subdivided into three categories:

I. Tyrosine kinases (TK): These kinases regulate several physiological mechanisms, including cell proliferation, differentiation, migration and metabolism, by transferring the ATP terminal phosphate to tyrosine residues of protein substrates [36].

II. Serine/Threonine kinases: They act on serine and threonine amino acids. Six other groups have been identified that primarily phosphorylate serine and threonine residues [37].

III. Histidine Kinases: They autophosphorylate a histidine moiety in the active site and then transfer the phosphate to an aspartate moiety [38].

It has been conclusively demonstrated that kinase alterations (especially hyperactivation, hyperproduction, or mutations) leading to the disruption of cell signaling cascades play important roles in several diseases, including diabetes, inflammation, neurological disorders and cancer [39]. Thus, kinases represent important targets for anticancer drug development.

* Corresponding author. Tel.: +20 1015643036; fax: +20 2 024051107.
E-mail address: saadnasser2003@yahoo.com (N.S.M. Ismail).
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[1] Fax: +20 064 3400363.

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Small molecules protein kinases inhibitors can be classified into three classes according to the activity state of the kinase they recognize, and their reversibility.

Type **I** inhibitors: bind to the ATP binding site through the formation of hydrogen bonds to the kinase "hinge" residues and through hydrophobic interactions in and around the region occupied by the adenine ring of ATP [40].

Type **II** inhibitors: act on the inactive (unphosphorylated) conformation of enzyme, by stabilizing the inactive conformation. Type I and II work as ATP competitive inhibitors and target ATP binding site [41].

Type **III** inhibitors: they are noncompetitive ATP inhibitors, they bind to the allosteric site of the protein distant from ATP pocket. They don't require any typical hinge binding motifs and can bind the enzyme regardless it's activation state [42].

2. Synthetic strategies

A plenty of synthetic methods (Fig. 1) have been outlined for the synthesis of pyrazolo[3,4-d]pyrimidines. 5-Amino-4-cyanopyrazole **I** has been used in the number of reactions to attain the synthesis of desired compounds through different routes. Reaction of **I** with formamide at 180 °C for 24 h afforded the corresponding compounds (route a) [43,44]. Two step reaction in which compound **I** is reacted with N,N-dimethylphosgeniminium chloride in dichloromethane under reflux for 2 h gave the corresponding dimethylcarbamimidic chloride derivative, which was then cyclized with hydrochloric acid to obtain the 4-chloro derivative of target compound (route b) [45]. Moreover, Rashad et al. [46] reported that treatment of compounds **I** with a mixture of hydrochloric acid/acetic acid (1:3) under reflux, gave pyrazolo[3,4-d]pyrimidine-4-one derivatives (route c). In addition, it was reported that treatment of **I** with triethyl orthoformate and acetic anhydride under reflux for 24 h. afforded the corresponding pyrazolo[3,4-d]pyrimidine derivatives at good yield (route d) [47,48].

Meanwhile, concentrated sulfuric acid mediated hydrolysis of compound **I** gave the corresponding 5-amino-4-pyrazolcarboxamide **II** derivative which underwent oxidative cyclization with various substituted aromatic aldehydes in the presence of equimolar molecular iodine as a mild Lewis acid and oxidant under neutral conditions in boiling acetonitrile to give a new series of pyrazolo[3,4-d]pyrimidine derivatives (route e) [12,49]. On the other hand, a microwave-assisted condensation employing 5-amino-4-pyrazolcarboxamide derivative **II** in formamide smoothly afforded the corresponding analogous 1H-pyrazolo[3,4-d]pyrimidines (route f) [50]. Pyrimidine derivatives can also used as a starting material for the synthesis of the corresponding pyrazolo[3,4-d]pyrimidines. It is reported that chlorination of barbituic acid using $POCl_3$ introduced the corresponding trichloro pyrimidine derivative **III** [51,52] that can be converted to pyrazolo[3,4-d]pyrimidines via reaction with 1-benzyl-4-hydrazinylpiperidine dihydrochloride in presence of tri-ethyl amine at 78 °C (route g) [52,53]. Soth et al. [54] carried out the reaction of tert-butyl carbazate (route h) with 4-chloro-5- cyano pyrimidine **IV** in ethyl amine to afford the target derivative. Also, the title compounds pyrazolo[3,4-d]pyrimidine were reported to be prepared by reacting 4-amino-6-aryl-2-phenyl pyrimidine-5-carbonitrile **V** derivatives and hydrazine hydrate in EtOH under reflux (route i) [55].

3. Anticancer activity

The biological investigations of pyrazolo[3,4-d]pyrimidines have revealed that substitution of various groups on the scaffold imparts anticancer activity through inhibition of different enzymes.

3.1. p38-α mitogen-activated protein kinases (MAPK) inhibitors

p38 Kinases are members of the mitogen-activated protein kinase family that transduce signals from various environmental stresses, growth factors, and steroid hormones. Four p38 MAP kinases, p38-α (MAPK14), -β (MAPK11), -γ (MAPK12/ERK6), and -δ (MAPK13/SAPK4), have been identified. p38 is highly expressed in aggressive and invasive breast cancer. Increased levels of activated p38 are markers of poor prognosis [56]. In 2007, Dhillon et al. [57] explored the role of MAPK pathways in cancer. Cancerous mutations in MAPK pathways are frequently affecting Ras and B-Raf in the extracellular signal-regulated kinase pathway. Stress-activated pathways, such as Jun N-terminal kinase and p38, largely seem to counteract malignant transformation. The balance and integration between these signals may widely vary in different tumors, but are important for the outcome and the sensitivity to drug therapy [58]. In 2011, Soth et al. [54] designed and established 3-amino-pyrazolo [3,4-d]pyrimidines as p38-α kinase inhibitors. Compounds **2**–**6** were tested through enzyme assay for inhibition of the p38-α -catalyzed phosphorylation of myelin basic protein. Potent inhibitors were identified from both the amide and amine subseries. The enzymatic assay revealed that the amide functionality has a mild effect on potency owing to the fact that it is away from the binding site whereas amine moiety showed high potency due to its tight binding to the active site. Compound **6** showed extremely high selectivity for p38-α over other kinases. That explained by the optimal fit of the 2,4-difluorophenyl ether into the back pocket of p38-α. This pocket is specifically bigger in p38-α than in many other kinases, due to its small gatekeeper residue (Thr106), also, the presence of a nonplanar front pocket side chain (the branched sulfone of 5), which is particularly well-tolerated by p38-α versus other kinases, for example, Lck, due to a larger open space in the front of the protein [59]. Finally, the methyl group of the sulfonic side chain of **6** fits more tightly into a lower hydrophobic pocket defined in part by Ala157 (Table 1).

3.2. Src kinase inhibitors

Src kinases are a family of nine different PTKs, including c-Src, c-Yes, Fyn, Lck, Lyn, Hck, Frk, Blk, and c-Fgr of which Src is the prototype [60]. SrcKs can regulate a number of signaling pathways that impact on the behavior of tumor cells, including proliferation, survival, migration, invasion, and angiogenesis [61]. Src tyrosine kinase expression is frequently elevated in a number of epithelial tumors including colon, breast, prostate, lung, ovary, and pancreas compared with the adjacent normal tissues. Interestingly, Src kinase can be considered as key modulator of cancer cell invasion and metastasis [62–65]. In 2011, Kumar et al. [43] explored the antiproliferative and proapoptopic activities of pyrazolo[3,4-d]pyrimidines (compounds **7**–**9**) as Src kinase inhibitors in human ovarian adenocarcinoma (SK-Ov-3), breast carcinoma (MDA-MB-361), and colon adenocarcinoma (HT-29) (Table 2). They concluded that pyrazolo[3,4-d]pyrimidines are involved in the stimulation of programmed cell death and decrease the Src phosphorylation. The SAR of the synthesized compounds revealed that the 3-phenyl group contributes significantly to Src kinase inhibitory activity through a hydrophobic interaction with a large hydrophobic pocket in the ATP-binding site [66]. In addition, the variation of N1 substitution in 3-phenylpyrazolopyrimidines with different 1,2,3-triazoles containing hydrophobic residues can occupy and/or interact with amino acids of the cavity of carbohydrate binding pocket of ATP and contribute to the enhancement of Src kinase inhibitory potency. It was found that compounds **7** and **8** exhibited modest inhibitory potency (IC_{50} = 6.2, 5.6 μM) against Src kinase. Structure–activity relationship studies suggested that the