Bioorganic & Medicinal Chemistry Letters 21 (2011) 3749–3754

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Identification and synthesis of *N*′-(2-oxoindolin-3-ylidene)hydrazide derivatives against c-Met kinase

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ARTICLE INFO

Article history:
Received 27 February 2011
Revised 9 April 2011
Accepted 13 April 2011
Available online 22 April 2011

Keywords:
c-Met kinase
N′-(2-Oxoindolin-3-ylidene)hydrazide
Pharmacophore-based virtual screening

ABSTRACT

A series of *N*′-(2-oxoindolin-3-ylidene)hydrazide derivatives were identified as moderately potent inhibitors against c-Met kinase by pharmacophore-based virtual screening and chemical synthesis methods. The structure–activity relationship (SAR) at various positions of the scaffold was investigated and its binding mode with c-Met kinase was analyzed by molecular modeling studies. In this study, two potent compounds D2 and D25, with IC_{50} value at 1.3 μM and 2.2 μM against c-Met kinase respectively, were identified. Finally, based on the clues extracted from this study, future development for the optimization of this scaffold was discussed.

MET (mesenchymal–epithelial transition factor) is a proto-oncogene that encodes tyrosine kinase receptor c-Met kinase, also known as hepatocyte growth factor receptor (HGFR). c-Met kinase, together with its ligand hepatocyte growth factor (HGF), is involved in cell proliferation, migration and invasion as well as is essential for normal embryonic development.[1] c-Met kinase can be activated via a variety of mechanisms, including ligand-dependent activation (autocrine or paracrine), gene amplification, gene mutation and cross-talk with other receptors (heterodimerization).[2] The activation gives rise to receptor dimerization and recruitment of several SH2 domain-containing signal transducers that activate a number of pathways including the Ras–MAPK, PI3k-Akt-mTOR cascades and so on.[3] When deregulated, the HGF/c-Met kinase pathway has been implicated in tumorigenesis and metastasis,[4] which implies the kinase as a potential target for cancer treatment. Given a growing number of drug candidates investigated in the clinical phase, c-Met kinase has risen as a kinase target of priority.[5]

As HGF/c-Met kinase signaling plays a role of significance in tumorigenesis and metastasis, several different strategies have been explored to inhibit c-Met kinase, each focusing on one of the serial steps that regulate c-Met kinase activation.[3] Up to now, several potential therapeutic strategies have been tried by targeting HGF/c-Met kinase pathway, including HGF antagonists, antibodies against HGF or c-Met kinase, and small molecular inhibitors against phosphorylation of the tyrosine residues in the tyrosine kinase domain. Among these strategies, inhibition of the tyrosine kinase activity mediated by an ATP-competitive small molecule (type I inhibitor) or non-ATP-competitive small molecule (type II inhibitor) is a pharmacologically attractive method, which has been demonstrated for other tyrosine kinases.[6]

To date, numerous efforts have been made toward the development of small molecular inhibitors against c-Met tyrosine kinase.[7–13] However, due to poor pharmacokinetic properties, quite a few small molecular inhibitors have been limited to in vitro assay or brief in vivo studies, and were not applicable for the clinical purposes. To identify more potent and novel scaffolds against c-Met kinase, in this study, pharmacophore-based virtual screening and molecular docking methods were performed to discover new c-Met kinase inhibitor leads. The procedure is shown in Figure 1, firstly, 24 reported compounds with a wide range of bioactivities and structural diversity were chosen to construct selective pharmacophore models.[7–13] Then, the 3D-QSAR pharmacophore generation implemented in Accelrys Discovery Sudio2.1 (Accelrys Inc., San Diego, CA) was performed to set up pharmacophore models. After the clustering and validation of these models, 4 rational models were extracted to screen the SPECS database. The SPECS database containing 190,000 chemicals (http://www.specs.net) was firstly filtered by the druglikeness and then adopted for

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doi:10.1016/j.bmcl.2011.04.064

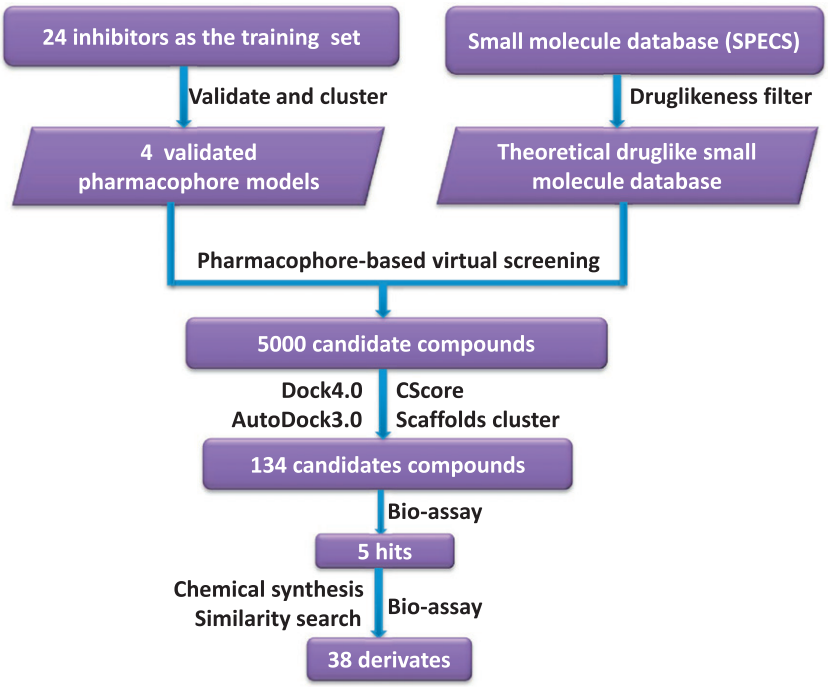
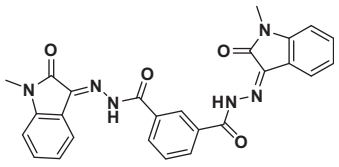
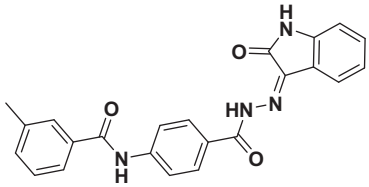
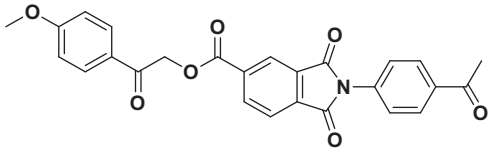
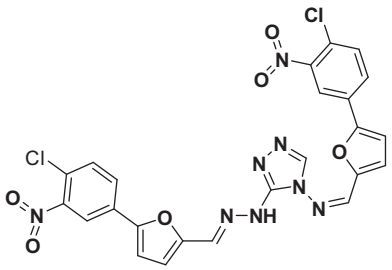
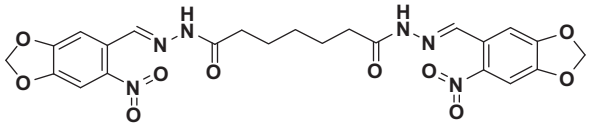


Figure 1. Flow chart of virtual and experimental screening strategy combined with chemical synthesis method for discovering c-Met kinase inhibitors.

Table 1
Molecular structures of 5 hits that show inhibitory activity against c-Met kinase (The compounds named with D* were purchased from the SPECS database, while the compounds named with S* were synthesized in this study.)

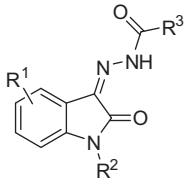
Code	Compound	Inhibition (%)	IC_{50} (μM)
D1		51.3	10.3
D2		56.4	1.3
D3		55.1	3.7
D4		61.4	3.3
D5		54.5	0.78

pharmacophore-based virtual screening. During the screening process, the BEST method was used, and the maximum number of conformers generated was set to 250. The pharmacophore models were employed one by one with the flexible search method to screen the database with Discovery Studio2.1. The molecular docking method via software DOCK4.0[14] and AUTODOCK3.05[15] was employed to evaluate the top 5000 small molecules from the pharmacophore-based database searching method. The active and inactive conformation of c-Met kinase crystal structure (PDB entries: 2RFS[10] and 3C1X[8]) were retrieved from the Protein Data Bank (http://www.rcsb.org/pdb) as the receptor models. Furthermore, 3000 compounds with the highest score by DOCK4.0[14] were scored by AUTODOCK3.05[15] program. Among the top 1000 molecules scored by AUTODOCK, the conformation with the highest score of each molecule was evaluated by CScore method implemented in Sybyl software package for predicting the binding score of the given protein-ligand complex structures. Taking account to the score and scaffold diversity classified by Pipeline program, 124 compounds among 50 scaffolds were purchased and evaluated for their ability to inhibit enzymatic activity of the c-Met kinase by in vitro assay which is described in the Supplementary data.

In vitro assay indicated that compounds D1 ~ D5 listed in Table 1 stand out as the most potent c-Met kinase inhibitors. At the concentration of 10 μM, their inhibition against c-Met kinase are >50.0%. Their IC_{50} values were also determined, ranging from 0.78 μM to 10.3 μM. Among the five hit compounds shown in Table 1, the compound D2 with isatin derivative scaffold stands out for further study because of its good druglikeness and moderate potency. Interestingly, we noticed that quite a few studies demonstrated these isatin derivatives were to be potential kinase inhibitors.[16–18] Therefore, the compound D2 with IC_{50} value at 1.3 μM against c-Met kinase, was chosen as the lead compound for further studies. The sub-structure similarity searching method against SPECS database and chemical synthesis were performed to obtain more analogues for the exploring their SAR. The synthesis method and spectrum data were applied in the Supplementary data. Finally, 37 non-ATP competitive compounds with moderate inhibitory activity against c-Met kinase were obtained in this study.

In the chemical synthesis procedure, we made our efforts to append diverse substitutents on the various positions of the *N*′-(2-oxoindolin-3-ylidene)hydrazide scaffold, influencing the c-Met kinase activity. Initially, we screened a wide range of hydrazide groups analogues listed in Table 2. We observed the inhibitory activity apparently abolished when the m-methylbenzoamine benzohydrazide (D2, IC_{50} = 1.3 μM) was changed to *p*-cyclopropane carbonyl amine benzohydrazide (D6). And the *p*-nitrobenzohydrazide analogue (D7) showed weak c-Met kinase activity with IC_{50} 28.3 μM. The 3-hydroxyl-2-naphthohydrazide analogue D8 displayed potent c-Met kinase activity with an IC_{50} value of 4.3 μM. Alkyl hydrazides analogues (D9 ~ D11) are not beneficial for c-Met kinase activity. Then, we turned our attention to those substituents on the benzene ring of isatin. The in vitro assay data indicated that the introduction of halogen in the 5-position was not favorable for the inhibitor's potency when the N-1-position was not substituted (Table 3). Moreover, a pool of substituents in the N-1-position were also attempted to identify more potent inhibitors (Table 4). It was observed that when N-1-position beared methyl group, *p*-nitro benzohydrazide analogue D12 revealed the inhibitory potency with an IC_{50} value of 12.3 μM, whereas *m*-nitro benzohydrazide analogue D13 abolished its inhibitory activity. In addition, the halogen analogues D14 and D15 totally abolished the inhibition against c-Met kinase. After *p*-nitro benzohydrazide group was kept, the introduction of more hydrophobic or hydrophilic substituents (D16, D17, D18 and D19) in the N-1-position abolished their inhibitory activity. In comparison of compound D17, it was very interesting that analogues D20 and D21 inhibited c-Met kinase activity with an IC_{50} value of 8.1 μM and 15.8 μM, respectively. Compound D22 and D23 weakly inhibited the activity of c-Met kinase.

Table 2
c-Met kinase inhibition activity for compounds D6–D11 of general structure



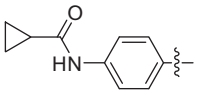
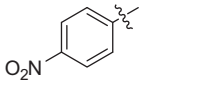
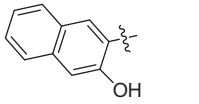
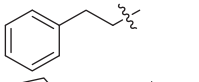
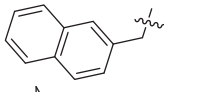
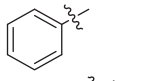
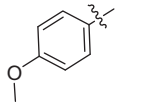
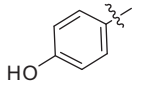
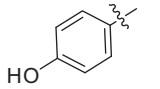
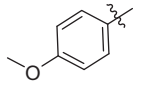
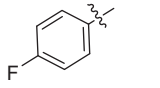
Code	Compound			Inhibition (%)	IC_{50} (μM)
	R^1	R^2	R^3		
D6	H	H		11.2	NA
D7	H	H		33.3	28.3
D8	H	H		75.2	4.3
D9	H	H		6.3	NA
D10	H	H		29.4	NA
D11	H	H		12.8	NA

Table 3
c-Met kinase inhibition activity for compounds S1–S6 of general structure

Code	Compound			Inhibition (%)	IC_{50} (μM)
	R^1	R^2	R^3		
S1	5-F	H		5.7	NA
S2	5-F	H		0	NA
S3	5-F	H		21.1	NA
S4	5-Cl	H		24.1	NA
S5	5-Cl	H		6.7	NA
S6	5-F	H		13.3	NA

To explore the SAR among these compounds, we further synthesized a series of isatins containing *N*-morpholinomethyl group in the N-1-position (Table 5). We found 5-Br and benzohydrazide substituted analogue D24 displayed potent c-Met kinase inhibition activity with an IC_{50} value of 3.5 μM. The corresponding analogue D25, appending an *p*-methoxy group on the benzene, improved activity slightly. The inhibitory activity of 5-F substituted analogues S7, S8 and S11 are significantly reduced. It was also observed that 5-Cl substituted analogues S9, S10 and S12 are weak inhibitors against c-Met kinase. These results indicate that the less negatively-charged halogen is beneficial for the potency of inhibitor.

To investigate the molecular basis of *N*′-(2-oxoindolin-3-ylidene)hydrazide derivatives interacting with c-Met kinase, molecular docking approach was performed to analyze the complex structures of compounds D2 and D25 with the kinase. As shown in Figure 2, the isatin core engages in key H-bonding interactions with the hinge region of the c-Met kinase. The NH atom in the backbone and the carbonyl of Met1160 H-bond with the oxygen atom of carbonyl and N1H atom of D2, respectively. The central phenyl ring of D2 which is flanked on one side by the gatekeeper residue (Leu1157) and on the opposite side by Phe1223 (DFG motif) involves in a π–π stacking interaction. The amide portion of D2 occupies the urea site and forms iterative H-bonds with c-Met kinase. The amide and carbonyl of D2 H-bond with the backbone of Asp1222 and the terminal of Lys1110, respectively. Finally, the terminal benzyl ring mostly resides in a hydrophobic site and interacts with a few aromatic residues including Ile1130, Phe1134 and Phe1200. To further investigate molecular basis of the derivatives with *N*-morpholinomethyl substitent interacting with receptor, the binding mode of the complex structure of D25 with c-Met kinase was analyzed. In the hinge region, the carbonyls of isatin core and hydrazide H-bond with the hydroxyl group of Tyr1159 and the NH atom in the backbone of Met1160. The bromine in

Table 4
c-Met kinase inhibition activity for compounds D12–D23 of general structure

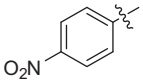
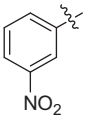
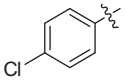
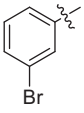
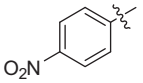
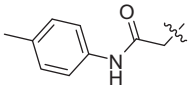
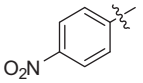
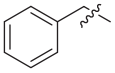
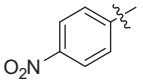
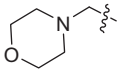
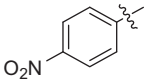
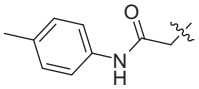
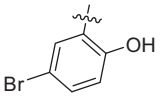
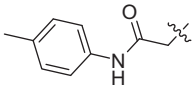
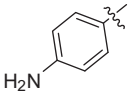
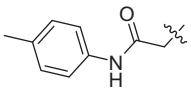
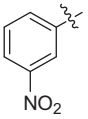
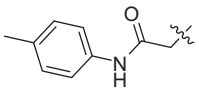
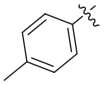
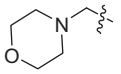
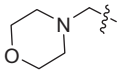
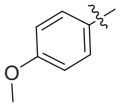
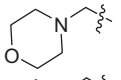
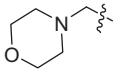
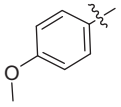
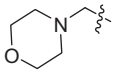
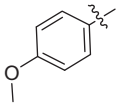
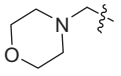
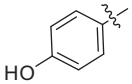
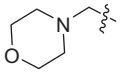
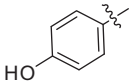
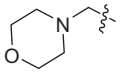
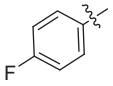
Code	Compound			Inhibition (%)	IC_{50} (μM)
	R^1	R^2	R^3		
D12	H	CH_3		57.8	12.3
D13	H	CH_3		12.3	NA
D14	H	CH_3		14.4	NA
D15	H	CH_3		25.9	NA
D16	H	CH_3CH_2		2.7	NA
D17	H			12.4	NA
D18	H			3.9	NA
D19	H			48.1	NA
D20	H			55.5	8.1
D21	H			58.6	15.8
D22	H			37.6	NA
D23	H			22.6	NA

Table 5
c-Met kinase inhibition activity for compounds D24–S12 of general structure

Code	Compound			Inhibition (%)	IC_{50} (μM)
	R^1	R^2	R^3		
D24	5-Br			85.4	3.5
D25	5-Br			73.6	2.2
S7	5-F			1.4	NA
S8	5-F			0.0	NA
S9	5-Cl			48	NA
S10	5-Cl			28.7	NA
S11	5-F			26.6	NA
S12	5-Cl			39.9	NA

the isatin probably matches the space of the binding pocket quite well compare with other halogens. The methoxy-benzene locates in the hydrophobic pocket surrounded by Val1092, Ala1108, Leu1140, Leu1157, Ala1221 and Phe1223. The *N*-morpholinomethyl group orients the solvent out of the binding pocket.

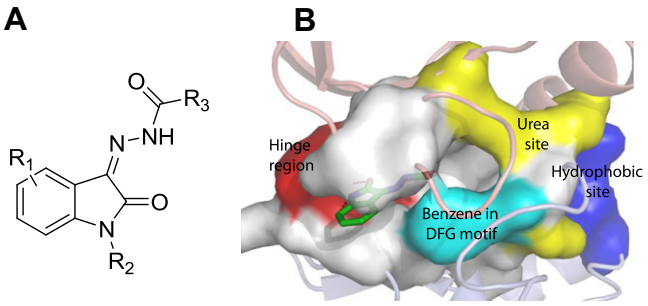


Figure 3. Future directions for designing c-Met kinase inhibitors with greater potency. (A) Base scaffold with three possible positions for modifications. (B) The scaffold in the ATP-binding site of the c-Met kinase. Regarding the whole pocket, the residues in 4 Å with the compound D2 are shown as surface. The hinge region, benzene ring in the DFG motif, urea site and hydrophobic site are colored red, cyan, yellow and blue respectively.

Based on the SAR and binding modes analysis aforementioned, the modifications on *N*′-(2-oxoindolin-3-ylidene)hydrazide scaffold were proposed to attempt to obtain more potent compounds. As shown in Figure 3, the hydrazide portion seems to be a linker without any interaction with c-Met kinase, which could be replaced with a few more drug-like groups. In the R^3 position, the terminal 3-methyl-benzene of compound D2 doesn't enter the hydrophobic site completely. Another amide group might be inserted before the 3-methyl-benzene in the urea site, which would afford the H-bond with the Glu1127 in α-helix and make the benzene deeper in the hydrophobic site. Meanwhile, different H-bond donors, acceptors and hydrophobic substituents could be attempted in the urea and hydrophobic sites. Considering the *N*-morpholinomethyl group into the R^2 position of the isatin derivatives, compound D23 seems to be a good lead compound for future inhibitor optimization. Meanwhile, the clues extracted from SAR

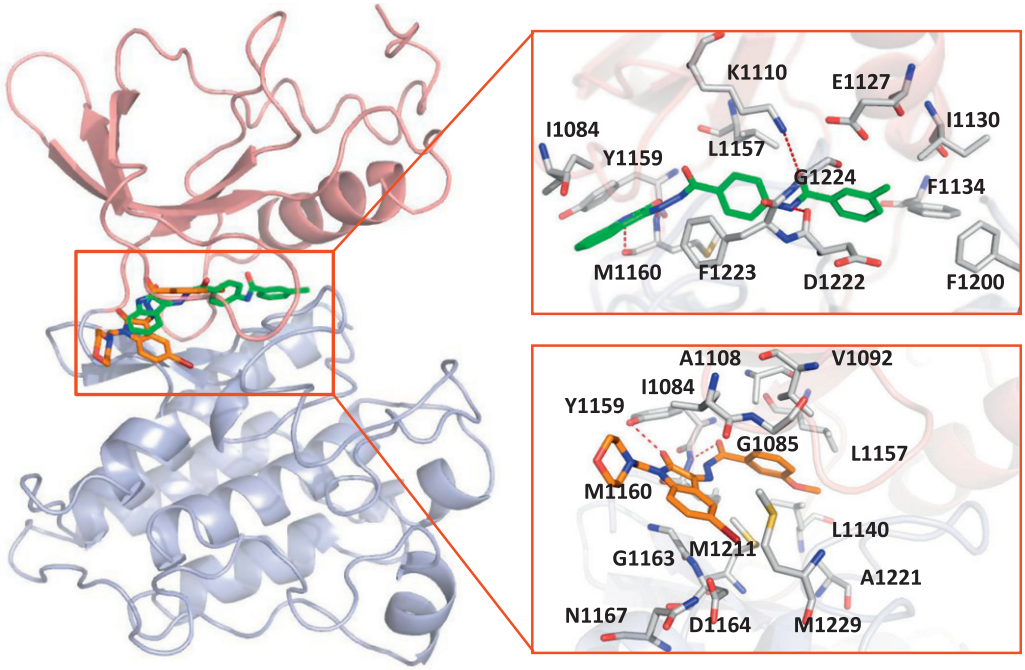


Figure 2. Binding mode analysis of compounds D2 and D25 with c-Met kinase at inactive conformation. The N lobe and C lobe of the c-Met kinase domain are red and blue, respectively. Compounds D2 and D25 are represented in green and orange sticks, respectively. Meanwhile, key residues in the pocket forming interactions with compounds are represented in gray sticks on the right.

of halogen analogues in R^1 position indicate that bromine is favored here. With the *N*-morpholinomethyl group into the nitrogen of the isatin kept in the hinge region, different H-bond donors, acceptors and hydrophobic substituents in the R^3 region could be beneficial for the inhibitor's potency as aforementioned. Taken together, our study demonstrates that an efficient and cost-effective virtual screening procedure can be used to identify a series of *N*′-(2-oxoindolin-3-ylidene)hydrazide derivatives against c-Met kinase. These studies gave rise to the discovery of potent compound D2 and D25, with the value of IC_{50} at 1.3 μM and 2.2 μM, respectively. Finally, the potentially helpful clues obtained in the in silico and chemical synthesis study on the isatin derivative scaffold will light up the road upon the future development of c-Met kinase inhibitors for the using as therapeutic agents against HGF/c-Met kinase signaling related tumorigenesis and metastasis.

Acknowledgments

We gratefully thank Dr. Manqing Hou from National Institutes of Health for her helpful comments. We also gratefully acknowledge the financial support from the National Natural Science Foundation of China (20972174, 81025017, 30725046, 91029704 and 21021063), the State Key Program of Basic Research of China grant (2009CB918502), Shanghai Committee of Science and Technology (10410703900), the Chinese Academy of Sciences (XDA01040305), National Science & Technology Major Project on (2009ZX09301-001). Guangdong S&T Dept. (2010A030100006) and Program of Shanghai Subject Chief Scientist (10XD1405100).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.064.

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