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De Novo Design of 2-Amino-4-Alkylaminoquinazoline-7-Carboxamides as Potential Scaffold for JAK1-Selective Inhibitors

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Janus kinase 1 (JAK1) is an intracellular nonreceptor tyrosine kinase that belongs to the JAK family kinases (JAK1, JAK2, JAK3, and Tyk2), which play key roles in transmitting proliferative and inflammatory signals through the JAK-STAT (signal transducer and activator of transcription) pathway. Consequently, a large compound screening campaign was conducted, and multiple chemical entities have been tested for their therapeutic potential against various indications including myeloproliferative diseases and inflammatory diseases.[1-3] In particular, due to the dominant role in signal transduction through γ_c-containing cytokine receptors, JAK1 has become an attractive target for the treatment of immunologic disorders such as rheumatoid arthritis (RA).[4-8] Several lines of evidence also supports that an ideal RA therapy would minimize inhibition of JAK2 due to a proven link between JAK2 deficiency and anemia.[9] Thus, in order to serve as an ideal therapeutic agent to target RA, a JAK1 inhibitor needs to achieve selective inhibition of JAK1 over other JAK isozymes.[10] Recently, several chemical entities have been identified with potent JAK1 inhibitory activities,[11-12] but with relatively low (6.4-20 fold) selectivity over JAK2.

In the course of our ongoing search for selective JAK1 inhibitors, structural modification of a previously reported tetracyclic pan-JAK inhibitor **1** (Fig. 1)[13-16] drew our attention; the tricyclic derivative **2** prepared by opening the imidazole ring of **1** showed potent JAK2 inhibitory activity along with significant JAK2-selectivity[17] (Fig. 1). Prompted by this result, we reasoned that removal of the fused fluorophenyl ring of **1** might provide different JAK-isozyme selectivity, which culminated in design of a bicyclic compound **3** with a 2,4-diaminoquinazoline-7-carboxamide core structure. By virtue of two free amino groups located at 2 and 4 positions (Fig. 1), various analogues of the compound **3** can be prepared by substitution at either or both of these functionalities, which would enable extensive structure-activity relationship study. In this proof-of-concept study, we investigated the feasibility of 4-monoalkylated 2,4-diaminoquinazoline-7-carboxamide as a novel scaffold for JAK1-selective inhibitors. Herein, we report synthesis of a series of 2-amino-4-alkylaminoquinazoline-7-carboxamide derivatives and evaluation of their JAK1-selective inhibition.

The title compounds **3a-3g** were prepared by employing a well-known five-step synthetic route starting from a commercially available dimethyl 2-aminoterephthalate (**4**) (Scheme 1).[18] Briefly, condensation of **4** with cyanamide in the presence of HCl provided a 2-aminoquinazolinone scaffold (**5**) in 85% yield. $POCl_3$-mediated chlorination of **5** smoothly underwent to provide 2-amino-4-chloroquinazoline **6** (63% yield), which, upon nucleophilic substitution with various amines followed by hydrolysis and amidation, provided the desired compounds **3a-3g** in 48%-72% yields[19] (three-steps) (Scheme 1).

In vitro inhibitory activity of the 2-amino-4-alkylaminoquinazoline-7-carboxamide derivatives (**3a-3g**) on the JAK isozymes was determined by Z′-LYTE™ Kinase Assay Kit-Tyr 6 Peptide (JAK1-JAK3) and Tyr 3 Peptide (Tyk2) (Invitrogen) according to the manufacturer's instruction. IC_{50} values of **3a-3g** against JAK isozymes are summarized in Table 1.

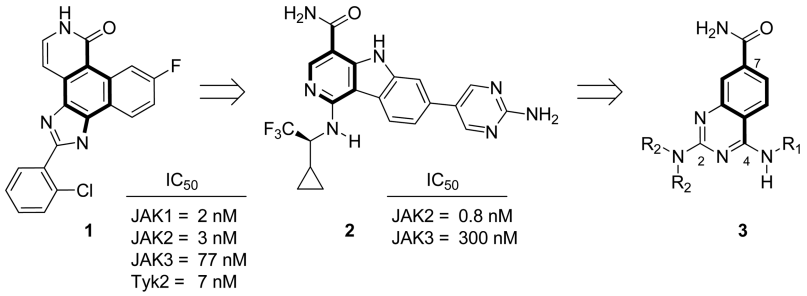
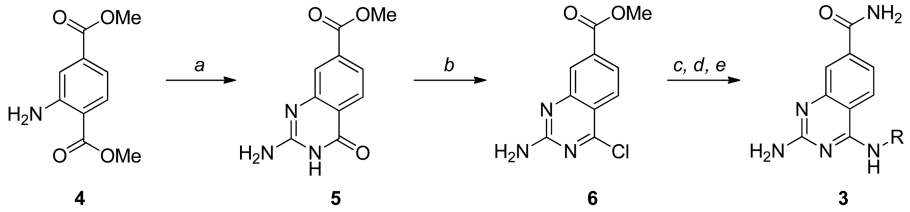


Figure 1. *De novo* design of 2-amino-4-alkylaminoquinazoline-7-carboxamide core (**3**).

[a]These authors contributed equally to this work.



3a R = $(CH_2)_2NH_2$
3b R = $(CH_2)_3NH_2$
3c R = Ph
3d R = 4-OH-Ph
3e R = 4-piperidine
3f R = 4-pyridine
3g R = 2-(1,1'-biphenyl)

Reagents & Conditions: (a) H_2N-CN, HCl, CH_3CN, 80 °C; (b) $POCl_3$, 110 °C; (c) RNH_2, TEA, EtOH, reflux; (d) LiOH, H_2O/MeOH (4:1), 40 °C; (e) EDC, NH_3-HOBT, DMF/CH_3CN

Scheme 1. Synthesis of the title compounds (**3a-3g**).

In general, the synthesized quinazoline derivatives showed only moderate to low inhibitory activity against the JAK isozymes. However, a clear structure-activity relationship observed for JAK1-selective inhibitors (**3a**, **3b**, **3d**, and **3e**) is noteworthy. Regarding selectivity for JAK1 in favor of JAK2, these inhibitors share the same structural features; the 4-amino functionalities of these inhibitors are connected with hydrogen bond donors through either linear (**3a** and **3b**) or cyclic (**3d** and **3e**) linkers. Others with a hydrogen bond acceptor (**3d**) or a hydrophobic aromatic substituent (**3c** and **3f**) showed low JAK1/JAK2 selectivity. Among the JAK1-selective quinazoline derivatives, only those with cyclic linkers (**3d** and **3e**) maintained JAK1 selectivity against Tyk2, but none of them showed selective JAK1 inhibition over JAK3.

The relationship between the quinazoline derivatives with a tethered hydrogen bond donor and their JAK1/JAK2 selectivity was further investigated by molecular modeling study. The compound **3e** with the most significant JAK1/JAK2 selectivity was therefore docked into the ATP-binding site of JAK1 (PDB ID = 4E5W)[11] and JAK2 (PDB ID = 2B7A)[20] by using the flexible ligand docking software Glide incorporated into the Schrödinger molecular modeling software suite (Fig. 2).

Due to similar structural features shared by the ligand-binding sites of JAK1 and JAK2, docking poses of the quinazoline **3e** show only a marginal difference: size of the quinazoline **3e** is well matched to those of the binding pockets of JAK1 (Fig. 2(a)) and JAK2 (Fig. 2(b)), and **3e** is shown to fit nicely into the ATP-binding sites of both of the JAK isozymes. Also, binding modes of **3e** to JAK1 (Fig. 2(c)) and JAK2 (Fig. 2(d)) are similar, in which **3e** forms hydrogen bonds with the backbone carbonyl (Leu959/Glu957 for JAK1 and Leu932/Glu930 for JAK2), side chain -COOH (Asn1008 for JAK1 and Asn981 for JAK2), and side chain -$CONH_2$ (Glu966 for JAK1) groups. In particular, the 4-piperazinyl moiety of **3e** forms a hydrogen bond with a side chain of Asn1008 of JAK1 (Fig. 2(c)), which provides a reasonable explanation for enhanced JAK1-inhibitory activity of the quinizoline derivatives of which 4-amino functionalities are connected with hydrogen bond donors (**3a**, **3b**, **3d**, and **3e**, Table 1). At this point, it should be noted that **3e** also forms a hydrogen bond with a side chain of Asn981 of JAK2 (Fig. 2(d)), but this observation is not in match with its JAK1-selective inhibitory activity. However, in the binding mode of **3e** to JAK2, 2,4-diaminoquinazoline moiety of **3e** is located at the unstable axial position of the piperidine ring at the expense of forming a hydrogen bond with Asn981 (Fig.

Table 1. Inhibitory activity (IC_{50}) of the title compounds against JAK isozymes

Compds	IC_{50} (μM)[a]				Selectivity[b]		
	JAK1	JAK2	JAK3	Tyk2	JAK1 /JAK2	JAK1 /JAK3	JAK1 /Tyk2
3a	8.8 ± 0.2	72.9 ± 0.8	27.7 ± 0.2	40.8 ± 0.4	8.3	3.1	4.6
3b	9.7 ± 0.6	72.4 ± 0.9	32.6 ± 0.5	35.7 ± 0.7	7.5	3.4	3.7
3c	25.9 ± 0.8	50.4 ± 0.7	26.7 ± 0.4	31.5 ± 0.5	1.9	1.0	1.2
3d	6.7 ± 0.2	52.8 ± 0.6	12.5 ± 0.8	48.6 ± 0.5	7.9	1.9	7.3
3e	5.6 ± 0.6	72.1 ± 0.8	15.7 ± 0.4	48.3 ± 0.3	12.9	2.8	8.6
3f	19.4 ± 0.4	54.2 ± 0.4	21.8 ± 0.7	50.6 ± 0.9	2.8	1.1	2.6
3g	10.5 ± 0.4	38.5 ± 0.2	35.4 ± 0.5	49.7 ± 0.6	3.7	3.4	4.7

[a]The means ± SD of three independent experiments are shown. [b]Selectivity = IC_{50} against JAK2, JAK3, or Tyk2/IC_{50} against JAK1

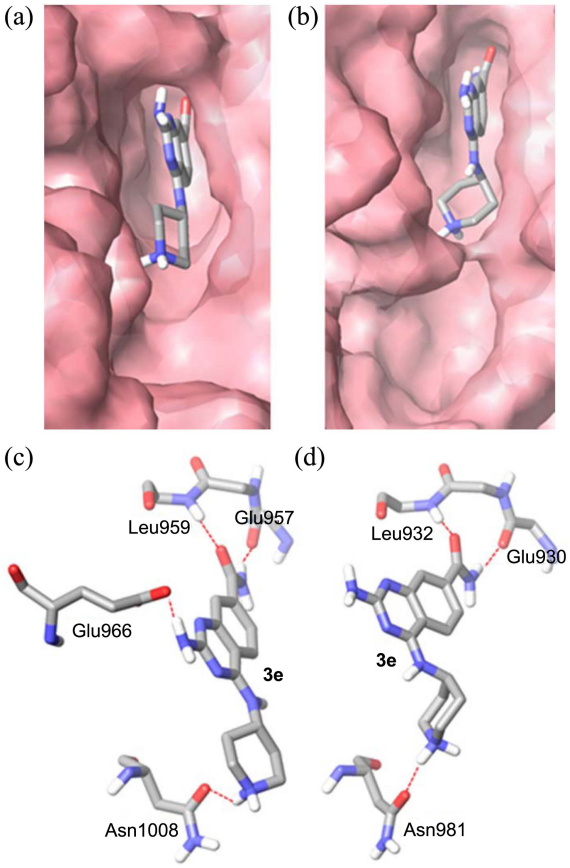


Figure 2. Docked structures of quinazoline **3e** into (a, c) JAK1 and (b, d) JAK2. Molecular surfaces of JAK1 and JAK2 are represented in (a) and (b), respectively, with bound **3e** (molecular representation in cylindrical tubes). Hydrogen bonds are noted as dotted lines.

2(d)). Thus, it is rational to suppose that the ATP-binding site of JAK2 is in inappropriate conformation for hydrogen bonding interaction with the hydrogen bond donors substituted at the 4-amino functionalities of the quinazoline scaffold, which results in reduced binding affinity and thereby lower inhibitory activity of **3e** against JAK2.

In summary, in order to develop JAK1-selective inhibitors, we designed a 2-amino-4-alkylaminoquinazoline-7-carboxamide scaffold by structural simplification of the tetracyclic pan-JAK inhibitor **1**. Evaluation of *in vitro* inhibitory activity against JAK isozymes identified the quinazoline derivatives of which 4-amino functionalities are connected with hydrogen bond donors (**3a**, **3b**, **3d**, and **3e**, Table 1) as selective JAK1 inhibitors. Molecular docking study of the most selective 2-amino-4-(4-piperazinyl)aminoquinazole derivative **3e** revealed that the hydrogen bond donor tethered at the 4-amino functionality of the quinazole scaffold plays an important role in differentiating the ATP-binding site of JAK1 from those of other isozymes. This result warrants further investigation of the quinazoline derivatives as selective JAK1 inhibitors and extensive structure-activity relationship study of 2,4-bisalkylated 2,4-diaminoquinazole-7-carboxamide derivatives is underway.

Experimental Section

Preparation of the Title Compound 3e. To a solution of methyl 2-amino-4-chloroquinazoline-7-carboxylate (**6**) (500 mg, 2.1 mmol) and 4-aminopiperidine (0.2 mL, 2.1 mmol) in EtOH (10 mL) was slowly added TEA (0.3 mL, 2.1 mmol). After stirring for 5 hours at 90 °C, the mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (10:1 = CH_2Cl_2:MeOH). The yellow powder, thus obtained, was dissolved in a mixture of MeOH (1 mL) and H_2O (4 mL), treated with LiOH (201 mg, 8.4 mmol) at 0 °C, and stirred for 4 h at 40 °C. After cooling to room temperature, the reaction mixture was acidified with 2 N HCl and then extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was dissolved in a 4:1 mixture of CH_3CN and DMF (8 mL/2 mL) and the resulting solution was treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (978 mg, 6.3 mmol) and HOBT-NH_3 (941 g, 6.3 mmol) at 0 °C. The reaction mixture was stirred at room temperature and the progress of reaction was monitored by TLC. After stirring for 4 hours at room temperature, the mixture was diluted with water and extracted three times with EtOAc. The combined organic layers were washed with saturated aqueous sodium bicarbonate solution and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure to give a crude product which was purified by column chromatography (SiO_2, CH_2Cl_2:MeOH:NH_4OH:H_2O = 80:20:1:1) to give **3e** (289 mg, 1.0 mmol, 48% yield) as a yellow powder; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32 (dd, J = 8.4, 2.1 Hz, 1H), 7.24 (d, J = 2.1 Hz, 1H), 6.56 (d, J = 8.4 Hz, 1H), 7.12 (brs, -NH_2), 6.83 (brs, -NH_2), 2.75-3.12 (m, 5H), 2.23-2.30 (m, 2H), 2.13-2.20 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.8, 162.6, 160.1, 153.5, 142.8, 135.4, 128.5, 123.6, 122.3, 110.5, 55.7, 47.1, 42.2.

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