Bioorganic Chemistry 76 (2018) 237–248

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

4-Aminopyridine based amide derivatives as dual inhibitors of tissue non-specific alkaline phosphatase and ecto-5′-nucleotidase with potential anticancer activity

Sidra Hassan [a], Syeda Abida Ejaz [a], Aamer Saeed [b,*], Muddasar Shehzad [b], Shafi Ullah Khan [a], Joanna Lecka [c,d], Jean Sévigny [c,d], Ghulam Shabir [b], Jamshed Iqbal [a,*]

[a] Centre for Advanced Drug Research, COMSATS Institute of Information Technology, 22060 Abbottabad, Pakistan
[b] Department of Chemistry, Quaid-I-Azam University, 45320 Islamabad, Pakistan
[c] Département de microbiologie-infectiologie et d'immunologie, Faculté de Médecine, Université Laval, Québec, QC, Canada
[d] Centre de Recherche du CHU de Québec – Université Laval, Québec, QC, Canada

ARTICLE INFO

Article history:
Received 16 August 2017
Revised 4 November 2017
Accepted 17 November 2017
Available online 21 November 2017

Keywords:
Ecto-5′-nucleotidase (e5′NT)
Alkaline phosphatases (APs)
4-aminopyridinederivatives
Mechanism of action
Molecular docking studies

ABSTRACT

Ecto-nucleotidase members i.e., ecto-5′-nucleotidase and alkaline phosphatase, hydrolyze extracellular nucleotides and play an important role in purinergic signaling. Their overexpression are implicated in a variety of pathological states, including immunological diseases, bone mineralization, vascular calcification and cancer, and thus they represent an emerging drug targets. In order to design potent and selective inhibitors, new derivatives of 4-aminopyridine have been synthesized (**10a-10m**) and their structures were established on the basis of spectral data. The effect of nature and position of substituent was interestingly observed and justified on the basis of their detailed structure activity relationships (SARs) against both families of ecto-nucleotidase. Compound **10a** displayed significant inhibition (IC_{50} ± SEM = 0.25 ± 0.05 μM) that was found ≈168 fold more potent as compared to previously reported inhibitor suramin (IC_{50} ± SEM = 42.1 ± 7.8 μM). This compound exhibited 6 times more selectivity towards *h*-TNAP over *h*-e5′NT. The anticancer potential and mechanism were also established using cell viability assay, flow cytometric analysis and nuclear staining. Molecular docking studies were also carried out to gain insight into the binding interaction of potent compounds within the respective enzyme pockets and herring-sperm DNA.

1. Introduction

Ecto-nucleotidases, membrane bound metalloenzymes, are involved in the regulation of purinergic signaling by maintaining extacellular nucleotides level. These extracellular nucleotides are responsible for the activation of purinergic receptors (P2); P2X and P2Y and ultimately control various physiological processes such as cell proliferation, motility, differentiation, neurotransmission, acute inflammation and neuroscretion [1,2]. Ecto-5′-nucleotidase, an important member of ecto-nucleotidase family, is a purine catabolic enzyme that primarily hydrolyzes the extracellular nucleoside monophosphate into adenosine and inorganic phosphate [3]. This adenosine maintains various physiological functions involving vasodilation, cytoprotection, stimulation of immunosuppression, angiogenesis and regulation of cell growth, differentiation and maturation [4]. Moreover, e5′NT also behave as an adhesive molecule that attaches with other cells and extracellular matrix such as fibronectin and laminin [5,6]. In cancer cells, the overexpression of e5′NT resulted in excessive adenosine production that facilitate proliferation of tumor cells. Cancer cell lines expressing e5′NT are more tumorigenic and invasive in nude mice, than e5′NT negative cell lines [7]. Overexpression of e5′NT is remarkably found in more aggressive gastric, pancreatic, breast and lymphoma cancer [8–12].

Another member of ectonucleotidase family, alkaline phosphatase (AP), a phosphate hydrolase uses two metal ions to catalyzes the transfer of inorganic phosphates to an acceptor substrate *i.e.*, nucleotides [13,14]. There are four isozymes of AP encoded in distinct gene loci *i.e.*, tissue specific and tissue non-specific AP. Tissue specific isozymes includes placental, germ cell and intestinal alkaline phosphastase, whereas, tissue non specific occurs in different part of body such as liver, bone and kidney

* Corresponding authors.
E-mail addresses: aamersaeed@yahoo.com (A. Saeed), drjamshed@ciit.net.pk (J. Iqbal).

https://doi.org/10.1016/j.bioorg.2017.11.013
0045-2068/

[15]. In bones, TNAP is involved in the hydrolysis of extracellular inorganic pyrophosphate (PPi) into inorganic phosphate and thus regulate the calcification process by maintaining the steady state level of PPi [16]. However, the overexpression of TNAP resulted in various pathophysiological abnormalities that can possibly lead to osteoarthritis, ankylosis, and vascular calcification [17,18]. Another isozyme, intestinal alkaline phosphatase (IAP) present at the apical brush border of intestine involves in the dephosphorylation of bacterial lipopolysaccharides (LPS) and reduces the bacterial toxicity and inflammation [19]. It has been found that overexpression of IAP results in hepatocellular carcinomas [20]. It has been described previously that AP dephosphorylates the protein participates in several mechanisms including apoptosis, cell growth, differentiation, and cell migration [21]. More than half of breast cancer patients usually develop bone metastasis and ultimately progresses into liver metastases. AP highly expressed in breast cancer patients with associated bone metastases and/or liver metastases, are often considered as a useful prognostic indicator [22,23]. Some of the known inhibitors of ecto-5′-nucleotidase and alkaline phosphatase are presented in Figs. 1 and 2, respectively.

Many inhibitors of APs and e5′NT have been identified, but they exhibited a non-selective behavior [24,25]. Therefore, there is a need to explore potent and selective inhibitors of APs and e5′NT that would be helpful in treating various relevant pathological conditions *i.e.*, bone mineralization, vascular calcification, inflammatory bowel diseases, immunological diseases and cancers. Keeping in view the importance of both enzymes in breast cancer progression and metastasis along with previously reported inhibitors structures, we decided to synthesize 4-aminopyridine derivatives for our study. Importantly, 4-aminopyridine, a pinacidil precursor [26], acts by blocking potassium channels and prolonging action potentials; thereby increasing neurotransmitter release at the neuromuscular junction [28]. Additionally, 4-aminopyridine molecule has been used clinically in the treatment of Lambert-Eaton myasthenic syndrome [27]. It has been shown to improve visual function, motor skills and relieve fatigue in patients with multiple sclerosis (MS). Moreover, 4-aminopyridine also works as a potent calcium channel activator and can improve synaptic and neuromuscular function by directly acting on the calcium channel beta subunit [29]. Here, we synthesized and tested 4-aminopyridine derivatives against both members of ecto-nucleotidase. The putative binding modes of these derivatives within the active site of enzyme were determined by molecular docking studies. Furthermore, the anticancer potential of potent derivatives were evaluated against different cell lines.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of N-(pyridin-4-yl)benzamides (*10a-m*)

Synthesis of the *N*-(pyridin-4-yl)benzamides **(10a-m)** was accomplished by the direct condensation of 4-aminopyridine with a variety of suitably substituted benzoic acids at low temperature using dicyclohexylcarbodiimide (DCC) as a coupling agent.

The reaction was monitored by TLC. The R_f value of amides was higher than either benzoic acids or 4-aminopyridine because of less polarity than either of reactants. The FTIR data of all synthesized amides showed the characteristic absorption band for (N—H) stretching in the range of 3248–3275 cm^{-1}, (C=O) stretching in the region of 1681–1694 cm^{-1} and (C=C) stretching in the region of 1571–1597 cm^{-1}. The ^1H NMR spectra represented a characteristic signal of one proton that appeared as singlet in the range of 8.59–11.5 ppm corresponding to the (NH) of amide. A doublet of two protons was observed in the range of 7.52–8.55 ppm and another doublet of two protons appeared in the range of 7.92–7.48 ppm which showed para-disubstituted pattern which is a characteristic of 4-aminopyridine structure. The ^{13}C NMR spectra of all the *N*-(pyridin-4-yl)benzamides **(10a-m)** showed characteristic signal for (C=O) amide carbon and were in the range of 174.1–167.8 ppm, signal for (4′) pyridine carbon and characteristic

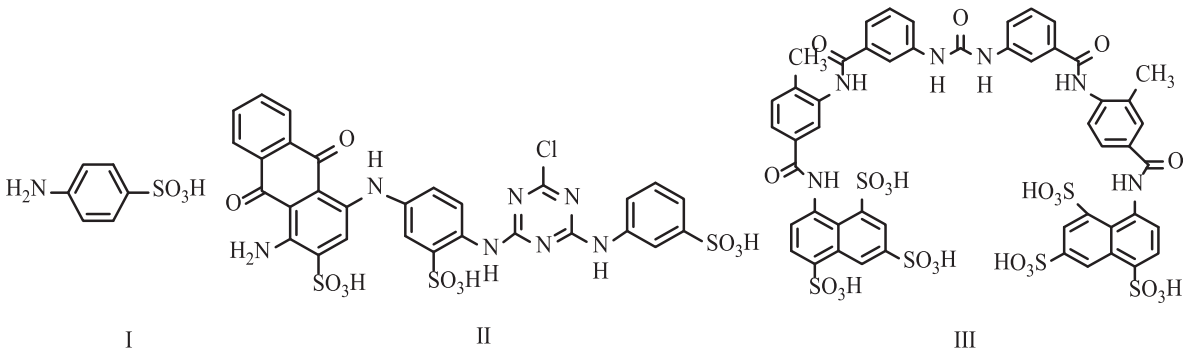

Fig. 1. Known Inhibitors of e5′NT. Sulfanilic acid **(I)** (1) Reactive blue **(II)** (2) Suramin **(III)** (3).

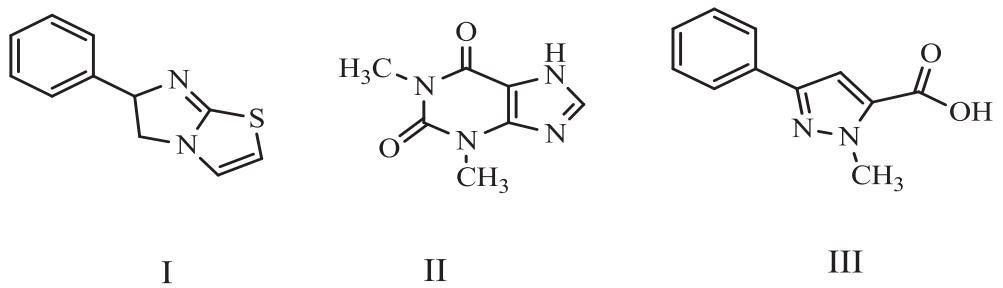

Fig. 2. Structures of known inhibitors of APs: levamisole (I), theophylline (II), 1-methyl-3-phenyl-1H-pyrazole-5-carboxylic acid (III) (4).