Design, Synthesis and Pharmacokinetic Evaluation of a Novel Series of Triazole-Based Src Kinase Inhibitors with Anti-proliferative Activity

Shaojun Chen[1], Chuansheng Guo[2], Shiting Shi[2], Yanyan Shi[2], Du Fang*[,1] and Houxing Fan*[,2]

[1]*Hunan University of Chinese Medicine, Changsha 410208, China*

[2]*Shanghai Sun-sail Pharmaceutical Science & Technology Co., Ltd., Shanghai 201203, China*

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Abstract: Src has been recognized as an important therapeutic target for the treatment of cancer. A novel series of triazole-based Src inhibitors were synthesized and evaluated for their anti-proliferative activities *in vitro* against human lung cancer A549 cells with Bosutinib as reference compound, most of the compounds showed more potent activity than Bosutinib. Compounds **6** and **8** were further subjected to pharmacokinetic performance assessment, both the compounds displayed low plasma concentrations and short half-time.

Keywords: Anti-proliferative activity, Biological activity, SKI-606, Src Kinase, Synthesis, Triazoly group.

INTRODUCTION

Src, a nonreceptor tyrosine kinase, is involved in the transduction and regulation of signaling pathways involved in normal cellular growth, proliferation, angiogenesis, adhesion, motility, and survival [1]. During tumorigenesis, these normal processes become aberrantly activated, leading to excessive growth, metastasis, and tissue invasion [2]. So Src has been recognized as an important therapeutic target in the cancer, even in other diseases including osteoporosis, stroke, and myocardial infarction [3-7]. and various classes of small molecule Src inhibitors have been synthesized including Dasatinib (**BMS354825**; Bristol Meyers Squibb Oncology) [8], Saracatinib (formerly **AZD0530**, AstraZeneca) [9], and Bosutinib (**SKI-606**; Wyeth Research) [10], which have reached Phase 1 or 2 clinical trials (Fig. **1**).

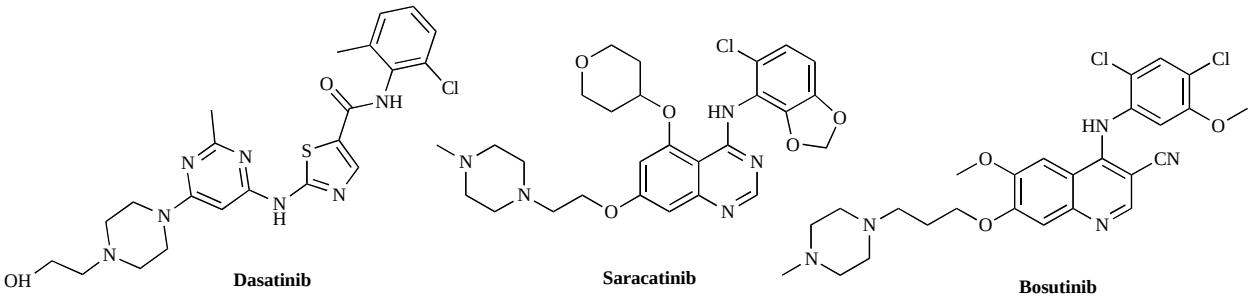

Fig. (1). Structures of known Src inhibitors.

Bosutinib (**SKI-606**), a Src kinase inhibitor with a 3-quinolinecarbonitrile core, is currently in Phase 2 clinical trials for the treatment of solid tumors. It is well known that triazoly group is a common substructure seen in some marketed drugs, which are examplified by Anastrozole and Letrozole (both used to treat breast cancer). In addition, in a series quinazoline vascular ebdothelial growth factor receptor tyrosine kinase (VEGFRr TK) inhibitors reported by Astra-Zeneca [11], the triazolyl group provided superior blood levels compared to a morpholine group. In order to find new Src kinase inhibitors with improved anticancer activity, we combined the structural properties of **SKI-606** and triazoly group into one molecule. In this paper, we report our efforts towards the synthesis and anti-proliferative activity of a novel series of analogues of **SKI-616** with introduction of substituted triazolyl groups. Furthermore, pharmacokinetic properties of the selected compounds **6** and **8** were also disclosed herein.

CHEMISTRY

As shown in Scheme **1**, condensation of 2,4-dichloro-5-methoxyaniline with cyanoacetic acid in the presence of 1,3-diisopropylcarbodiimide gave the acetamide derivative **1** [12]. Treatment of compound **1** with 3-iodo-4-methoxyaniline in the presence of triethylorthoformate and iso-propanol as solvent provided **2**, which upon subsequent phosphorusoxychloride-mediated ring closure reaction in acetonitrile and methanol resulted in formation of the desired 7-iodo-3-quinolinecarbonitrile **3**. Compound **3** readily underwent standard Sonogashira coupling reaction employing TMS-Acetylene (TMS = trimethylsilyl) led to the formation

*Address correspondence to this author at the Department of Medicinal Chemistry, Shanghai Sun-sail Pharmaceutical Science & Technology Co., Ltd., No. 1690 Cailun Road, Shanghai, 201203, People's Republic of China; Tel: +86-(0)21-50278490-8061; Fax: +86-(0)21-50278483;
E-mails: 1125fd@sina.com, hxfan@sun-sail.cn

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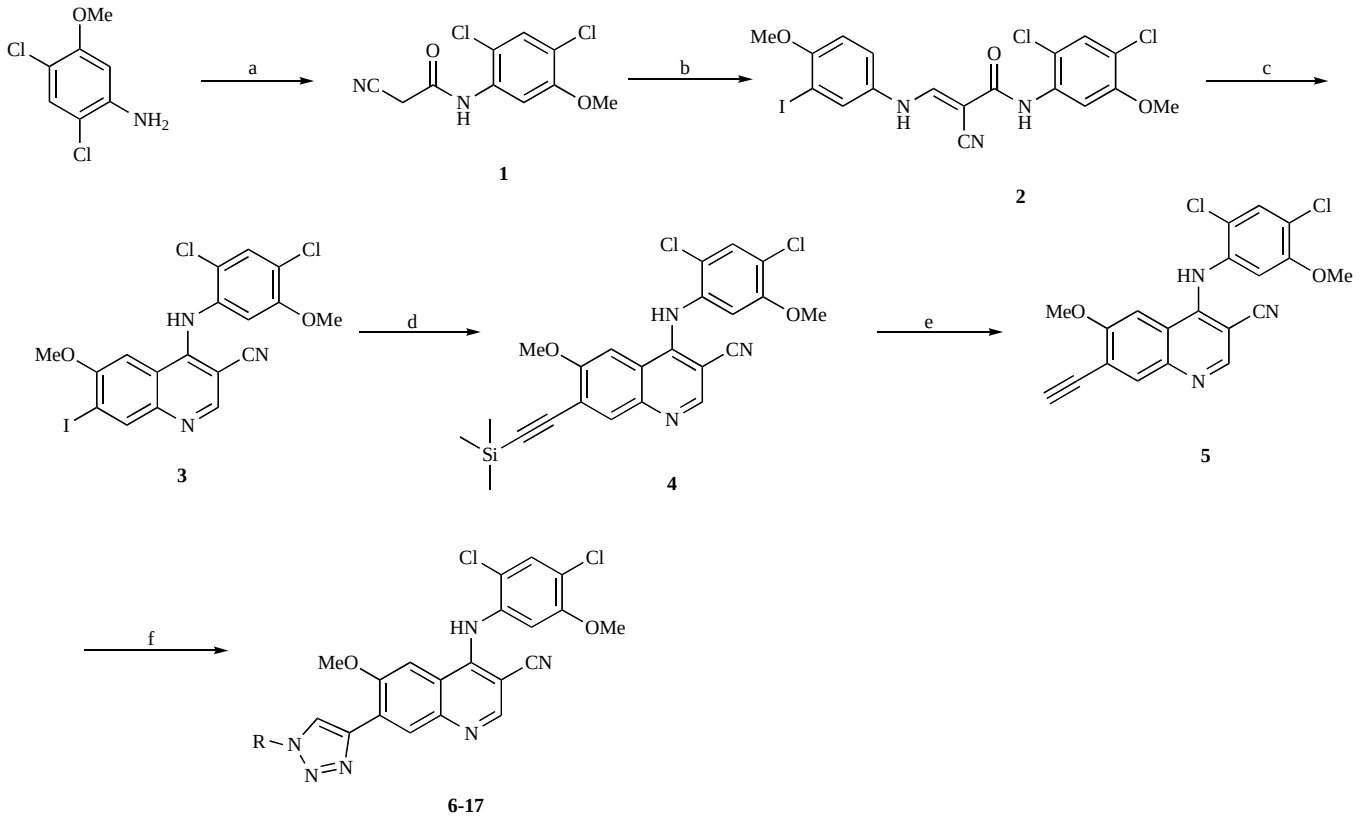


Scheme 1. Reagents and conditions: (**a**) cyanoacetic acid, 1,3-diisopropylcarbodiimide, THF, reflux, 1 h; (**b**) triethylorthoformate, *i*-PrOH, reflux, 20 h; (**c**) $POCl_3$, CH_3OH-CH_3CN, reflux, overnight; (**d**) Trimethyl silyl acetylene, diisopropylanmine, CuI, $Pd(PPh_3)_2Cl_2$, THF, reflux, 4 h; (**e**) TBAF, THF, r.t, 15 min; (**f**) RN_3, $CuSO_4.5H_2O$, Sodium Ascorbate, DMF, 60 ^oC, 3 h.

of TMS-protected alkyne **4** in 90% yield. The desired building block **5** was obtained in 80% yield by deprotection of **4** with TBAF in THF at room temperature. Finally, compound **5** underwent cycloaddition with azide catalyzed by $CuSO_4.5H_2O$ and sodium ascorbate to give the target compounds **6-17** in good yield and regioselectivity [13]. Structures of the desired compounds were confirmed by ^{1}HNMR and Ms (ESI) or HRMS (ESI) (Table **1**).

BIOLOGY

Anti-Proliferative Activities

The anti-proliferative activities of the target compounds were examined using human lung cancer A549 cell (GIBCO, Grand Island, NY, USA)with Bosutinib as reference compound [9, 14]. Cells in 96-well plates were treated in triplicate with equivalent concentration with Bosutinib of compounds at 37 ^oC for 72 h in 5% CO_2 atmosphere. The cell viability was assessed by measuring the conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a colered product. Briefly, cells were incubated with MTT (5 mg/mL, 20 μL/well). After 4 h, the colored product in the cells was dissoloved in DMSO and was measured at 570 nm using a multiskan spectrum (Thermo Electron Co., Vantaa, Finland). The inhibition rate on cell proliferation was calculated for each well as (A570 control cells – A570 treated cells) / A570 control cells × 100%. The average IC_{50} values were determined by Logit method from at least three independent tests.

Pharmacokinetics

Compounds **6** and **8** were subjected to pharmacokinetic properties study in healthy male Swiss mice (18-20 g weight, 15 mice in each group). The test compounds were administered orally at the dose of 20 mg/kg. Series specimens (300 μL) were collected *via* retrobulbar vein and quantitation was performed by LC-MS, pharmacokinetic parameters were calculated from the mean plasma concentration by non-compartmental analysis.

RESULTS AND DISCUSSION

The result of *in vitro* inhibition ratio of compounds **6-17** against human lung cancer A549 cells in 10 μM concentration is summarized in Table **2**. It clearly showed that all compounds expect compound **7** displayed more potent anti-proliferative activity than the positive control **SKI-606**. Especially, compound **16** showed the best activity with inhibition ratio of 91.1% compared to that 78.5% of **SKI-606**. In order to investigate the effect of the length of the chain on anti-proliferative activity, compounds **10** and **17** were prepared, both of the compounds exhibited potent activity at the same level. In generally, piperidine derivatives (compounds **12-16**) showed more potent activity than that of azetidine derivatives (compounds **6-9**), but there is not significant difference between them.

The IC_{50} values of the selected compounds against human lung cancer A549 cells are also listed in Table **2**, most

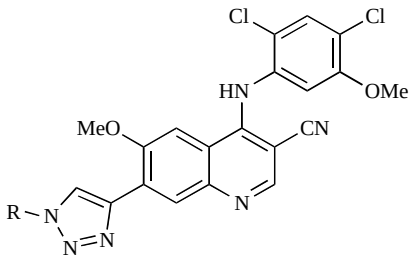
Table 1. Physical and Spectral Properties of Target Compounds 6-17

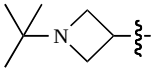
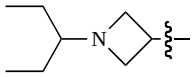
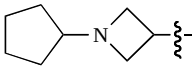
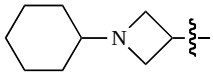
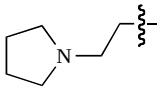
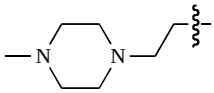
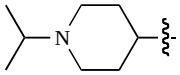
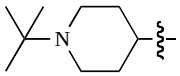
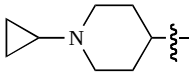
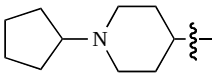
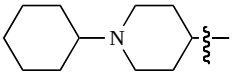
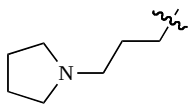
Compd	Ms (ESI) or HRMS (ESI) m/z	m.p.(˚C)	^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm)	Yield (%)[a]
6	HRMS (ESI) calcd for $C_{27}H_{28}N_7O_2Cl_2$ [M + H^+]: 552.1682, found: 552.1675.	238-240	9.87 (s, 1H), 8.67-8.70 (m, 3H), 8.02 (s, 1H), 7.78 (s, 1H), 7.40 (s, 1H), 4.10 (s, 3H), 3.87 (s, 3H), 3.67 (d, *J* = 6.4 Hz, 4H), 1.00 (s, 9H).	21
7	566. 5 $[M+H]^+$	112-114	9.86 (s, 1H), 8.72 (s, 1H), 8.65 (s, 1H), 8.46 (s, 1H), 8.00 (s, 1H), 7.76 (s, 1H), 7.39 (s, 1H), 5.26-5.30 (m, 1H), 4.08 (s, 3H), 3.86 (s, 3H), 3.74 (s, 2H), 3.45 (s, 2H), 2.19-2.23 (m, 1H), 1.22-1.37 (m, 4H), 0.81 (t, *J* = 14.4 Hz, 6H).	28
8	HRMS (ESI) calcd for $C_{28}H_{28}N_7O_2Cl_2$ [M + H^+]: 564.1682, found: 564.1689.	238-240	9.92 (s, 1H), 8.72 (s, 1H), 8.63 (s, 1H), 8.46 (s, 1H), 8.00 (s, 1H), 7.81 (s, 1H), 7.36 (s, 1H), 5.27-5.31 (m, 1H), 4.09 (s, 3H), 3.50 (s, 3H), 3.74 (t, *J* = 7.6 Hz, 2H), 3.47 (t, *J* = 6.8 Hz, 2H), 2.86-2.89 (m, 1H), 1.63-1.65 (m, 2H), 1.57-1.60 (m, 4H), 1.43-1.49 (m, 2H).	21
9	578.6 $[M+H]^+$	223-225	9.86 (s, 1H), 8.73 (s, 1H), 8.66 (s, 1H), 8.47 (s, 1H), 8.02 (s, 1H), 7.78 (s, 1H), 7.40 (s, 1H), 5.29-5.33 (m, 1H), 4.07 (s, 3H), 3.87 (s, 3H), 3.77 (t , *J* = 6.6 Hz, 2H), 3.50 (t , *J* = 3.3 Hz, 2H), 2.19-2.21 (m, 1H), 1.69 (d, *J* = 1.2 Hz, 4H), 1.51-1.56 (m, 1H), 1.18-1.25 (m, 3H), 0.99-1.07 (m, 2H).	12
10	538.5 $[M+H]^+$	197-199	9.89 (s, 1H), 8.66-8.69 (m, 2H), 8.47 (s, 1H), 8.02 (s, 1H), 7.76 (s, 1H), 7.41 (s, 1H), 4.59-4.61 (m, 2H), 4.09 (s, 3H), 3.87 (s, 3H), 3.15-3.21 (m, 2H), 2.55-2.58 (m, 4H), 1.36-1.69 (m, 4H).	25
11	552.25 $[M+H]^+$	126-128	9.93 (s, 1H), 8.64-8.66 (m, 2H), 8.47 (d, *J* = 6.0 Hz, 1H), 8.05 (s, 1H), 7.76 (s, 1H), 7.40 (s, 1H), 4.58 (t, *J* = 6.0 Hz, 2H), 4.09 (s, 3H), 3.86 (s, 3H), 3.20 (dd, J_1 = 4.8 Hz, J_2 = 7.5 Hz, 2H), 2.83 (t, *J* = 7.5 Hz, 2H), 2.42-2.48 (m, 6H), 2.22 (s, 3 H).	13
12	566.3 $[M+H]^+$	163-165	9.91 (s, 1H), 8.66-8.69 (m, 2H), 8.46 (s, 1H), 8.01 (s, 1H), 7.76 (s, 1H), 7.39 (s, 1H), 4.60-4.63 (m, 1H), 4.09 (s, 3H), 3.89 (s, 3H), 3.00-3.04 (m, 2H), 2.86-2.90 (m, 1H), 2.40-2.45 (m, 2H), 2.11-2.17 (m, 4H), 1.05 (d, *J* = 6.6 Hz, 6H).	14
13	580.5 $[M+H]^+$	156-158	9.88 (s, 1H), 8.67 (s, 1H), 8.64 (s, 1H), 8.45 (s, 1H), 8.00 (s, 1H), 7.73 (d, *J* = 5.3 Hz, 1H), 7.38 (s, 1H), 4.59-4.62 (m, 1H), 4.09 (s, 3H), 3.86 (s, 3H), 3.29 (t, *J* = 0.6 Hz, 2H), 2.35-2.42 (m, 2H), 2.18 (s, 4H), 1.12 (s, 9H).	21
14	565.47 $[M+H]^+$	220-222	9.83 (s, 1H), 8.68 (s, 1H), 8.65 (s, 1H), 8.46 (s, 1H), 7.98 (s, 1H), 7.76 (s, 1H), 7.39 (s, 1H), 4.60-4.64 (m, 1H), 4.09 (s, 3H), 3.86 (s, 3H), 3.06 (d, *J* = 8.0 Hz, 2H), 2.35-2.41 (m, 2 H), 2.02-2.08 (m, 4H), 1.66-1.69 (m, 1H), 0.43-0.47 (m, 2H), 0.31-0.34 (m, 2H).	39
15	592.5 $[M+H]^+$	256-257	9.86 (s, 1H), 8.69 (s, 1H), 8.60 (s, 1H), 8.44 (s, 1H), 7.99 (s, 1H), 7.75 (s, 1H), 7.37 (s, 1H), 4.56-4.60 (m, 1H), 4.09 (s, 3H), 3.86 (s, 3H), 3.04-3.12 (m, 2H), 2.50-2.57 (m, 1H), 2.11-2.14 (m, 6H), 1.81-1.87 (m, 2H), 1.60-1.65 (m, 2H), 1.51-1.56 (m, 2H), 1.36-1.41 (m, 2H).	28
16	606.6 $[M+H]^+$	186-188	9.94 (s, 1H), 8.67 (s, 1H), 8.64 (s, 1H), 8.49 (s, 1H), 8.06 (s, 1H), 7.78 (s, 1H), 7.41 (s, 1H), 4.88-4.92 (m, 1H), 4.22 (s, 3H), 3.87 (s, 3H), 3.39-3.55 (m, 2H), 3.17-3.29 (m, 3H), 2.46-2.50 (m, 4H), 2.08 (s, 2H), 1.82-1.85 (m, 2H), 1.61-1.64 (m, 1H), 1.42-1.45 (m, 2H), 1.24-1.34 (m, 2H), 1.14 (t, *J* = 8.0 Hz, 1H).	39
17	552.45 $[M+H]^+$	141-143	9.89 (s, 1H), 8.62-8.64 (m, 2H), 8.43 (d, *J* = 6.6 Hz, 1H), 8.01 (s, 1H), 7.73 (d, *J* = 4.4 Hz, 1H), 7.34 (d, *J* = 4.4 Hz, 1H), 4.51 (t, *J* = 4.4 Hz, 2H), 4.09 (s, 3H), 3.84 (s, 3H), 3.48-3.57 (m, 2H), 2.40-2.45 (m, 4H), 2.02-2.12 (m, 2H), 1.66-1.75 (m, 4H).	42

[a]the yields are for last step.

of the compounds displayed more potent activity than **SKI-606** against human lung cancer A549 cells. It clearly showed that piperidine derivatives was inferior to azetidine derivatives, it is not consistent with the situation indicated by cell inhibition ratio. Compound **6** exhibited the most potent anti-proliferative activity with IC_{50} value of 0.42 μM compared to that 2.12 μM of **SKI-606**, in addition, compound **8** also showed potent activity with IC_{50} value of 0.48 μM.

Table 2. ***In Vitro*** **Anticancer Activity of Synthetic Compounds**



Compounds	R	Inhibition ratio(%) [a, b]	IC_{50} (uM) [b]
6		89.8 ± 2.5	0.42 ± 0.18
7		53.3 ± 27.2	ND [c]
8		87.1 ± 3.1	0.48 ± 0.22
9		86.2 ± 4.3	ND
10		90.8 ± 1.6	1.36 ± 0.54
11		87.4 ± 4.5	ND
12		87.5 ± 4.6	ND
13		88.2 ± 3.8	1.65 ± 0.83
14		81.4 ± 6.3	ND
15		88.3 ± 3.3	1.81 ± 0.69
16		91.1 ± 0.5	2.48 ± 0.57
17		89.8 ± 0.7	ND
Bosutinib (SKI-606)		78.5 ± 17.7	2.12 ± 0.58

[a]against human lung cancer A549 cell, in concentration of 10 μM, [b]values are means of three experiments, [c]not determined.

Compounds **6** and **8**, two of the most potent compounds, were further subjected to pharmacokinetic properties study. As shown in Table **3**, both of the compounds exhibited low plasma concentrations and AUC. Compounds **6** and **8** exhibited the maximum plasma concentrations of 133 and 150 ng/mL, and the AUC_{0-t} is 548 ng.h/mL and 450 ng.h/mL respectively. In a word, the pharmacokinetics result was not good enough to support the further study. We can decide

Table 3. Pharmacokinetics Properties of Compounds 6 and 8 in Mice

Compounds	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/mL)	$AUC_{0-\infty}$(ng.h/mL)	MRT (h)	$t_{1/2}$ (h)
6	20	1.00	133	548	3.95	2.06
8	20	0.50	150	450	4.46	3.62

whether the compounds go to the next step based the pharmacokinetics paramaters. In an orally dose of 20 mg/kg in mice, if the compound displayed C_{max} > 500 ng/mL, AUC_{0-t} > 3000 ng.h/mL and $t_{1/2}$ > 2.0 h, we will do a further research. The poor pharmacokinetic performance may be due to their poor cell permeability that results from the relatively large molecular size.

CONCLUSION

In summary, a series of novel Triazole-based Src kinase inhibitors were synthesized and evaluated for their antiproliferative activities against human lung cancer A549 cells. Most of the compounds showed potent *in vitro* anticancer activities, among them compounds **6** and **8** were further subjected to pharmacokinetic performance study, unfortunately, the result was not desirable. Further optimization of this new series analogues to improve their PK profiles is ongoing in our laboratory.

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