Bioorganic & Medicinal Chemistry 26 (2018) 4310–4319

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Selective inhibition of human cathepsin S by 2,4,6-trisubstituted 1,3,5-triazine analogs

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ARTICLE INFO

Keywords:
Cathepsins
Cysteine protease
Protease inhibitors
1,3,5-Triazine analogs
Microwave irradiation

ABSTRACT

We report herein the synthesis and biological evaluation of a new series of 2,4,6-trisubstituted 1,3,5-triazines as reversible inhibitors of human cysteine cathepsins. The desired products bearing morpholine and *N*-Boc piperidine, respectively, were obtained in three to four steps from commercially available trichlorotriazine. Seventeen hitherto unknown compounds were evaluated in vitro against various cathepsins for their inhibitory properties. Among them, compound **7c** (4-(morpholin-4-yl)-6-[4-(trifluoromethoxy)anilino]-1,3,5-triazine-2-carbonitrile) was identified as the most potent and selective inhibitor of cathepsin S (Ki = 2 ± 0.3 nM). Also **7c** impaired the autocatalytic maturation of procathepsin S. Molecular docking studies support that **7c** bound within the active site of cathepsin S, by interacting with Gly23, Cys25 and Trp26 (S1 subsite), with Asn67, Gly69 and Phe70 (S2 subsite) and with Gln19 (S1′ pocket).

1. Introduction

Lysosomal cysteine cathepsins (Cat) have been involved in many physiological and pathological processes such as alzheimer's disease,[1] cancer,[2] Stroke[3] and Ebola.[4] Eleven human cysteine cathepsins, i.e. cathepsins (B, C, F, H,…) have been identified.[5,6] These proteases share similar three-dimensional structures[7], catalytic mechanism, and substrate specificity.[8,6] Cathepsins are primarily involved in cellular turnover and degradation of endocytosed proteins. Moreover, a growing body of evidence supports that cathepsins play specific functions during numerous pathophysiological events. For instance, Cat K, that is a critical bone resorbing protease, corresponds to a clinically relevant target for osteoporosis and bone metastasis treatment,[9–12] while Cat S is an attractive target for drugs in autoimmune diseases (e.g. rheumatoid arthritis), emphysema or neuropathic pain.[13,14]

Inhibitors of cysteine cathepsins frequently contain an electrophilic functional group, capable of interacting with the nucleophilic cysteine residue located within the enzyme active site. Such groups include aldehydes or vinyl sulfones. Since nitrile-based heterocycles have been identified as potent and selective inhibitors of Cat K and Cat S, a special attention was paid for this scaffold.[15]

On our ongoing efforts to develop reversible cathepsin inhibitors, we have previously reported the synthesis and the biological evaluation of 1,3,5-triazines substituted by a nitrile function and a cyclohexylamine or a piperazine moiety.[16] Among the synthesized compounds, some cyclohexylamine derivatives exhibited a highly potent inhibitory effect (nM range) against both Cat B/K/L/S (similarly to other reported cyclohexylamine derivatives)[17] more, we have shown by docking studies that the piperazine moiety of the best inhibitors fits into the S1′ subsite and interacts with the side chain of Gln19 through a hydrogen bond. Based on these results, we decided to substituted the cyclohexylamine and the piperazine moiety by a morpholine and a *N*-piperidine as new pharmacophores, to conserve hydrogen bond interactions with the S1′ pocket of cathepsins (Fig. 1).

Thus, we report herein the synthesis and biological evaluation of two series of *hitherto unknown* 1,3,5-triazines substituted by either a nitrile, a morpholine or a piperidine moiety (Fig. 2.).

2. Results and discussion

The synthesis of those new 2,4,6 trisubstituted triazines was based on our recently reported procedure,[16] from commercially available cyanuric chloride **1**. (Scheme 1). The mono-substituted products **2** and **3** with morpholine and *N*-Boc piperidine respectively, were obtained in

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https://doi.org/10.1016/j.bmc.2018.07.032
Received 4 April 2018; Received in revised form 10 July 2018; Accepted 18 July 2018
Available online 19 July 2018
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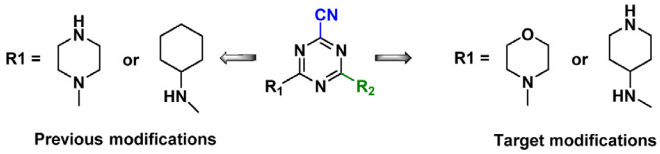

Fig. 1. Modifications of 1,3,5-triazine scaffold.

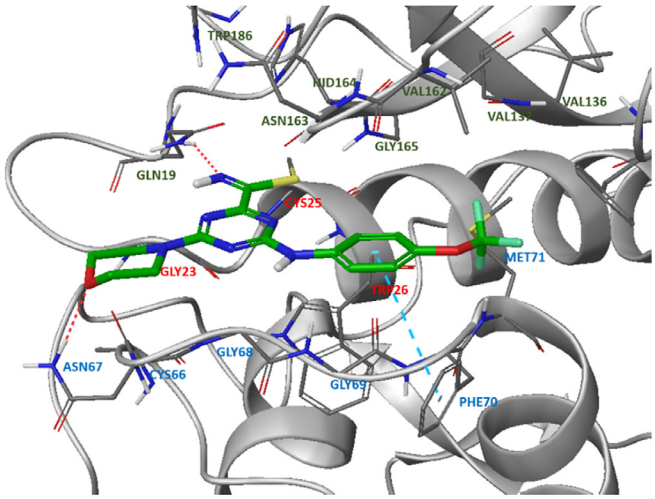

Fig. 2. Binding pose of compound 7c in the active site of Cat S (PDB ID: 3OVX). Residues within a distance of 4 Å from the inhibitor have been shown. Hydrogen bond and stacking interactions between Cat S and **7c** are in red and blue, respectively. Residues forming S1 subsite of Cat S are labelled in red, S2 residues in blue and S1′ residues in green.

good yields, by nucleophic substitution of cyanuric chloride **1** in presence of 1.1 equiv of diisopropylethyl amine (DIPEA) in dichloromethane at 0–5 °C for 2–4 h. The second nucleophilic substitution was carried out with appropriate amines in presence of 1.1 eq. of DIPEA, in acetonitrile, under microwave activation (MW) during 60–90 min, to afford the desired products **4a-d**, **4g-j** and **5a-h**, in 59% to 89% yield. However under these conditions, starting from compound **2** in presence of amine derivatives bearing electron withdrawing group (NO_2 at *para* or *meta* position for **e** and **f** respectively), products **4e** and **4f** were not obtained. Thus, in order to obtain **4e** and **4f**, we decided to introduce first the two amines, **e** and **f**, in presence of potassium carbonate (K_2CO_3) in acetone at 0–5 °C for 1 h to afford products **6e-f** in good yields. A second nucleophilic substitution with morpholine using the same conditions led to **4e** and **4f**.

The treatment of triazine **4a-j** and **5a-d,f-h**, with 1.1 equiv of potassium cyanide (KCN) in the presence of 0.2 equiv of 1,4-diazabicyclo [2.2.2]octane (DABCO) in dimethylsulfoxide DMSO, provided the corresponding nitrile products **7a-j** and **8a-d,f-h** in good yield. Finally, the deprotection of Boc group of compounds **8a-d,f-h** was achieved under acidic condition and gave the desired piperidine series **9a-d,f-h**.

2.1. Inhibition of cysteine cathepsins

In the current study, the SAR of the triazines derivatives bearing nitrile group **7** and **9**, where the substitution of the phenyl amine moiety, by fluoro, nitro, methoxy or trifluoromethoxy groups at meta or para position, were evaluated for their inhibitory properties (IC_{50}) towards human Cat B, K, L and S. The enzyme inhibition data of the screening were expressed as IC_{50} values (50% inhibitory concentration) and summarized in Table 1.

After titration by E-64, human cathepsins B, L, K and S (1 nM) were incubated in the presence of triazine derivatives as described in details elsewhere (section 4.6), using Z-Phe-Arg-AMC (benzyloxycarbonyl-phenylalanyl-arginine-4-methylcoumarin, 20 μM) as substrate for cathepsins B (K_m = 180 μM), L (K_m = 0.8 μM) and K (K_m = 9.7 μM), and Z-LR-AMC (20 μM) as substrate for Cat S (K_m = 23 μM). The enzyme inhibition data of the screening were expressed as IC_{50} values (average values). N.I.: no inhibition. Furthermore, the inhibition constant (K_i) of the most potent inhibitor (compound **7c**) was determined using the Cheng-Prusoff equation.[18] K_i values are expressed as mean ± SEM (n = 3). A newly described substrate-derived and reversible azaGly cathepsin inhibitor (compound **10**) was used as control.[19]

Among the synthesized compounds, morpholine analogs (**7a-j**) exhibited an inhibition for Cat S at the noticeable exception of **7i** and **7j** bearing (*R*)-or (*S*)-2-hydroxyl-2-phenylethylamine. Conversely, they did not inhibit Cat B and Cat L (IC_{50} > 1000 nM). Moreover these morpholine derivatives demonstrated a weak inhibitory activity towards Cat K, except for **7g** and **7h** having respectively a fluoro electron withdrawing group at para- or meta- position of aromatic ring ($IC_{50} \leq$ 40 nM). If some compounds (**7c**, **7e**, **7h**), exhibiting potent activity for CatS ($IC_{50} \leq$ 18 nM), only **7c** showed high selectivity with an IC_{50} of 4 nM for Cat S and no activity for Cat B, K and L ($IC_{50} \geq$ 1800 nM). The substitution of morpholine for the compound **7c** by piperidine moiety (compound **9c**) resulted in loss of activity for Cat S (IC_{50} > 150 nM). The piperidine moiety is better tolerated by Cat K and Cat L and decrease selectivity for Cat S inhibitory activity for compounds **9f** and **9g**.

2.2. Docking studies

To elucidate the binding mode of **7c**, molecular docking to the crystal structure of cathepsin S from its complex with a potent inhibitor (PDB ID 3OVX, resolution 1.49 Å) was performed. The protein structure was prepared in Protein Preparation Wizard,[20] under default settings (assigning bond orders, adding hydrogens, creation of disulfide bonds, deleting waters beyond 5 from het groups, H-bond assignment and restrained minimization), whereas three-dimensional structure, conformation and protonation states (at pH 7.4) of **7c** were generated by LigPrep (force field used OPLS2005, retention of specified chiralities and generation of only one low energy ring conformation).[21] Finally, Glide[22,23] was used for covalent docking (Cys25 was the reactive residue, sampling nitrogen inversion, sampling ring conformations with energy window equal to 2.5 kcal mol^{-1}, penalizing nonplanar conformation of amides, up to 100 steps during energy minimization and performing post-docking optimization of each conformer to the enzyme model. Each pose was ranked according to affinity score, and the highest scored pose was further analyzed. The binding mode of **7c** within the active site of Cat S was similar to that published previously,[16] i.e. besides covalently bound Cys25, the imine nitrogen formed a hydrogen bond with the side chain of Gln19 (S1′pocket) and triazine part interacted mainly with S1 subsite residue Gly23. However, compared to the previously published piperazine derivatives[24,25] more interactions of **7c** with the S2 site were observed; in particular, morpholine oxygen formed a hydrogen bond with NH backbone of Asn67 (hydrogen bond distance: 2.29 Å). It caused that *p*-trifluoromethoxyphenyl group was located in S2 site not as deeply as mentioned piperazine derivatives but had contacts with Gly69, and was close enough to Phe70 to be stabilized by π-π interactions.

2.3. Effect of 7c on the autocatalytic maturation of procathepsin S

In addition to neutrophile elastase and matrix metalloproteinases, Cat S has a highly potent elastinolytic activity and readily participates to degradation and/or turnover of the extracellular matrix (ECM). Interestingly, lung chronic inflammation and tissue remodelling result partly from an imbalance between proteolytic enzymes and their inhibitors in favour of proteolysis.[26,27] Cat S was mostly found as its proform (zymogen) in human inflammatory bronchoalveolar lavage fluids (BALFs).[28] BALF procathepsins could be activated