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Anilinopyrazole as Selective CDK2 Inhibitors: Design, Synthesis, Biological Evaluation, and X-ray Crystallographic Analysis

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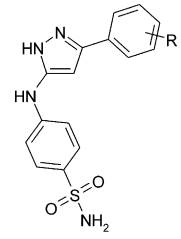
Abstract—A novel series of anilinopyrazoles has been designed based on the X-ray crystal structure analysis. Most compounds from this series not only show sub-nanomolar IC_{50} values for CDK2, but also demonstrate almost 1000-fold selectivity to other kinases including CDK1.

Total 518 protein kinases identified from the human genome sequence and 130 protein phosphatases exert precise and reversible control on protein phosphorylation.[1] Through this function, they play pivotal roles in regulating aspects of metabolism, gene expression, cell growth, cell division and cell differentiation. The regulation of the cell cycle is a well orchestrated pairing of different family members of the Cyclin Dependent Kinases (CDKs) and cyclin regulatory subunits.[2,3] The inhibition of CDK2 should arrest cells in G1 phase and prevent them from entering the cell cycle. Therefore, inhibitors of CDK2 may have utility in the treatment of proliferative diseases such as cancer and psoriasis.[4] When dealing with protein kinases, one major concern is selectivity among the different kinases due to the markedly conserved structure of the catalytic domain. Regarding CDKs, structural information indicates that the catalytic center includes a core of ca. 300 amino acids which possesses a high degree of homology between the different members of this protein kinase family.[5] CDK inhibitors acting as ATP competitive ligands have been thoroughly reviewed in the literature.[3] Achieving selectivity between CDK1 and CDK2 remains a challenge since these enzymes share 64% identity overall and 90% identity in ATP binding pocket.[6]

Generation of a highly selective CDK2 inhibitor series emerged from our kinase system based research.[7] A crystal structure of **1a** (where **R** = ***m*-Br**), composed of cyclin A/CDK2, was successfully obtained with good resolution and this co-crystallized structure allowed a good understanding of the necessary selectivity requirements. This communication describes our efforts in exploring a selective CDK2 inhibitor, represented by structure **1**, which not only shows excellent selectivity against other kinases such as VEGFR2, SRC, and GSK3 but also shows excellent selectivity against CDK1 (Scheme 1).

The initial objective of our program was to generate diverse kinase inhibitors using a system-based-research strategy,[7] from which a CDK2 hit compound **1a**, was identified with good activity and selectivity. To this end, synthetic methods to explore the activity and selectivity



Scheme 1. Anilinopyrazole **1**.

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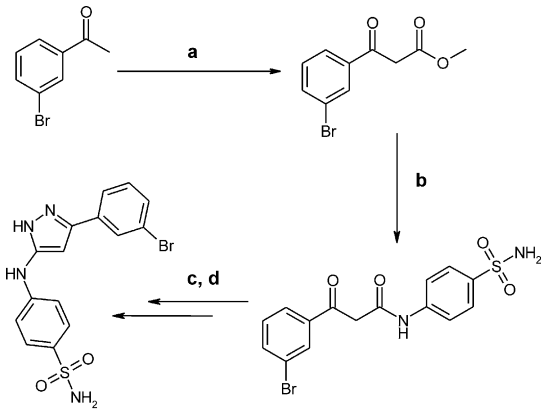
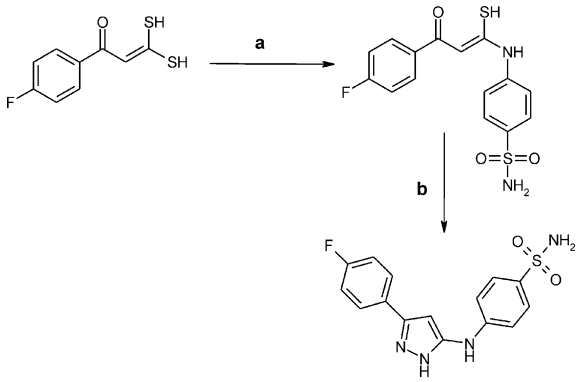


Scheme 2. Reagents: (a) NaH, carbonic acid dimethyl ester (excess), 75 °C, 20 min; (b) 4-aminobenzene sulfonamide, toluene/DMF = 3/1, 130 °C, 12 h; (c) Lawesson's reagent, toluene, 140 °C, 1 h; (d) hydrazine monohydrate, HOAc, EtOH, 85 °C, 12 h.



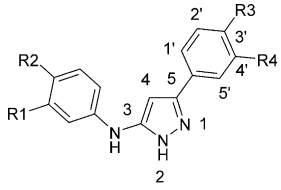
Scheme 3. Reagents: (a) 4-aminobenzene sulfonamide, toluene, 140 °C, 3 h; (b) hydrazine monohydrate, HOAc, EtOH, 85 °C, 12 h.

of the anilinopyrazoles have been established and are summarized in Schemes 2–4.

Preparation of the anilinopyrazole compounds was generally achieved by the reaction of substituted acetophenone with carbonic acid dimethyl ester, followed by treatment with aniline (Scheme 2). The corresponding thiopropionamide was obtained by reaction with Lawesson's reagent, followed by treatment with hydrazine to give the desired analogues. This protocol was used to provide compounds **1a** and, **2** as indicated in Table 1. Commercially available 1-(4-fluoro-phenyl)-3,3-dimercaptopropenone could also be used as starting materials for the preparation of anilinopyrazole analogues (Scheme 3). Compounds **1b** and, **3** were obtained with this method. An alternative procedure as shown in Scheme 4 involved the reaction of acetophenone with an isothiocyanate, followed by the same cyclization approach with hydrazine to afford the corresponding desired compounds **1c**–**1g** and **1i**–**1k**, which are exemplified in Table 1. Further transformations of the above compounds can be carried out to explore other functionalization. For example, anilinopyrazole sulfonamide derivative **1o** can be prepared via a reduction of the nitro- intermediate with Na_2S, followed by a reaction with benzenesulfonyl chloride as indicated in Scheme 4.[8]

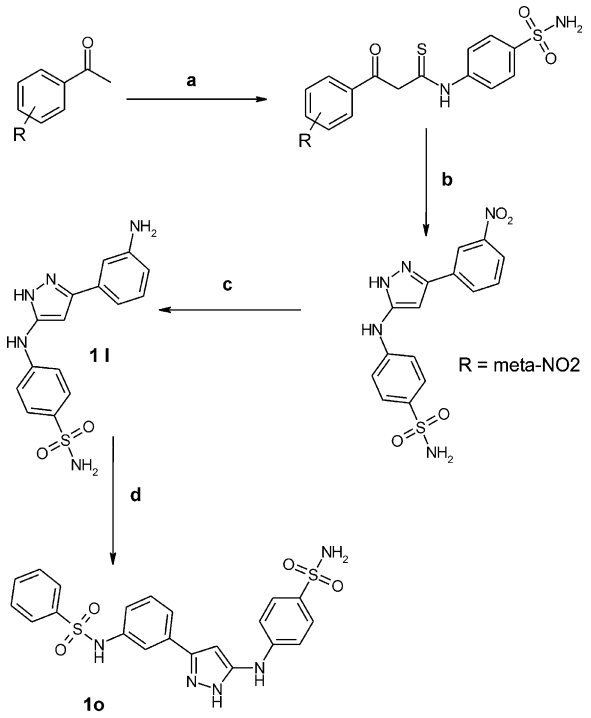
The crystal structure of CDK2 complexed with **1a** was determined with high resolution. The inhibitor bound into the ATP binding pocket is shown in Figure 1. Two hydrogen bonds were formed between the aminopyrazole moiety of compound **1a** and CDK2. Specifically, the sulfonamide NH and amide carbonyl oxygen were

Table 1. CDK1 and CDK2 inhibitory activities of **1a**–**1o**, **2**, and **3**



Compd	R1	R2	R3	R4	Kinase IC_{50} (nM)	
					CDK1	CDK2
1a	H	$-SO_2NH_2$	H	Br	ND	23.4
2	H	$-NHSO_2CH_3$	H	Br	ND	602
3	$-SO_2NH_2$	H	F	H	ND	871
1b	H	$-SO_2NH_2$	F	H	ND	17.4
1c	H	$-SO_2NH_2$	Br	H	126	10
1d	H	$-SO_2NH_2$	$-N(CH_3)_2$	H	ND	>2000
1e	H	$-SO_2NH_2$	$-OCH_3$	F	ND	407
1f	H	$-SO_2NH_2$	–OH	F	200	12.6
1g	H	$-SO_2NH_2$	$-NO_2$	H	ND	0.33
1h	H	$-SO_2NH_2$	$-NH_2$	H	501	0.34
1i	H	$-SO_2NH_2$	H	–Morpholine	ND	490
1j	H	$-SO_2NH_2$	H	–OMe	398	24.5
1k	H	$-SO_2NH_2$	H	–OH	398	9.1
1l	H	$-SO_2NH_2$	H	$-NH_2$	ND	166
1m	H	$-SO_2NH_2$	H	$-NHSO_2CH_3$	501	10.7
1n	H	$-SO_2NH_2$	H	–NHCONH–(2-fluoro-5-trifluorophenyl)	794	61.6
1o	H	$-SO_2NH_2$	H	$-NHSO_2$-phenyl	501	10.7

both H-bonded with Asp86. The *para*-sulfonamide on the 3-anilino group proved to be an essential functionality, based on the fact that all other substitutions dramatically decreased the inhibitory potency. For example, compound **2** where methanesulfonamide replaces sulfonamide demonstrated a 25-fold reduction in inhibitory activity. Furthermore, attaching sulfonamide at the *meta*-position (Table 1, compound **3**) dramatically reduced the activity. In the ligand bound crystal structure, the bromophenyl ring of compound **1a** occupies a pocket near Lys33 composed of Lys33, Glu51, Asp145, Val64 and Phe80. This area including Phe80, is smaller in size, relative to many other kinases such as GSK3 and VEGFR2, and confers a very important opportunity for selectivity. We propose that if this space could be perfectly filled by a substituent, this will result in not only high activity but also excellent selectivity. The 1′- and 2′-positions at the backside of the bromophenyl ring reside near the surface of Val64 and Phe80 suggesting that there is no space for accommodating substituents of any size. The 3′-substituent on the phenyl ring would occupy a small cavity close to Glu51 and a large substituent would be expected to encounter unfavorable steric interactions with the enzyme in this position. The 4′-position projects toward a cleft composed by Lys33 and Asp145, and into the region near the sugar pocket. This structural characteristic suggested a large substituent could be tolerated.



Scheme 4. Reagents: (a) 4-isothiocyanato-benzenesulfonamide, $LiN(TMS)_2$, −78 °C to rt, THF, 12 h; (b) hydrazine monohydrate, HOAc, EtOH, 85 °C, 12 h; (c) Na_2S aq, EtOH, 80 °C, 5 h; (d) benzenesulfonyl chloride, pyridine, 0 °C to rt, 12 h.

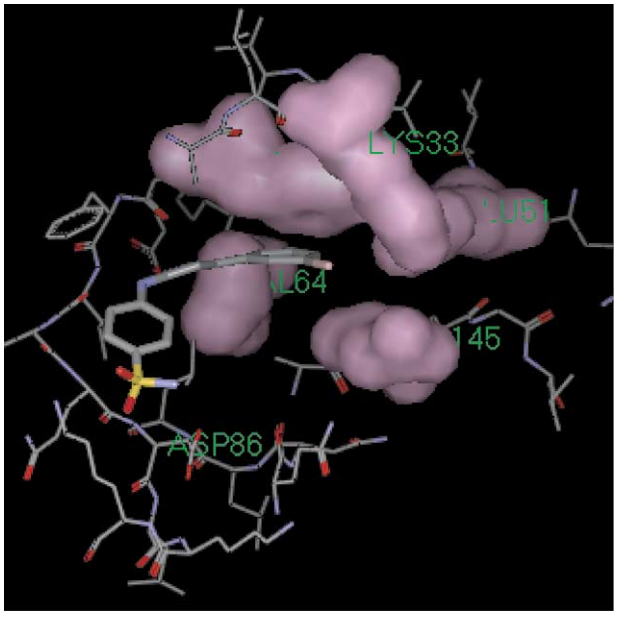


Figure 1. Crystal structure of **1a** bound to activated CDK2. Surface representation of CDK2 illustrating the Lysine pocket composed of Lys33, Glu51, Asp145, Val64 and Phe80. The sulfonamide functionality was observed to be positioned at the opening to the ATP binding cleft. Inhibitor atoms colored as follows: C, gray; N, blue; O, red; S, yellow; Br, purple.

The SAR of the anilinopyrazoles proved very consistent with these structural considerations. Accordingly, substituents R3 were size limited as evidenced by the inhibitory activities of compounds **1b**–**1h** (Table 1). Compounds **1g**, **1h** with a particularly favorable steric fit for the cavity showed the most potent inhibition of CDK2 with IC_{50}s in the sub-nanomolar range and excellent selectivity relative to other kinases including CDK1. A slightly larger substituent such as the dimethylamino group showed >100-fold less potent CDK2 inhibition. Many compounds containing R4 substituents have also been studied. A morpholine substituent (compound **1i**) was anticipated to interact unfavorably with the cleft composed by Lys33 and Asp145, and this substitution dramatically decreased the activity. Favorable substituents like bromo, methoxy, hydroxy (compounds **1a**, **1j**, **1k**) showed more potent inhibition with IC_{50} values less than 25 nM. In addition, the amino group (compound **1l**) was found to show moderate inhibition of CDK2 and derivation to give sulfonamide and urea substituents provided potent CDK2 inhibitors in contrast to the parent compound.

The compounds in the disubstituted pyrazole series described here were generally about 100-fold more potent as inhibitors of CDK2 than other kinases. For example, compound **1h** bearing an amino group at the 3′-position, which was considered to fill the Lys33 pocket best, showed more than 1000-fold selectivity against other kinases screened [kinases, IC_{50} (nM)]: GSK-3, 10,000; SRC, 3981; VEGFR-2, 6300; EGFR, >10,000; ERBB-4, >10,000; TIE-2, 6300.[9]

In conclusion, we have identified a series of novel, potent CDK2 inhibitors. This series also exhibits an excellent selectivity profile against other kinases screened to date.

Acknowledgements

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8. Analytic data of potent CDK2 inhibitors **1a**: ^{1}H NMR (400 MHz, DMSO-d_6) ppm 6.44 (s, 1H), 7.06 (s, 2H), 7.42 (m, 3H), 7.55 (d, 1H, $J=7.6$ Hz), 7.64 (d, 2H, $J=8.8$ Hz), 7.76 (d, 1H, $J=7.6$ Hz), 8.00 (t, 1H, $J=1.8$ Hz), 9.10 (s, 1H), 12.76 (s, 1H). LC/MS: m/z 393, 395 $(M+1)^+$. **1h**: ^{1}H NMR (400 MHz, DMSO-d_6) ppm 5.34 (s, 2H), 6.05 (s, 1H), 6.59 (d, 2H, $J=8.6$ Hz), 7.03 (s, 2H), 7.38 (d, 2H, $J=8.6$ Hz), 7.43 (d, 2H, $J=8.8$ Hz), 7.61 (d, 2H, $J=8.8$ Hz), 8.96 (s, 1H), 12.23 (s, 1H). LC/MS: m/z 330 $(M+1)^+$, 328 $(M-1)^-$. **1l**: ^{1}H NMR (400 MHz, DMSO-d_6) ppm 5.19 (s, 2H), 6.12 (s, 1H), 6.55 (d, 1H, $J=7.6$ Hz), 6.86 (m, 2H), 7.05 (s, 2H), 7.08 (t, 1H, $J=7.6$ Hz), 7.44 (d, 2H, $J=8.8$ Hz), 7.63 (d, 2H, $J=8.8$ Hz), 9.02 (s, 1H), 12.48 (s, 1H). LC/MS: m/z 330 $(M+1)^+$, 328 $(M-1)^-$.
9. The procedures for crystallography and enzymology have been described in ref 3.