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Synthesis and SAR of 1,9-dihydro-9-hydroxypyrazolo[3,4-*b*]quinolin-4-ones as novel, selective c-Jun N-terminal kinase inhibitors

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Abstract—A novel class of 1,9-dihydro-9-hydroxypyrazolo[3,4-*b*]quinolin-4-ones as c-Jun-N-terminal kinase (JNK) inhibitors is described. These compounds were synthesized via the condensation of 2-nitrobenzaldehydes and hydroxypyrazoles. The structure–activity relationships (SAR) and kinase selectivity profile of the inhibitors are also discussed. Compound **16** was identified as a potent JNK inhibitor with good cellular potency.

Type 2 diabetes has become the most prevalent metabolic disease worldwide.[1] Patients with type 2 diabetes are insulin resistant, a condition in which the body fails to respond to insulin properly. c-Jun N-terminal kinase-1 (JNK1), a member of the mitogen-activated protein (MAP) kinase family, has recently emerged as an attractive target for diabetes therapy, since JNK1 is believed to play a key role in linking obesity and insulin resistance.[2] JNK1 is activated by inflammatory cytokines, and negatively regulates insulin signaling via serine (307) phosphorylation of the insulin receptor substrate (IRS-1) which leads to the degradation of IRS-1.[2,3] $JNK1^{-/-}$ mice show marked reduction in both plasma glucose and insulin concentrations relative to their wild-type littermates, and thus are protected from diet-induced obesity.[2a] In addition, JNK1 activity is elevated in adipocytes of type 2 diabetic patients.[4] Inhibitors of JNK1 can potentially increase insulin sensitivity, and therefore could be useful as therapeutics for the treatment of type 2 diabetes, as well as serving as valuable tools for the evaluation of other clinical benefits that these inhibitors may provide.[5]

Research related to JNK inhibitors has gained increasing attention in the past few years, and as a result a number of JNK inhibitors have recently appeared in the literature.[6] We wish to report here our efforts toward the identification of 1,9-dihydro-9-hydroxypyrazolo[3,4-*b*]quinolin-4-ones as novel ATP competitive, pan-JNK inhibitors. A high-throughput screening (HTS) of the Abbott compound collection identified two 9-hydroxypyrazoloquinolinones **1**, **2** (Fig. 1) as ATP-competitive JNK1 inhibitors with IC_{50} values around 1 μM. JNK1 inhibitors need to be cell permeable to reach the intracellular targets, therefore a phospho c-Jun cell-based ELISA assay was also utilized to test the compounds cellular potency.[7] In this assay, compound **2** was marginally active with an IC_{50} of 16.4 μM. Additionally, both compounds showed enzymatic IC_{50}s of greater than 50 μM against p38 and ERK2, two closely related MAP kinases. The novelty of the core structure[8] as well

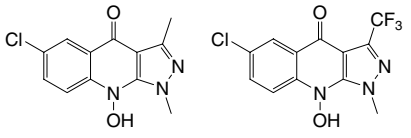

Figure 1. 1,9-Dihydro-9-hydroxypyrazolo[3,4-*b*]quinolin-4-ones identified from HTS.

Keywords: JNK; Diabetes; Obesity; Pyrazoloquinolinones.
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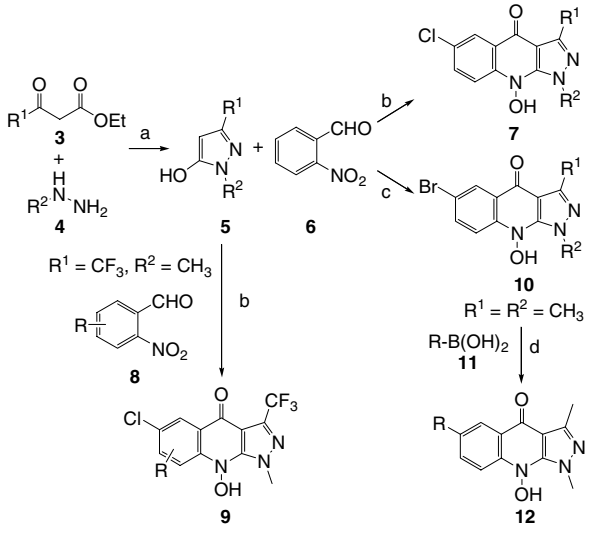
as its good selectivity profile motivated us to initiate hit to lead chemistry to explore the activity of this class of compounds as JNK1 inhibitors.

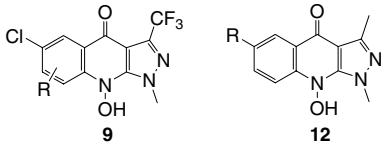
9-Hydroxypyrazoloquinolinones were prepared in a straightforward manner through the condensation of 2-nitrobenzaldehydes (**6**) and hydroxypyrazoles (**5**) in the presence of concentrated hydrochloric acid (Scheme 1). This method allowed for easy modification of the N-1 and C-3 positions of the pyrazole ring, as large numbers of hydroxypyrazoles were accessible via either commercial sources or the condensation of β-keto esters (**3**) with substituted hydrazines (**4**) (Scheme 1). A number of substituted nitrobenzaldehydes (**8**) reacted smoothly with hydroxypyrazoles (**5**) providing the desired products in good yield. However, 5-substituted nitrobenzaldehydes reacted poorly, which resulted in very little or no product. Similarly, 6-bromosubstituted analogs (**10**) were prepared using hydrogen bromide in acetic acid as the solvent. The bromide served as a handle for further fuctionalization at the 6-position of the aromatic ring (Scheme 1). For example, Suzuki coupling of **10** with arylboronic acids yielded **12**. In most cases only a simple filtration was needed for purification of the final product.



Scheme 1. Reagents and conditions: (a) cat. cont. HCl, EtOH, reflux, overnight; (b) HCl/HOAc, 85 °C, 5 h, 50–60%; (c) HBr (33% in HOAc)/H_2O, 85 °C, 5 h, 52%; (d) FibreCat 1032 (5 mol%),[9] K_2CO_3, EtOH/H_2O, microwave 120 °C, 20 min, 50% plus reduced product (**12a**, R = H).

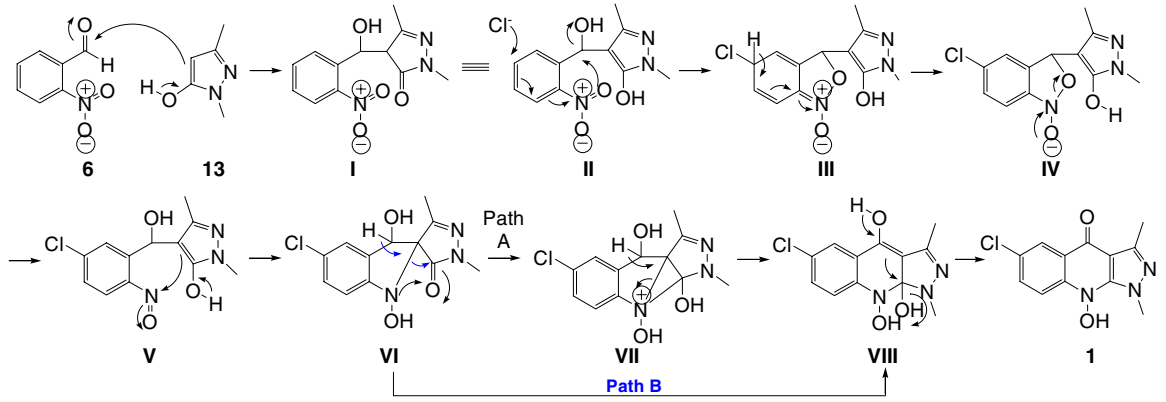
The reaction mechanism for the formation of **1** is proposed in Scheme 2. In the presence of an acid, hydroxypyrazole **13**, an enol, reacted with the aldehyde to give **II**. As previously reported by Loudon in a mechanistically similar system,[10] the next step was the addition of chloride to the aromatic ring with subsequent rearomatization in acidic media providing the *N*-hydroxyisoxazoline derivative **IV**.[11] This step was consistent with the observation that 5-substituted nitrobenzaldehydes did not react or react poorly with hydroxypyrazoles, since the addition site was blocked by the substituents at that position. Ring opening of **IV** produced nitroso intermediate **V**, which was subjected to intramolecular addition of the hydroxypyrazole to give the tricyclic system **VI**. Finally, intramolecular cyclization of **VI**, proton abstraction at the benzylic position of **VII**, and subsequent dehydration of **VIII** would form the desired pyrazoloquinolone product **1** (path A).

Table 1. Enzymatic and cellular activity of analogs with aromatic ring modifications



Compound	R	JNK1 IC_{50} (μM)	Pc-Jun IC_{50} (μM)
1	—	1.22	>30
2	—	0.98	16.4
10	—	0.92	>30
9a	5-Cl	>10	NT[a]
9b	7-Cl	5.14	>30
9c	8-OMe	>10	NT[a]
9d	7-N(Me)$_2$	>10	NT[a]
12a	H	>10	NT[a]
12b	Ph	>10	NT[a]
12c	1*H*-Pyrazol-3-yl	>10	NT[a]

[a] Compounds with enzymatic IC_{50}s greater than 10 μM were not tested in the cellular assay.



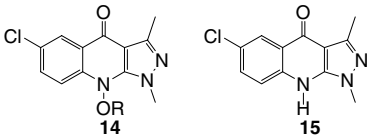
Scheme 2. Proposed mechanism for the formation of 9-hydroxypyrazolo[3,4-*b*]quinolin-4-one **1**.

Alternatively, proton abstraction at the benzylic position could occur first, followed by rearrangement to give **VIII** directly, which then lead to the formation of the final product **1** (path B).

All the compounds were tested in our JNK1 enzymatic inhibition assay as well as a cell-based assay measuring inhibition of TNFα stimulated phosphorylation of c-Jun in HepG2 cells.[12] The effect of different substitution on the phenyl ring was studied first, and these results are summarized in Table 1. Analog **12a**, in which chlorine was replaced with hydrogen, was not active against JNK1, whereas there was only a slight decrease in activity when chloride was replaced with bromide (**10**). However, replacement of chlorine by an aryl group at the same position gave only inactive compounds (**12b**,**c**). Most analogs (**9a**–**9d**) with substitution at the 5, 7, and 8 positions of the ring were inactive as well.

The role of the *N*-hydroxyl group was investigated next. As summarized in Table 2, O-alkylated analogs generally maintained or slightly decreased (2- to 4-fold) binding potency when R was small alkyl groups (**14a**–**14c**, Table 2), whereas bulky R group resulted in com-

Table 2. Enzymatic and cellular activity of 9-(alk)oxy analogs



Compound	R	JNK1 IC_{50} (μM)	Pc-Jun IC_{50} (μM)
1	H	1.22	>30
14a	Me	4.59	19.4
14b	Et	2.78	10.6
14c	Pr	5.43	5.6
14d	*i*-Pr	>10	NT[a]
14e	Cyclopentyl	>10	NT[a]
14f	Bn	>10	NT[a]
15	—	>10	NT[a]

[a] Compounds with enzymatic IC_{50}s greater than 10 μM were not tested in the cellular assay.

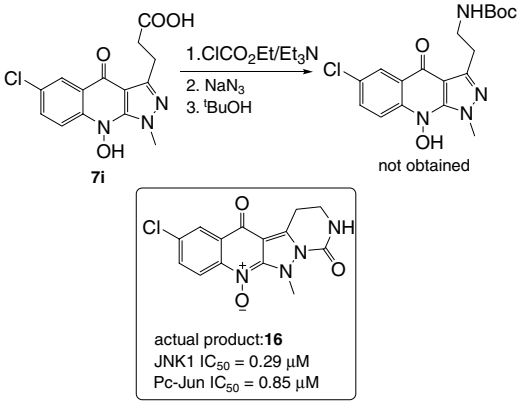
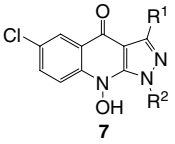


Figure 2. Cell permeable JNK inhibitor **16**.

Table 3. Enzymatic and cellular activity of analogs with C-1 and N-3 modifications



Compound	R_1	R_2	JNK1 IC_{50} (μM)	Pc-Jun IC_{50} (μM)
1	Me	Me	1.22	>30
7a	Et	Me	0.96	32
7b	*n*-Pr	Me	1.31	26.6
7c	*n*-Bu	Me	0.52	>30
7d	*i*-Pr	Me	2.64	>30
7e	*t*-Bu	Me	>10	NT[a]
7f	COOMe	Me	>10	NT[a]
7g	4-NH_2–Ph	Me	>10	NT[a]
7h	CH_2CH_2OMe	Me	2.41	14.7
7i	$(CH_2)_2CO_2H$	Me	>10	NT[a]
7j	$(CH_2)_3CO_2H$	Me	2.74	>30
7k	$CH_2CH_2NH_2$	Me	2.83	>30
7l	$(CH_2)_3CONHMe$	Me	0.75	>30
7m	$(CH_2)_2NHCOMe$	Me	0.78	>30
7n	$(CH_2)_3NHCONHEt$	Me	0.50	>30
7o	$(CH_2)_2NHCO_2Et$	Me	0.43	>30
7p	Me	CH_2CO_2H	4.41	>30
7q	Me	CH_2CO_2Et	7.64	>30
7r	Me	$CH_2CONHMe$	8.77	>30
7s	Me	CH_2CH_2OH	7.67	>30
7t	Me	Et	4.19	>30
7u	Me	Ph	1.75	>30

[a] Compounds with enzymatic IC_{50}s greater than 10 μM were not tested in the cellular assay.

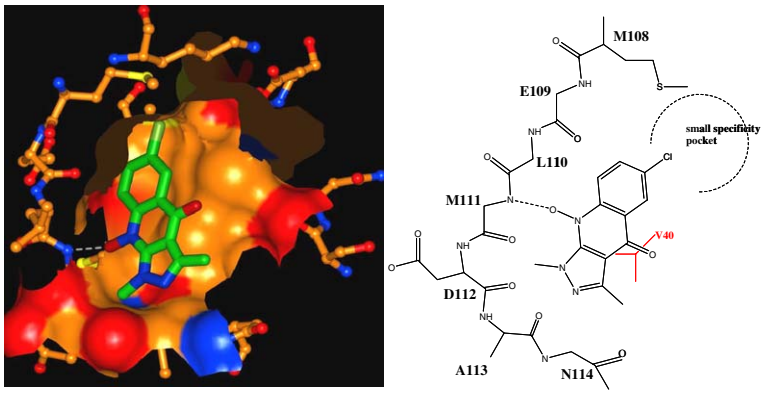


Figure 3. X-ray structure of compound **1**.

plete loss of activity (**14d**–**14f**). Interestingly, compounds with small alkyl groups exhibited increased cellular activity, especially compound **14c** (R = Pr), which showed an almost 4-fold improvement in cellular potency presumably due to increased cell permeability in the absence of the acidic hydroxyl proton. Removal of the hydroxyl group yielded inactive compound **15**, indicating that the hydroxyl group may serve as a H-bond donor within the active site of the enzyme.

A number of compounds with different substitution at the N-1 and C-3 positions of the pyrazole ring were also prepared and tested (Table 3). Elongation of the C-3 methyl group to ethyl (**7a**), propyl (**7b**), and butyl (**7c**) groups appeared to have some impact on potency, in particular **7c** gave a 2.5 fold boost in JNK1 activity. Branching at the α-carbon was not favorable, leading to decreased JNK1 activity or inactive analogs (**7d**–**7g**). To further optimize the SAR at the C-3 positions, a number of ethers, acids, amines, amides, ureas as well as carbamates were prepared (see representative examples **7h**–**7o** in Table 3), which yielded several potent compounds with submicromolar potency. However, most of these analogs showed no or minimal cellular activity possibly due to low C log *P* values (<1.00) which may lead to low cell permeability. On the other hand, the effect of N-1 substitution appeared to be less profound. A variety of substituents (**7p**–**7u**) including carboxylates, amides, alcohols, and other hydrophobic groups were tolerated at this position, but the fact that all analogs exhibited similar IC_{50}s seemed to suggest that it might not be a critical point of contact with the enzyme.

We also serendipitously discovered a novel tetracyclic analog **16**, which was obtained under Curtius rearrangement conditions in an attempt to convert carboxylic acid **7i** to amines (Fig. 2). To our delight, compound **16** showed binding IC_{50} of 0.29 μM with submicromolar cellular potency, again suggesting that the molecule is more cell permeable in the absence of the acidic hydroxyl proton.

An X-ray structure of **1** bound into the ATP site of JNK1 has been obtained (Fig. 3)[13]. In the crystal structure, the N-9 oxygen binds to the hinge region of JNK1 via a hydrogen bond with Met 111. The chlorine of the aromatic ring extends toward the specificity pocket,[14] thus additional interaction with the protein may result from the substituents at C-5 and C-6, and possibly produce JNK1-selective inhibitors over JNK2 and JNK3. On the other hand, the pyrazole ring protrudes from the binding pocket, with N-1 and C-3 substituents having minimal contact with the enzyme, therefore the modification of these two positions showed minimal impact on potency.

The kinase selectivity of several pyrazoloquinolinone JNK inhibitors was evaluated as well, and they generally showed no significant activity at 10 μM against a panel of 81 kinases.[15] These compounds were also metabolically stable, for example, 100% of parent compound **1** was recovered after 30 min incubation with human liver microsome. The oral bioavailability for compounds **1** and **2** was 15% and 56%, respectively.[16]

In summary, we have discovered a novel series of 1,9-dihydro-9-hydroxypyrazolo[3,4-*b*]quinolin-4-ones as JNK inhibitors with submicromolar potency, good pharmacokinetic profiles, and cell permeability. The one-step procedure for the synthesis of 9-hydroxypyrazoloquinolinones greatly facilitated the SAR study for this series of compounds. X-ray crystal structure data suggest that further extension at the C-5 or C-6 position of the aromatic ring, or core modification of this ring to introduce H-bond acceptors may lead to more potent and selective JNK inhibitors.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.02.046.

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12. Some of the potent analogs were also tested in a JNK2 inhibition assay. In general, they are about few folds less potent against JNK2 as compared to JNK1. For example: JNK2 IC_{50}s for compounds **1** and **2** were 3.1 and 3.0 μM, respectively.
13. Co-crystals of JNK1 complexed with **1** belong to space group $P3_221$ with cell constants $a = b = 150.6$, $c = 119.0$ Å and diffracted weakly to about 3.5 Å, using synchrotron radiation (Advanced Photon Source, APS, IMCA-CAT, 17-ID). The structure was solved by molecular replacement and refined by conventional methods. Crystallographic coordinates of JNK1 complexed with **1** have been deposited at the Protein Data Bank (www.rcsb.org) with Accession code 2g01.
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15. A detailed kinase selectivity table was attached in the Supplementary material for reference.
16. See Supplementary material for detailed PK data for compounds **1** and **2**.