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Structure-based design, synthesis and evaluation of 2,4-diaminopyrimidine derivatives as novel caspase-1 inhibitors

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ABSTRACT

Interleukin-1β converting enzyme contributes in various inflammatory and autoimmune diseases by maturing pro-inflammatory cytokines IL-1β, IL-18 and IL-33. Therefore, inhibition caspase-1 may provide a potential therapeutic strategy for the treatment of chronic inflammatory diseases. Here we have reported structure-based design, synthesis and biological evaluation of 2,4-diaminopyrimidine derivatives (**6a-6w**) as potential caspase-1 inhibitors. Six compounds **6m, 6n, 6o, 6p, 6q** and **6r** showed significant enzymatic inhibition with IC_{50} ranging from 0.022 to 0.078 μM. These compounds also displayed excellent cellular potency at sub-micromolar concentration. Moreover, molecular docking studies provided the useful binding insights specific for caspase-1 inhibition. All these results indicated that compounds **6m, 6n** and **6o** could be potential leads for the development of newer caspase-1 inhibitors as anti-inflammatory agents.

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1. Introduction

Caspases are cysteine-dependent aspartate-specific proteases, primarily responsible for proinflammatory cytokine maturation and apoptosis. These endoproteases possess cysteine as a nucleophile for hydrolysing peptide bonds that occurs mainly after C-terminus of Asp residues [1]. Fourteen human caspases have been identified and classified based on their biological functions and length of regulatory pro-domains: (I) Inflammatory caspases (Caspases-1, 4, 5 and 11), (II) Initiator caspases (Caspases-2, 8, 9, 10 and 12) and (III) Effector caspases (Caspases-3, 6, 7 and 14) [2,3]. Generally, they exist as inactive precursors which acquired enzymatic activity by auto activation or by amplification cascade, a highly regulated process. The active caspase is homodimer of heterodimers (containing p10 and p20 subunits) with two active sites located in close proximity model.

Interleukin-1β-converting enzyme (ICE, caspase-1) is mainly responsible for post-translational processing of the pro-inflammatory cytokines such as IL-1β, IL-18 and IL-33. The biologically inactive pro-IL-1β (31 kDa) undergoes proteolytic cleavage at Asp^{116}-Ala^{117} which, releases the mature cytokine, IL-1β (17.5 kDa) [4]. IL-1β plays a key role in physiological process such as inflammation, cell proliferation, differentiation and pyroptosis. Excessive secretion of IL-1β contributes to the pathophysiology of various inflammatory and autoimmune diseases such as rheumatoid arthritis (RA), osteoarthritis (OA), artherosclerosis and sepsis [5]. Literature also reveals that caspase-1 might be involved in unscheduled programmed cell death in many degenerative diseases [6]. As the key component of inflammation induction, caspase-1 has emerged as an important therapeutic target for modulating various inflammatory diseases.

Large numbers of anti-inflammatory drugs only improve the disease related symptoms and show side effects of NSAIDs [7]. Currently used IL-1 receptor antagonist (Kineret) or anti-IL-1b antibody (canakinumab) also exhibit some severe allergic reactions [8,9]. A positional scanning library identifies Ac-YVAD-CHO, tetrapeptide fragment as a potent, selective caspase-1 inhibitor (Ki 5 nM) [10]. Unfortunately, this peptide suffers from poor pharmacokinetic profile and modest selectivity within the enzyme family. Based on these requisites, two peptidomimetics VX-740 (pralnacasan) and VX-765 were developed and progressed into late-stage of clinical trials [11,12]. However, several pharmacological constrain limits their clinical utility. Thus, ICE represents an opportunity for the development of small molecules as novel anti-inflammatory agents.

The X-ray crystal structure of caspase-1 and their subsequent peptide inhibitors provided clear premises for structure based design of newer caspase-1 inhibitors [13]. Further the molecular

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docking study revealed the key residual interactions, which includes polar interactions with Arg179, His237 and Cys285 at P1site; H-bonding interactions with Ser339 and Arg341 at P2-P3 site and hydrophobic interactions with Trp 340, His342, Pro343 and Arg383 at P4 site. A summary of the protein-ligand interactions of co-crystalized ligand and 6 m with 1rwx is displayed in Fig. 1. It clearly emphasized that both compounds occupy the same spatial area and position in to the binding site of caspase-1. Based on these computational results newer series of 2,4-diaminopyrimidine was designed, synthesized and tested against caspase-1 enzyme. Further, the cellular potency of the most active compounds was determined by THP-1 cell based assay.

2. Results and discussion

2.1. Chemistry

The synthesis of targeted compounds **6a-6w** was accomplished by the synthetic route outlined in Scheme 1. The cyclocondensation of guanidine hydrochloride (**1**) and ethyl acetoacetate (**2**) afforded 2-amino 4-hydroxy 6-methyl pyrimidine (**3**) which was heated with phosphorus oxychloride to get the key intermediate 2-amino-4-chloro-6-methyl pyrimidine (**4**) [14,15]. Compound (**4**) was refluxed with various substituted amines in the presence of catalytic conc. HCl in ethanol to yield compounds (**5a-i**) [16,17]. Finally, 2-amino-(4-substituted phenylamino)-6-methylpyrimidins **(5a-i)** were reacted with substituted phenoxy acetyl chloride in dry DMF gave targeted compounds (**6a-6w**). All the final compounds were characterized by IR, MS, ^{1}HNMR and ^{13}C NMR spectroscopic techniques.

2.2. Caspase-1 enzyme assay

The synthesized compounds were evaluated for their caspase-1 inhibition potential using luminescence based assay [18]. Compounds were tested in three different concentrations, in comparison to Ac-YVAD-CHO peptide as a standard drug. The Z-WEHD-AMC was used as a substrate and enzyme concentration was adjusted at one unit for catalysing the peptide bond. As shown in Table 1, compounds demonstrated good inhibitory potential with various substituents at both phenyl rings, IC_{50} range from 0.022 to 12.48 μM. Firstly, un-substituted phenoxy acetamide **(6a,** IC_{50} = 12.48 ± 0.08 μM) was evaluated for its less potency compared to –CH_3 substituted derivatives (**6b, 6c**, IC_{50} = 4.83 ± 0.05, 4.51 ± 0.14 μM). Compound bearing electron withdrawing substituent at R_1 ring (**6d**, IC_{50} = 1.86 ± 0.77 μM) showed improved enzymatic inhibition. However, *p*-CH_3 (**6e,** IC_{50} = 8.25 ± 0.06 μM) and *p*-OCH_3 (**6f,** IC_{50} = 8.73 ± 0.15 μM) substituents at R phenyl showed dramatic loss in the potency. When the R ring was fixed with *p*-methyl/methoxy and R_1 phenyl ring substituted with *o/m* –CH_3 group (**6g, 6 h,** IC_{50} = 3.78 ± 0.21, 3.56 ± 0.77 μM) displayed 2 fold more potency compare to *p*-CH_3 derivative (**6i,** IC_{50} = 6.85 ± 0.08 μM). Replacement of methyl group with *p*-Cl and *p*-F substituents at R_1 phenyl ring showed 4 fold increase in the activity compare to **6i** (**6j, 6k,** IC_{50} = 1.65 ± 0.16 μM, 1.72 ± 0.12 μM). However, 3-Cl, 4-CH_3 substituents at R1 ring did not produce any improvement in the activity (**6l,** IC_{50} = 2.80 ± 0.20 μM). The electron withdrawing substituents at remote R phenyl ring demonstrated highest enzymatic inhibition (**6m, 6n, 6o,** IC_{50} = 0.035 ± 0.007, 0.027 ± 0.005, 0.022 ± 0.013 μM). Incorporation of alkyl substituent at various positions of R1 phenyl also exhibited comparable potency with **6m-6o** (**6p, 6q, 6r,** IC_{50} = 0.078 ± 0.015, 0.052 ± 0.008 & 0.045 ± 0.010 μM). Whereas, the electronegative substituents at *para* position of both the phenyl ring caused decrease in the activity (**6s**, IC_{50} = 1.75 ± 0.21 μM)**.** Specifically, adding the *m*-CH_3 on R_1 and *o*-F substituent on R ring exhibited 2 fold increase in the potency (**6t**, IC_{50} = 0.95 ± 0.13 μM). Likewise, 2,3-dimethyl substituents on R phenyl ring led to more than 10 fold decrease in the activity. (**6u, 6v, 6w**, IC_{50} = 10.57 ± 0.19, 10.81 ± 0.54, 9.78 ± 0.30 μM). These results suggested electron withdrawing substituents at the *para* position of R phenyl and *o*/*m*-CH_3 substituent at R1 phenyl ring influence the activity.

2.3. Cell based assay

Compounds exhibiting significant enzymatic inhibition were also evaluated for their cellular potency [19]. As shown in Table 2, most of the compounds displayed comparable cellular potency to the Ac-YVAD-CHO and MG-132. Compound having halogen substituent at *para* position of R phenyl ring (**6m**, IC_{50} = 6.25 ± 0.06 μM) showed 2 fold decrease in the potency compare to *ortho* and *meta* positions (**6n, 6o**, IC_{50} = 3.55 ± 0.02, 3.20 ± 0.15 μM). The *o*/*m*-CH_3 substituent on R_1 phenyl (**6q, 6r** IC_{50} = 6.84 ± 0.16, 6.37 ± 0.21 μM) was more tolerated than *p*-CH_3 substituent (**6p**, IC_{50} = 8.0 ± 0.3 μM). This, poor correlation between enzymatic and cell-based activity is major concern in discovery of newer caspase-1 inhibitors with important therapeutic potential.

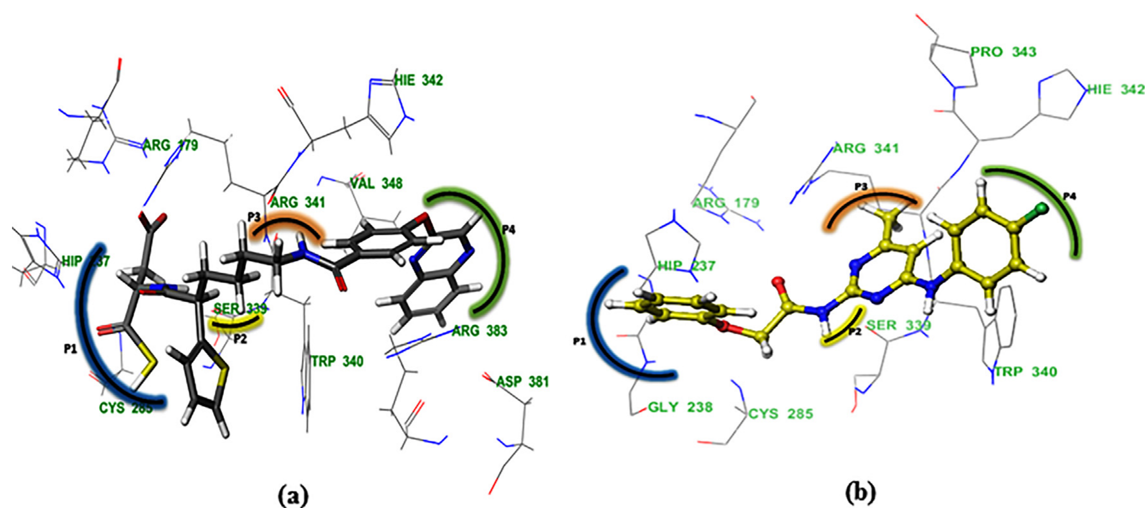

Fig. 1. (a) The key residual interactions of 1rwx with co-crystallized ligand (b) The key residual interactions of 1rwx with 6 m.

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