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RESEARCH ARTICLE

Design and Synthesis of Some Novel 3*H*-[1, 2, 3]triazolo[4, 5-d]pyrimidines as Potential c-Met Inhibitors

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ABSTRACT

c-Met receptor tyrosine kinase has been under extensive basic and preclinical investigation, and is now known to be a "druggable" target with promising results of early phase clinical results of c-Met targeting agents emerging. On the basis of structure of two c-Met inhibitors, PF-04217903 and JNJ-38877605, some novel 3*H*-[1,2,3]triazolo[4,5-d]pyrimidines were rationally designed using the strategies of bioisosterism, synthesized and evaluated as novel c-Met inhibitors.

KEYWORDS

c-Met inhibitor, *3H*-[1, 2, 3]triazolo[4, 5-d]pyrimidines, Bioisosterism principles, *Suzuki* coupling reaction, Design and synthesis

INTRODUCTION

The c-Met RTK family is the only known high-affinity receptor for hepatocyte growth factor (HGF), also known as scatter factor (SF), which is produced by stromal and mesenchymal cells and acts primarily on c-Met-expressing epithelial cells in an endocrine and/or paracrine fashion. c-Met and HGF are widely expressed in a variety of tissues, and their expression is normally confined to cells of epithelial and mesenchymal origin, c-Met is expressed by most carcinomas and its elevated expression relative to normal tissue has been detected in a number of cancers including lung, breast, colorectal, prostate, pancreatic, head and neck, gastric, hepatocellular, ovarian, renal, glioma, melanoma, and a number of sarcomas.[1-5]

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Activation of the HGF/c-Met signaling pathway has been shown to lead to a wide array of cellular responses including proliferation, survival, angiogenesis, wound healing, tissue regeneration, scattering, motility, invasion and branching morphogenesis. Since c-Met is at the crossing of many channels leading to tumorigenesis and metastasis, targeting this receptor could be a relatively simple way to interfere with many pathways simultaneously. HGF/c-Met signaling pathway is now known to be a "druggable" target with promising results of early phase clinical results of c-Met targeting agents emerging.[6-11]

In our previous study, we discovered some novel c-Met inhibitors 1-3 using Pfizer's *3H*-[1,2,3]triazolo[4,5-b] pyrazine derivatives PF-04217903 and Janssen's *3H*-[1,2,4]triazolo [4,3-b]pyridazine derivatives JNJ-38877605 as leading compounds (Fig. 1).[12-16]

In an ongoing effort to discovery novel c-Met inhibitors and build diversification of chemical library, we continued to develop structurally

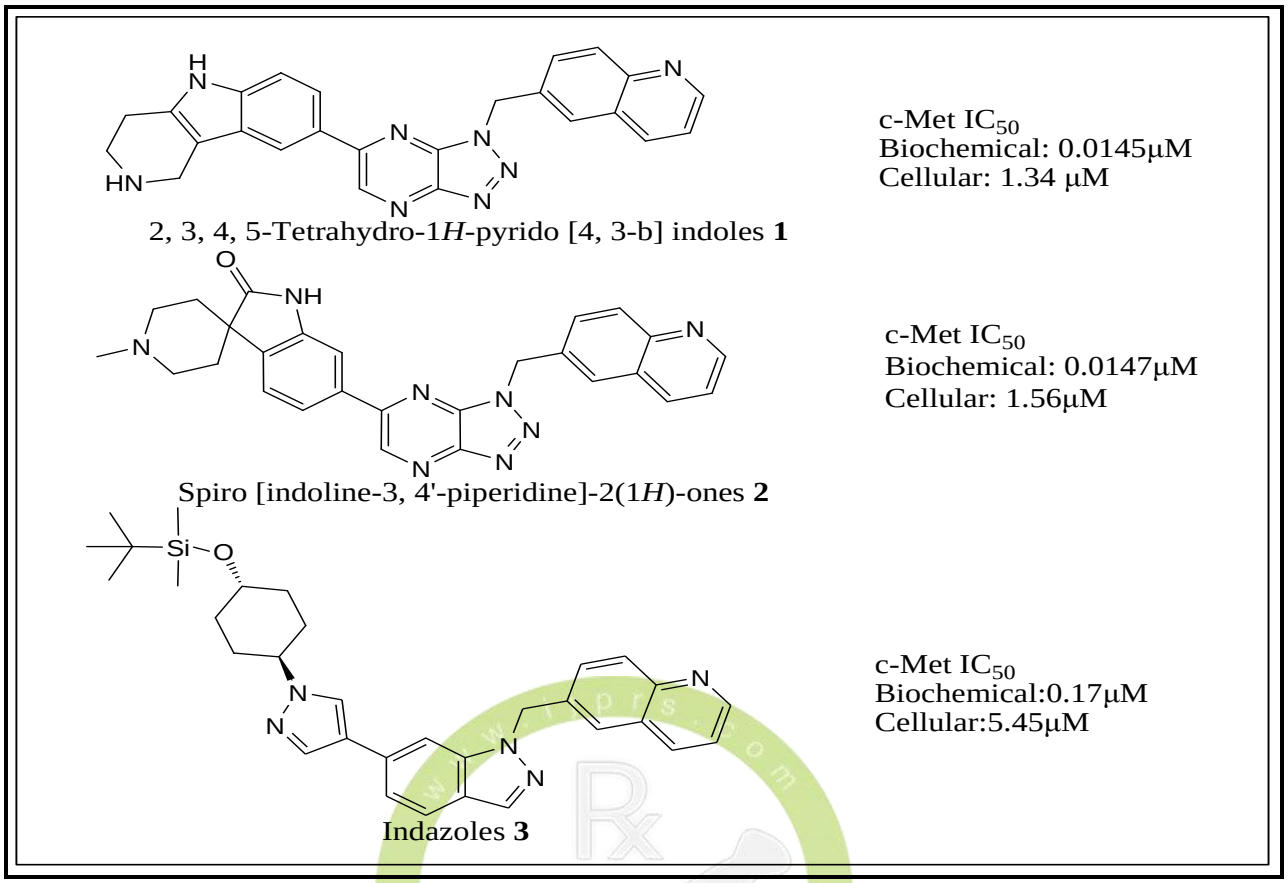


Figure 1: Our reported c-Met inhibitors

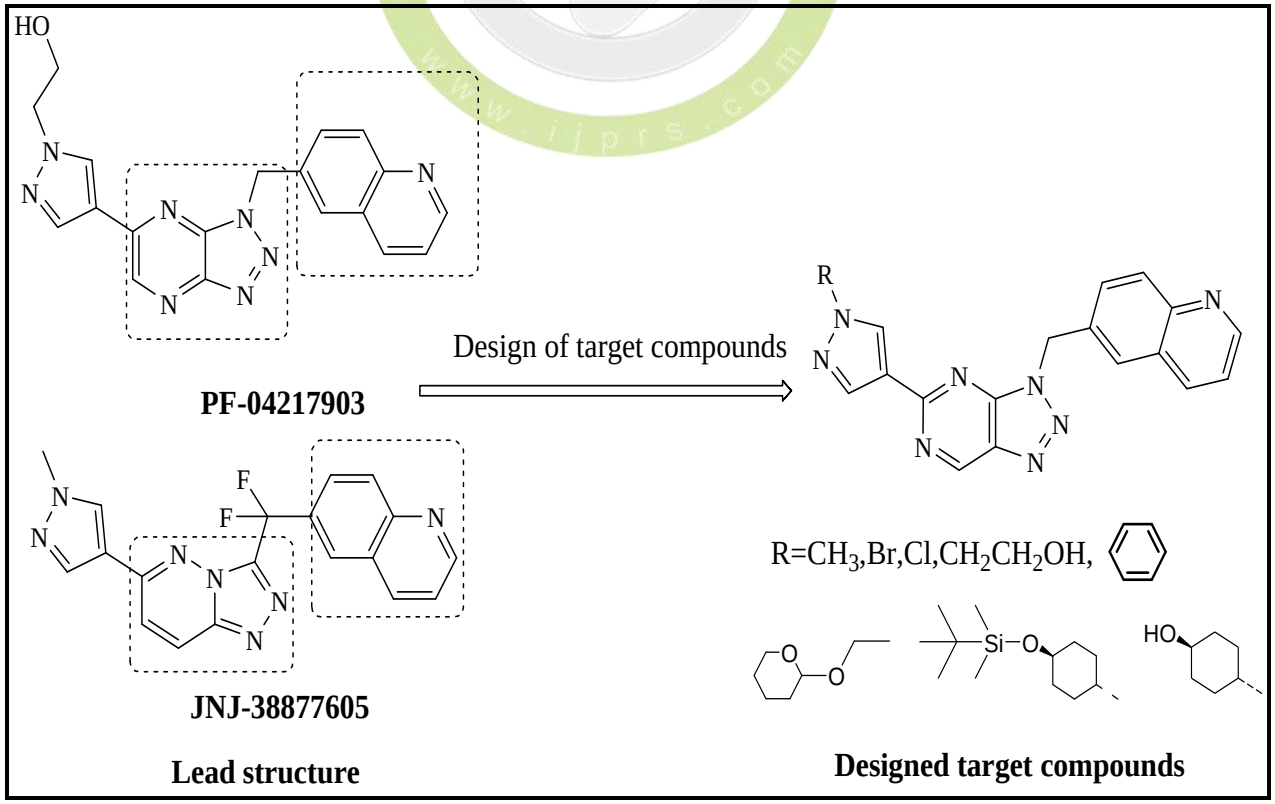
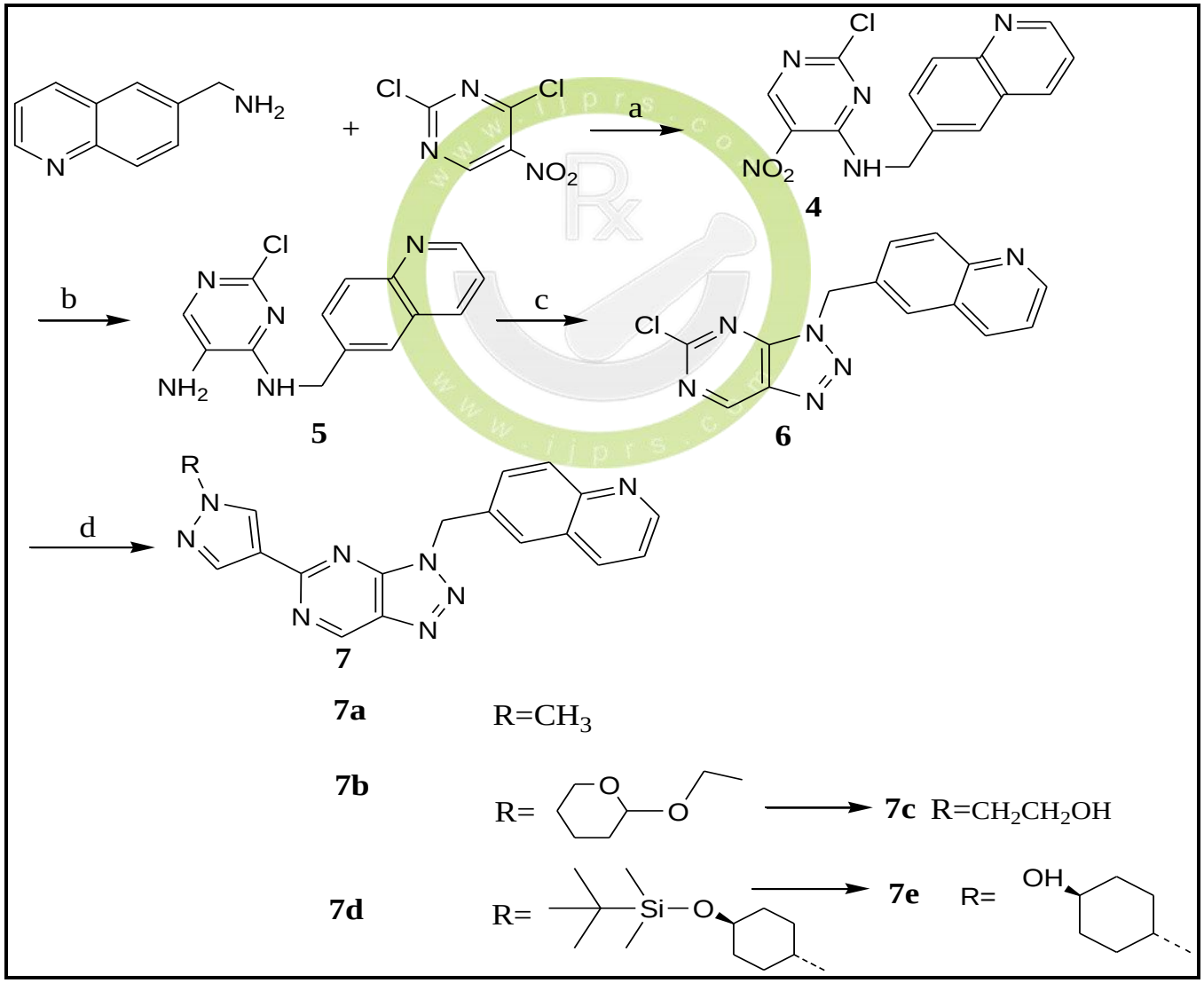


Figure 2: Structure-based biosterism design of 3*H*-[1,2,3]triazolo[4,5-d]pyrimidines derivatives

relevant compounds 7a-7e by replacing *3H*-[1,2,3]triazolo[4,5-b]pyrazine of PF-04217903, *3H*-[1,2,4]triazolo[4,3-b]pyridazine of JNJ-38877605 with *3H*-[1,2,3]triazolo[4,5-d] pyrimidine by means of biological isostere principle, combination principles and molecular docking (Fig. 2), and generated the scores of designed compounds and ligand (PDB ID: 3dkf) using Surflex-Dock program (Table 1), then synthesized these compounds through directional synthesis and other method of chemical synthesis. Compounds 7a-7c have been reported.[17] we investigated inhibitions of synthesized compounds on c-Met to obtain more active compounds.

Table 1: Results of Molecular Docking

Compound	Score
3dkf-ligand	5.86
7a	8.75
7b	11.45
7c	10.39
7d	10.53
7e	11.33



Scheme: 1 Synthesis of target compounds. Reagents and conditions: a) DIPEA, THF, r.t., 3h;b)$SnCl_2$, EtOH, reflux, 5 h; c) isomyl nitrite, DMF, 50℃, 2 h; d)$Pd(PPh_3)_4$, DMF/H_2O, K_2CO_3, 80℃, 18h

MATERIALS AND METHOD

Chemistry

All chemicals were obtained from Aladdin or J&K. Solvents were purified and dried by standard procedures, and stored over 3-Å molecular sieves. Reactions were followed by TLC using SILG/UV 254 silica-gel plates. Flash chromatography (FC): silica gel (SiO_2; 40μm, 230-400 mesh). ^{1}H-NMR Spectra: Bruker Digital NMR Spectrometer, rep. δ in ppm, J in Hz. EI-MS: Waters ZQ4000.

Method

General Synthetic Procedure for Compounds

Preparation of 4

6-Aminomethylquinoline (1.582 g, 10 mmol), diisopropanolamine (1.78 mL, 10 mmol) in 4 mL THF was added drop-wise to a solution of 2,4-dichloro-5-nitropyrimidine (1.94 g, 10 mmol) in 10 mL THF and stirred at room temperature for 3 h. The solvent was removed under reduced pressure, and the crude product was purified by FC with EtOAc/hexanes to obtain the target products 2-chloro-5-nitro-N-(quinolin-6-yl methyl)pyrimidin-4-amine. Yield 72%: ^{1}H-NMR ($CDCl_3$, 400 MHz): δ(ppm) 5.06 (d, J = 6.0 Hz, 2 H), 7.48 (dd, J = 4.4, 8.4 Hz, 1 H), 7.74 (dd, J = 2.0, 9.2 Hz, 1 H), 7.83 (d, J = 1.2 Hz, 1 H), 8.21 (d, J = 8.4 Hz, 1 H), 8.22 (d, J = 6.80 Hz, 1 H), 8.80 (m, br, 1 H), 8.95 (dd, J = 1.2, 4.0 Hz, 1 H), 9.09 (s, 1 H)。 EI-MS: 315.93 [M+H$^+$, ^{35}Cl], 317.97 [M+H$^+$, ^{37}Cl].

Preparation of 5

A solution of 2-chloro-5-nitro-N-(quinolin-6-yl methyl)pyrimidin-4-amine (**4**; 1.579 g, 5 mmol) and $SnCl_2$ (4.74 g, 25 mmol) in EtOH (40 mL) was refluxed for 5 h, then cooled to room temperature. The solvent was removed under reduced pressure. The solution was adjusted to pH 9 by $NaHCO_3$, and 50 mL EtOH was added. The mixture was filtered and concentrated to obtain 2-chloro-N^4-(quinolin-6-ylmethyl) pyrimidine-4,5-diamine, which was used in the next reaction directly. Yield 50%: EI-MS: 286.06 [M+H$^+$, ^{35}Cl], 288.04 [M+H$^+$, ^{37}Cl].

Preparation of 6

The mixture of 2-chloro-N^4-(quinolin-6-ylmethyl) pyrimidine-4,5-diamine (**5**; 1.73 g, 6.19 mmol), isoamyl nitrite (1 mL, 7.32 mmol), DMF (20 mL) was heated at 55℃ for 2 h, cooled and quenched with saturated solution of Na_2SO_3(15 mL). Water (30 mL) was added to dissolve the precipitate, followed by DCM (60 mL). The organic layer was separated and the aqueous layer was extracted with DCM (3×40 mL). The combined organic extracts were washed with saturated solution of $NaHCO_3$ and brine, dried over Na_2SO_4. The solvent was evaporated and the resulting residue was dried to give the desire product 6-((5-chloro-3*H*-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl) methyl) quinoline. Yield 74%: ^{1}H-NMR ($CDCl_3$, 400 MHz): δ(ppm) 6.05 (s, 2 H), 7.49 (dd, J = 4.4, 8.4 Hz, 1 H), 7.85 (dd, J = 1.6, 8.8 Hz, 1 H), 7.95 (d, J = 1.6 Hz, 1 H), 8.19-8.24 (m, 2 H), 8.95 (dd, J = 1.6, 4.4 Hz, 1 H), 9.42 (s, 1 H). EI-MS : 296.97 [M+H$^+$, ^{35}Cl], 298.95 [M+H$^+$, ^{37}Cl].

Compound 7a-7c was prepared according to Ref. 17.[17]

Preparation of 7a

The Pd $(PPh_3)_4$ (88.2 mg, 0.108 mmol) was added portion wise to a solution of the 6-((5-chloro-3*H*-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl) methyl)quinoline (**6**; 550 mg, 1.86 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazole (581 mg, 2.79 mmol), Cs_2CO_3 (1.82 g, 5.57 mmol) in DMF/H_2O (4/1, 4 ml), and nitrogen was bubbled through the mixture for 5 min. Then, the mixture was stirred for 18 h at 80℃ (LC-MS control), then cooled to r. t., H_2O (7 ml) was added, and the mixture was extracted with CH_2Cl_2 (3 × 10 ml). The organic layer was dried (1 g of Na_2SO_4), concentrated to obtain crude product, which was purified by FC with MeOH/CH_2Cl_2, then to get the compound 5-(1-methyl-1*H*-pyrazol-4-yl)-3-(quinolin-6-ylmethyl)-3*H*-[1,2,3]triazolo[4,5-d]pyrimidine **7a**. Yield 65%: ^{1}H-NMR ($CDCl_3$, 400 MHz): δ(ppm) 3.97 (s, 3 H), 5.83 (s, 2 H), 7.43-8.32 (m, 7 H), 8.87 (m, 1 H), 9.38 (s, 1 H); EI-MS:

343.1 [M+H$^+$]. Compound **7b** was prepared in analogy to **7a**. 5-(1-(2-(tetrahydro-2H-pyran-2-yloxy)ethyl)-1H-pyrazol-4-yl)-3-(quinolin-6-ylmethyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidine **7b**: Yield 79%: ^{1}H-NMR ($CDCl_3$, 400 MHz): δ(ppm) 1.26-1.78 (m, 6 H), 3.38-4.61 (m, 7 H), 5.97 (s, 1 H), 7.39 (m, 1 H), 7.78-7.81 (m, 1 H), 7.89 (m, 1 H), 8.04-8.13 (m, 2 H), 8.27(s, 1 H), 8.32 (s, 1 H), 8.88-8.91 (m, 1 H), 9.43 (s, 1 H); EI-MS: 457.2 [M+H$^+$].

Preparation of 7c

A solution of 5-(1-(2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl)-1*H*-pyrazol-4-yl)-3-(quinolin- 6-ylmethyl)-3*H*-[1,2,3]triazolo[4,5-d]pyrimidine (**7b**; 308 mg, 0.676 mmol), p-toluenesulfonic acid monohydrate (155 mg, 0.82 mmol) in 20 mL MeOH was stirred at room temperature for 3 h. The solvent was removed under reduced pressure. The crude product was purified by FC with MeOH/DCM to obtain the target product 5-[1-(2-hydroxyethyl)-1*H*-pyrazol-4-yl]-3-(quinolin-6-ylmethyl)-3*H*-[1,2,3]triazolo[4,5-d]pyrimidine **7c**.

Yield 65%. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ(ppm) 3.69 (dd, 2 H), 4.15 (t, 2 H), 4.87 (t, 1 H), 5.97 (s, 2 H), 7.43 (m, 1 H), 7.72 (m, 1 H), 7.88-7.92 (m, 2 H), 8.14 (m, 2 H), 8.29 (s, 1 H), 8.45(s, 1 H), 8.87 (m, 1 H), 9.59 (s, 1 H); EI-MS: 373.2 [M+H$^+$]; Compound **7d** was prepared in analogy to **7a.** 3-(quinolin-6-ylmethyl)-5-{1-[(1*R*,4*R*)-4-(tert-butyldimethylsilyloxy)cyclohexyl]-1*H*-pyrazol-4-yl}-3*H*-[1,2,3]triazolo[4,5-d]pyrimidine **7d:** Yield 83%: ^{1}H-NMR ($CDCl_3$, 400 MHz): δ(ppm) 0.09 (s, 6 H), 0.87 (s, 9 H), 1.42-1.51 (m, 2 H), 1.76-1.85 (m, 2 H), 1.94-2.01 (m, 2 H), 2.14-2.20 (m, 2 H), 3.62 (m, 1 H), 3.95 (m, 1 H), 6.12 (s, 2 H), 7.43(m, 1 H), 7.69(m, 1 H), 7.89-7.95 (m, 2 H), 8.03 (m, 2 H), 8.19 (s, 1 H), 8.31(s, 1 H), 8.87 (m, 1 H), 9.53(s, 1 H); EI-MS: 541.34 [M+H$^+$].

Preparation of 7e

4M 1, 4-dioxane solution of HCl (1 mL) was added drop-wise to a solution of 3-(quinolin-6-ylmethyl)-5-{1-[(1*R*,4*R*)-4-(tert-butyldimethylsilyloxy)cyclohexyl]-1*H*-pyrazol-4-yl}-3*H*-[1,2,3]triazolo[4,5-d]pyrimidine (**7g**; 54 mg, 0.1 mmol) in 3 mL THF and stirred at room temperature for 3 h.

The solvent was removed under reduced pressure. 10 mL DCM and 5 mL 10% NaOH was added to the residue and shaken. The organic layer was isolated, washed by NaCl and concentrated to obtain the crude product, which was purified by FC with MeOH/DCM to obtain the target product 3-(quinolin-6-yl methyl)-5-[1-((1*R*,4*R*)-4-hydroxy-cyclohexyl)-1*H*-pyrazol-4-yl]-3*H*-[1,2,3]triazolo[4,5-d]pyrimidine **7e**. Yield 98%: ^{1}H-NMR ($CDCl_3$, 400 MHz): δ(ppm) 1.46(dq, 2 H), 1.89(dq, 2 H), 2.11-2.15 (m, 2 H), 2.31-2.54 (m, 2 H), 3.77 (m, 1 H), 4.24 (m, 1 H), 5.67 (s, 2 H), 7.38(m, 1 H), 7.69(m, 1 H), 7.87-7.93 (m, 2 H), 8.05 (m, 2 H), 8.27 (s, 1 H), 8.39(s, 1 H), 8.87 (m, 1 H), 9.47(s, 1 H); EI-MS: 427.20 [M+H$^+$].

c-Met Kinase Assay

The c-Met kinase activities were investigated according to Ref. 12. The IC_{50} values of compounds were determined using an IMAP Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET) assay. 50 nM 6 His-tagged recombinant human c-Met residues 974-end (Millipore) was cultured with a 25 µl solution of 20 mM Tris, 10 mM $MgCl_2$, 2.5 mM $MnCl_2$, 0.01% Tween 20 and 2 mM DTT with 5 µM ATP and 200 nM 5FAM-KKKSPGEYVNIGFG-NH_2 for 60 min at ambient temperature. Compounds were tested at 10 concentrations starting from 20 µM at a final concentration of 1% DMSO.

The reaction was terminated by addition of 50 µl of IMAP stop solution containing 60%Buffer A: 40%Buffer B and a 1 in 400 dilution of beads and Terbium reagent. Plates were read after an overnight incubation at 4 ℃ on an Analyst HT reader. Reported IC_{50}'s are from a minimum of 2 experiments (n = 2). Data analysis was carried out using a four parameter curve fit. The standard errors of the mean were calculated and expressed as a percentage of the mean IC_{50}. The average for this value was 12%.

Molecular Docking

The molecular docking was performed according to Ref. 14. The docking experiment was performed using Surflex-Dock program with Sybyl 7.3 soft. The programs adapted an empirical scoring function and a patented searching engine. [17-18] A crystal structure of the c-Met complex with SGX523 was obtained from the protein data bank (pdb entry: 3dkf). Ligand was docked into the corresponding protein's binding site guided by protomol, which is an idealized representation of a ligand that makes every potential interaction with binding site. Default values were chosen to finish this work except that the threshold was 1 when the protomol was generated. Beginning of docking, all the water and ligands were removed and the random hydrogen atoms were added. Then the receptor structure was minimized in 10000 cycles with Powell method in sybyl7.3. All the compounds were constructed using Sketch Molecular module. Hydrogen and Gasteiger-Hückel charges were added to every molecular. Then their geometries were optimized by conjugate gradient method in TRIPOS force field. The energy convergence criterion was 0.001kcal/mol.

RESULTS AND DISCUSSION

Chemistry

The synthetic route of **7a-7e** was shown in Scheme 1. The important intermediate **6** reacted with different pinacol borane esters to get the designed compounds **7a**, **7b** and **7d** respectively via *Suzuki* coupling reaction, which gave good yields under basic conditions using DMF/H_2O as a solvent and Pd $(PPh_3)_4$ as a catalyst and provided a range of applicability.[18] Compounds 7f and 7h could be obtained in high yields by the hydrolysis of **7b** and **7d**. The reaction of 6-aminomethylquinoline with **2**, 4-dichloro-5-nitropyrimidine gave compound 4, which was transformed to compound **5** under reduction of $SnCl_2$. The compound 6 was prepared from compound **5** by treatment with isoamyl nitrite in 65% yield. The synthesized compounds were characterized by ^{1}H-NMR and ESI-MS. ^{1}H-NMR spectral data were found in agreement with the assigned molecular structure.

Evaluation of Biological Activity

All the title compounds were evaluated for their anti-c-Met enzyme activities in vitro. The results of anti-c-Met enzyme activities were presented in Table 2. On the whole, these compounds did not demonstrate excellent anti-c-Met enzyme activity(IC_{50}>30 μM) as we expected, but this study provided important information for build diversification of chemical library and these compounds can be used as lead structure to be optimized. Further research works are currently under investigation and will be reported in due course.

Table: 2 Inhibition of Designed Compounds on c-Met

Compound	c-Met IC_{50}(μM)
7a	56
7e	35
7f	47
7g	41
7h	36

CONCLUSION

In conclusion, our strategy to replace replacing 3*H*-[1,2,3]triazolo[4,5-b]pyrazine of PF-04217903, 3*H*-[1,2,4]triazolo[4,3-b]pyridazine of JNJ-38877605 with 3H-[1,2,3]triazolo [4,5-d]pyrimidines moiety was unsuccessful in leading to compounds, which demonstrated inhibition of c-Met kinase activity. But these compounds would be useful as lead compounds of developing c-Met inhibitors.

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