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Discovery of 4-(dihydropyridinon-3-yl)amino-5-methylthieno[2,3-*d*] pyrimidine derivatives as potent Mnk inhibitors: synthesis, structure–activity relationship analysis and biological evaluation

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ABSTRACT

Phosphorylation of the eukaryotic initiation factor 4E (eIF4E) by mitogen-activated protein kinase (MAPK)-interacting kinases (Mnks) is essential for oncogenesis but unnecessary for normal development. Thus, pharmacological inhibition of Mnks may offer an effective and non-toxic anti-cancer therapeutic strategy. Herein, we report the discovery of 4-(dihydropyridinon-3-yl)amino-5-methylthieno [2,3-*d*]pyrimidine derivatives as potent Mnk inhibitors. Docking study of **7a** in Mnk2 suggests that the compound is stabilised in the ATP binding site through multiple hydrogen bonds and hydrophobic interaction. Cellular mechanistic studies on MV-4-11 cells with leads **7a**, **8e** and **8f** reveal that they are able to down-regulate the phosphorylated eIF4E, Mcl-1 and cyclin D1, and induce apoptosis.

1. Introduction

Dysregulation of protein synthesis is implicated in the progression of various pathologies, most notably cancer [1]. As a key rate-limiting step in protein synthesis, initiation of cap-dependent translation is contingent on the availability and activity of the eukaryotic initiation factor 4E (eIF4E) [2]. This general translation factor is regulated by mitogen-activated protein kinase (MAPK)-interacting kinases (Mnks) through phosphorylation at Ser209 [3]. The phosphorylation process is necessary for oncogenic transformation [4,5]. Perturbation of the process by mutating eIF4E at Ser209 [6], silencing Mnk1 with small hairpin RNA (shRNA) [7] or knocking out Mnk1/2 [7] attenuated or even prevented tumour formation. Conversely, activation of Mnk1 promoted tumourigenesis in a manner similar to eIF4E [8]. In contrast with their pivotal role in tumourigenesis, Mnks seem to be dispensable for normal development [4,5]. For example, neither cap-dependent translation nor global protein synthesis was influenced in Mnk-deficient embryonic fibroblasts [9]. Taken together, these biological studies provide a rationale for the potential application of specific Mnk inhibition as an effective and non-toxic therapeutic strategy against cancer. Hence, there is a pressing demand in discovering pharmacologically selective Mnk inhibitors.

Regrettably, little progress has been made in the discovery of pharmacological Mnk inhibitors since the first isolation and identification of the two kinases in 1997 [3,10]. The known Mnk inhibitors include CGP052088 [11], CGP57380 [12,13], cercosporamide [14] and hypothemycin derivatives [15–17] (Fig. 1). These small molecules inhibit Mnks at nanomolar to micromolar concentrations. However, all of them target multiple protein kinases. Earlier this year, a series of 5-(2-(phenylamino)pyrimidin-4-yl)thiazol-2(3*H*)-one derivatives was reported as potent Mnk2 inhibitors with sub-micromolar affinity, but the kinase selectivity remains unconquered [18].

The high structural similarity of the ATP binding pockets among kinases renders selective Mnk inhibition by exogenous inhibitors challenging. Previous crystallographic analyses of Mnks reveal two distinct structural features: (i) a noncanonical DFD motif (*vs.* a common DFG motif in other kinases) and (ii) three Mnk-specific inserts (*i.e.*, insertions I1, I2 and I3) at the catalytic domain [19,20]. In the absence of a ligand, Mnks predominantly adopt an

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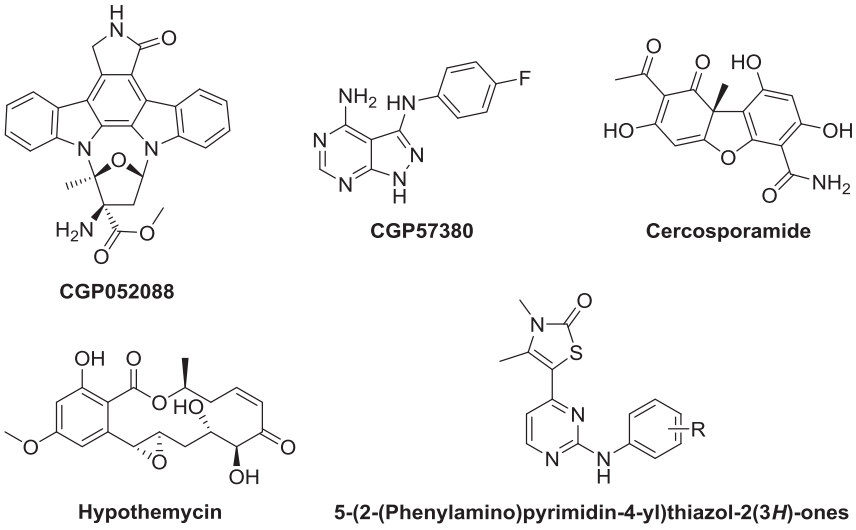

Fig. 1. Chemical structures of known Mnk inhibitors.

unusual DFD-out conformation, in which the Phe residue flips into the ATP binding pocket, thus preventing the accessibility of ATP [21]. Exploiting the above characteristics would assist in the rational design of highly selective Mnk inhibitors. Nevertheless, the lack of structural details on the ATP-bound Mnks has hampered the development of specific Mnk inhibitors.

In our earlier attempt to identify novel selective Mnk inhibitors, the ChemBridge database consisting of approximately 572,000 molecules was pre-filtered according to molecular weights, and the remaining 387,000 compounds with molecular weights ranging from 300 to 450 were subjected to a comprehensive virtual screening using both protein- and ligand-based approaches. In the former method, the only available staurosporine-bound Mnk2 (D228G) crystal structure (PDB ID: 2HW7) was adopted to create a binding site for the protein-ligand docking. In the latter approach, CGP57380 and cercosporamide, two most extensively studied Mnk inhibitors, were selected as query molecules for the ligand-based alignment. Successful deployment of these two methods was followed by structure-guided optimisation and the evaluation of Mnk inhibitory activity of the remaining candidates using biochemical assays to reveal 4-aminothieno[2,3-*d*]pyrimidine as a valuable chemical scaffold for Mnk inhibitors [22]. Using analogue-based drug design, we further explored the utility and versatility of this scaffold. Herein, we describe the synthesis and biological evaluation of 4-(dihydropyridinon-3-yl)amino-5-methylthieno[2,3-*d*]pyrimidine derivatives.

2. Results and discussion

2.1. Chemistry

The synthetic route deployed to prepare methyl 4-((1-alkyl-2-oxo-5-methyl-1,2-dihydropyridin-3-yl)amino)-5-methylthieno [2,3-*d*]pyrimidine-6-carboxylates (**6a** and **6b**) and their derivatives (**7a**, **7b** and **8a**–**f**) is delineated in Scheme 1. *N*-Alkylation of 5-methyl-3-nitropyridin-2(1*H*)-one **1** with iodomethane or 1-bromo-2-methoxyethane in the presence of potassium carbonate gave 1,5-dimethyl-3-nitropyridin-2(1*H*)-one **2a** or 1-(2-methoxyethyl)-5-methyl-3-nitropyridin-2(1*H*)-one **2b** respectively in good yields (**2a**: 95% and **2b**: 60%), which underwent palladium-catalysed hydrogenation to afford the corresponding 3-amino-1,5-dimethylpyridin-2(1*H*)-one **3a** or 3-amino-1-(2-methoxyethyl)-5-methylpyridin-2(1*H*)-one **3b** in excellent yields (**3a**: 94% and **3b**: 100%). Chlorination of methyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylate **4** with thionyl chloride in the presence of *N*,*N*-dimethylformamide (DMF) yielded methyl 4-chloro-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate **5** in a yield of 89%. Each of amines **3a** and **3b** was individually coupled with chloride **5** in the presence of *p*-toluenesulfonic acid under microwave irradiation to give the corresponding methyl 4-((1-alkyl-2-oxo-5-methyl-1,2-dihydropyridin-3-yl)amino)-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylates **6a** and **6b** in good yields (**6a**: 86% and **6b**: 54%), which were subsequently subjected to saponification in an alkaline methanolic solution to yield the carboxylic acids **7a** and **7b** respectively in excellent yields (**7a**: 100% and **7b**: 84%). Amination of carboxylic acids **7a** and **7b** with appropriate amines was effected in anhydrous DMF in the presence of the coupling reagent 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) and *N*,*N*-diisopropylethylamine (DIPEA) to give the corresponding amides **8a**-**f** in moderate to good yields (36–80%).

The above synthetic approach was successfully applied to the preparation of methyl 4-((1-methyl-6-oxo-1,6-dihydropyridin-3-yl)amino)-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate (**12**) and its derivatives (**13** and **14**) (Scheme 2). In brief, 5-nitropyridin-2(1*H*)-one **9** sequentially underwent alkylation and reduction to give 5-amino-1-methylpyridin-2(1*H*)-one **11**. Due to its instability in the air, amine **11** was directly used in the next synthetic step without further purification and characterisation; *p*-toluenesulfonic acid-catalysed substitution of chloride **5** with amine **11** was achieved by microwave irradiation to give the desired methyl 4-((1-methyl-6-oxo-1,6-dihydropyridin-3-yl)amino)-5-methylthieno [2,3-*d*]pyrimidine-6-carboxylate **12**. Ester **12** was readily hydrolysed to afford carboxylic acid **13** in a yield of 88%, which was subsequently aminated with ammonia to give amide **14** in 54% yield.

2.2. Structure–activity relationship analysis

Up-regulation of eIF4E is observed in acute myeloid leukaemia (AML) [23] and targeting eIF4E by pharmacologic inhibition of its activating kinases Mnks may provide a new approach for the treatment of AML. A recent study demonstrated that the suppressive effects of cercosporamide on AML correlated with its Mnk