Bioorganic & Medicinal Chemistry Letters 26 (2016) 765–768

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Identification of benzothiophene amides as potent inhibitors of human nicotinamide phosphoribosyltransferase

Wei Chen [†], Guoqiang Dong [†], Shipeng He [†], Tianying Xu, Xia Wang, Na Liu, Wannian Zhang, Chaoyu Miao [*], Chunquan Sheng [*]

School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, People's Republic of China

ARTICLE INFO

Article history:
Received 27 September 2015
Revised 17 December 2015
Accepted 29 December 2015
Available online 29 December 2015

Keywords:
Nampt inhibitors
Antiproliferative activity
Structure–activity relationship

ABSTRACT

Nicotinamide phosphoribosyltransferase (Nampt) is an attractive therapeutic target for cancer. A Nampt inhibitor with novel benzothiophene scaffold was discovered by high throughput screening. Herein the structure–activity relationship of the benzothiophene Nampt inhibitor was investigated. Several new inhibitors demonstrated potent activity in both biochemical and cell-based assays. In particular, compound **16b** showed good Nampt inhibitory activity (IC_{50} = 0.17 μM) and in vitro antitumor activity (IC_{50} = 3.9 μM, HepG2 cancer cell line). Further investigation indicated that compound **16b** could efficiently induce cancer cell apoptosis. Our findings provided a good starting point for the discovery of novel antitumor agents.

Cancer is a disease of genetic heterogeneity, but the metabolic alternation is the common future of malignant cells.[1,2] Although Warburg suggested that energy generation in cancer cells switch form the Krebs (tricarboxylic acid) cycle to glycolysis to sustain robust proliferation as early as in 1924,[3,4] it is only recently that targeting cancer metabolism has emerged as a therapeutic strategy for the development of antineoplastic agents.[5] Among the metabolic targets, considerable attention has been given to the biosynthetic pathways leading to nicotinamide adenine dinucleotide (NAD).[6,7] NAD is an important cofactor in redox reactions and serves as the substrate for poly (ADP-ribose) polymerases (PARPs),[8] mono (ADP-ribose) transferases (ARTs),[2] and sirtuins,[9] which convert NAD to nicotinamide (NAM). In cancer cells, NAD is rapidly consumed because of increased demand for ATP and high activity of NAD-consuming enzymes such as PARPs and Sirtuins.[9–11] Thus, malignant cells are more sensitive to NAD availability as compared with normal cells. Rapidly proliferating cancers requires constant re-synthesis of NAD in order to maintain sufficient levels for cell survival. Biochemically, there are three main pathways to generate NAD in eukaryotic cells: a de novo pathway using tryptophan (Trp) as the precursor, the primary salvage pathway in which NAD is recycled from NAM, and the alternative salvage pathway using nicotinic acid. The main source of cellular NAD is from the primary salvage pathway using NAM as the precursor.[12] Nicotinamide phosphoribosyltransferase (Nampt) catalyzes the rate-limiting step.[13,14] Given that Nampt plays a key role in the replenishment of NAD, inhibition of Nampt can dramatically impact NAD metabolism and cancer proliferation. Thus, Nampt is considered as an attractive target for the development of new cancer therapies.

To date, several classes of Nampt inhibitors have been reported in the scientific and patent literatures.[2,12,15] The most advanced compounds FK866, CHS828 and its prodrug GMX1777 (Fig. 1) have entered the human clinical trials.[16–18] FK866 is in phase 2 clinical trials for the treatment of cutaneous T-cell lymphoma (CTCL), while CHS828 is in phase 1 trials against metastatic melanoma. However, the clinical development of FK866 has been hampered by dose-limiting thrombocytopenia.[19] CHS828 was also reported with thrombocytopenia and various gastrointestinal symptoms.[20] With regard to limited classes of small-molecule Nampt inhibitors, it is highly desirable to discover new inhibitors with novel chemical scaffolds and potent antiproliferative activity (e.g. compound **1**).[15,21,22]

The pharmacophoric model (Fig. 1) of the Nampt inhibitors consists of a cap group (typically a *meta* or *para* substituted pyridine), a connecting unit, a linker and a tail group.[2] Previously, we described the identification of compound **2** (Fig. 1) as a novel Nampt inhibitor by high throughput screening.[15] It showed submicromolar activity against Nampt (IC_{50} = 0.15 μM). Interestingly, the benzothiophene inhibitor **2** did not contain a pyridine or nitrogen-containing heterocycle moiety in the cap group. This molecule demonstrated that a non-nitrogen heterocycle NAM mimetic can

* Corresponding authors. Tel.: +86 21 81871271 (C.M.), +86 21 81871239 (C.S.).
E-mail addresses: cymiao@smmu.edu.cn (C. Miao), shengcq@hotmail.com (C. Sheng).
† These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bmcl.2015.12.101
0960-894X/

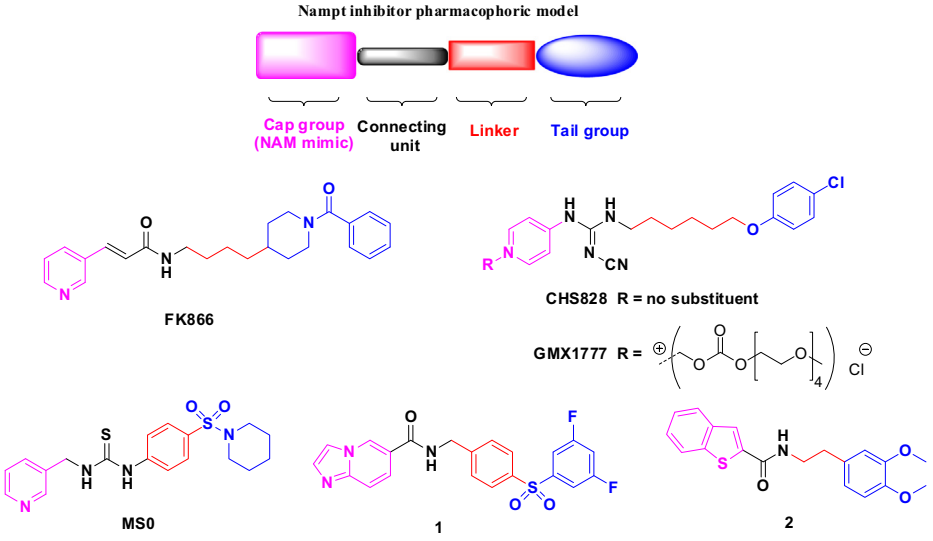

Figure 1. Pharmacophoric model and chemical structures of the Nampt inhibitors.

be tolerated in the Nampt binding site. However, the in vitro antitumor potency of inhibitor **2** was poor (Table 1) and its structure–activity relationships (SAR) is still unknown. Thus, a series of novel benzothiophene Nampt inhibitors were designed, synthesized and assayed.

The synthetic routes of the target compounds were shown in Schemes 1–3. As depicted in Scheme 1, commercially available compound **3** was reacted with the substituted carboxylic acids in the present of HBTU and Et_3N to give target compounds **7**, **8a–c**, and **9**. Compounds **13a–b** were synthesized according to the procedures outlined in Scheme 2. Treatment of compound **3** and (*tert*-butoxycarbonyl)glycine (**10**) via condensation reaction afforded the key intermediate **11**. After the removal of the Boc protecting group of **11**, amide **12** was coupled with carboxylic acids (**5a–b**) to give the target compounds **13a–b**. Finally, the compounds bearing the imidazole tails (**15a–b**, **16a–c**, **17**) were synthesized using the condition similar to that of compound **7** (Scheme 3). Condensation of 3-(1*H*-imidazol-1-yl)propan-1-amine (**14**) with carboxylic acids afforded the target compounds **15a–b**, **16a–c**,and **17**.

Our previously described fluorometric assay was used to evaluate the Nampt inhibitory activity.[15] As shown in Table 1, most of the benzothiophene derivatives exhibited moderate to good anti-Nampt activity. The replacement of the benzothiophene scaffold with benzofuran (compound **7**) led to significant decrease of the Nampt inhibitory activity. The removal of the benzene ring of the benzothiophene scaffold afforded the substituted thiophene derivatives. Introduction of methyl group at the thiophene C-5 position yielded several good Nampt inhibitors. Compounds **8b**, **13b** and **16b** had an IC_{50} value of 0.86 μM, 0.95 μM and 0.17 μM, respectively. Compound **16b** showed comparable Nampt inhibitory activity to lead compound **2** (IC_{50} = 0.15 μM). However, compounds with a nitro group at the C-5 position (**8c** and **16c**) lost their inhibitory potency in both biochemical and cell-based assays. Pyridine is a classical cap group in the known Nampt inhibitors. However, when benzothiophene was replaced by the *meta* substituted pyridine, compounds **9** and **17** were inactive in the anti-Nampt and antiproliferative assay. Thus, the SAR of the benzothiophenes might be different from that of classical Nampt inhibitors.

The synthesized compounds were also evaluated for antiproliferative activities against the HCT116 (colon cancer cells), MDA-MB-231 (breast cancer cells) and HepG2 (liver cancer cells) human cancer cell lines using the standard MTT assay.[23] As shown in Table 1, these compounds showed better activity against HepG2 than the other two cell lines. Moreover, most target compounds exhibited better antiproliferative potency than lead compound **2**. Among them, compound **16b** exhibited the best antiproliferative activity against the HepG2 cell line (IC_{50} = 3.9 μM). Despite good in vitro Nampt inhibitory potency, the 5-methylthiophenes **8b** and **13b** lost activity for cancer cell lines. In consistent with the Nampt inhibitory activities, compounds containing the pyridine in cap group (**9** and **17**) were completely inactive in the antitumor assays.

Table 1
Nampt inhibition and in vitro antitumor activity of target compounds

Compd	IC_{50} (μM)			
	Nampt	HCT116	MDA-MB-231	HepG2
7	1.4 ± 0.11	159 ± 11	>371	137 ± 14
8a	5.3 ± 0.32	169 ± 21	191 ± 14	99 ± 8.7
8b	0.86 ± 0.05	>307	>307	>307
8c	>10	86 ± 9.2	>328	80 ± 9.0
9	>10	>401	>401	>401
13a	>10	196 ± 15	>328	47 ± 4.2
13b	0.95 ± 0.08	>401	>401	>401
15a	1.1 ± 0.12	25 ± 1.9	36 ± 2.8	5.2 ± 0.45
15b	1.0 ± 0.09	61 ± 5.7	10 ± 1.2	11 ± 1.3
16a	>10	198 ± 16	>276	102 ± 12
16b	0.17 ± 0.01	>320	>320	3.9 ± 0.33
16c	>10	>390	>390	60 ± 5.5
17	>10	>276	>276	>276
2	0.15 ± 0.02	281 ± 20	>293	82 ± 7.9

Molecular docking studies were performed using GOLD 5.0[24] to investigate the binding mode of compounds **2** and **16b** with Nampt. The crystal structure of Nampt in complex with FK866 was obtained from protein database bank (PDB ID: 2GVJ)[25] and prepared for molecular docking analysis in Discovery Studio 3.0[26] by removing the ligand from the binding pocket, merging nonpolar hydrogens, adding polar hydrogens, and rendering Gasteiger charges to each atom. Conformations were generated by genetic algorithm and scored using GoldScore as fitness function. The best conformation was chosen to analyze the ligand–protein interaction. As depicted in Figure 2, the benzothiophene scaffold of compound **2** was sandwiched between the side chains of Phe193 and Tyr18 and formed π–π interactions. The oxygen atom of amide formed a hydrogen bond with the hydroxyl group of Ser275. The phenyl and alkyl group interacted with Val242, Ala244 and