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Naphthyl Ketones: A New Class of Janus Kinase 3 Inhibitors

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Abstract—Potent inhibition of Janus kinase 3 was found for a series of naphthyl(β-aminoethyl)ketones (e.g. **7**, $pIC_{50} = 7.1 \pm 0.3$). Further studies indicated that these compounds fragment in less than 1 h by retro-Michael reaction in the Jak3 in vitro ELISA assay procedure. The breakdown product of **7**, 2-naphthylvinyl ketone (**22**, $pIC_{50} = 6.8 \pm 0.3$) showed very similar inhibitory activity to **7**. Compounds **7** (in neutral buffer) and **22** will be useful pharmacological tools for the investigation of the Janus tyrosine kinase Jak3.

Organ transplant surgery has become a standard treatment for organ failure, and the success of this surgery has depended on the selection of appropriate donor organs and drug treatment to control host rejection of the new organ. The macrocyclic fungal metabolite cyclosporin A[1] has been the mainstay of this drug therapy, and rapamycin has recently shown[2] good results in transplantation trials. The discovery of synthetic small molecule immunosuppressive agents with minimal side effects is a key objective in immunotherapy, and herein we report an approach involving inhibition of the Janus tyrosine kinase Jak3.[3,4]

Cytokines are involved in the growth, differentiation and function of many cell types. They bind to cell surface receptors triggering intracellular signalling pathways, and this results in the activation of the Janus kinase family of tyrosine kinases. Jak3 is a recently discovered[4] member of this family, which also includes Jak1, Jak2 and Tyk2. Whilst other family members are expressed relatively ubiquitously, Jak3 is mainly found[4] in lymphoid cells, and appears only to be stimulated by activation of cytokine receptors containing the χc subunit (IL-2R, IL-4R, IL-7R, IL-11R, IL-13R), but not by T cell receptor activation. Therefore it is predicted that Jak3 inhibition should lead to immunosuppression by blocking the T cell mitogenic signal mediated by the cytokine IL-2. This view is well supported: the clinical efficiency of IL-2Rα antibody in transplant rejection,[5] the lack of response to IL-2 in patients with deficiencies in Jak3,[6] and reports[6] that Jak3 knockout mice are immunodeficient. Thus Jak3 inhibition represents an attractive and novel means of achieving immunosuppression.

There are few known structural classes of small molecule Jak3 inhibitors, with two of these being non-selective inhibitors. Thus WHI-P131 **1**,[7] is within a known[8] family of EGF-R tyrosine kinase inhibitors, and AG 490 **2**, has been reported both as a Jak2[9] and a Jak3 inhibitor.[10] Weak Jak3 inhibition (pIC_{50} 4.8 ± 0.3) was detected[11] for **2**, and it was used as our standard inhibitor. Indolone inhibitors **3**, were recently described.[12]

As part of a search for novel, selective inhibitors of Jak3, >200,000 compounds from the Alderley Park collection were screened[11] for Jak3 inhibition in an ELISA assay at test compound concentrations of 10 μM. Amongst the confirmed active inhibitors found (criteria for activity was inhibition better than $pIC_{50} = 4.5$) was a series of 2-aminoethylketones (AEKs **4**–**11**, Table 1).

Chemistry

Compounds **4**–**11** (Table 1), and **13**, **14**, **21** (Table 2) were prepared[13] by conventional Mannich reactions of the corresponding aryl ketones with amine hydrochlorides and formaldehyde. The oxime **15** was synthesized by reaction of hydroxylamine with **4**. 2-Acetyl naphthalene was dimethylated with MeI in the presence of KHMDS to give the corresponding isopropyl ketone, which was transformed to **16** in a Mannich reaction with morpholine (Scheme 1). A known[14] aldehyde **17**, was converted to **18** by reductive amination (Scheme 2).

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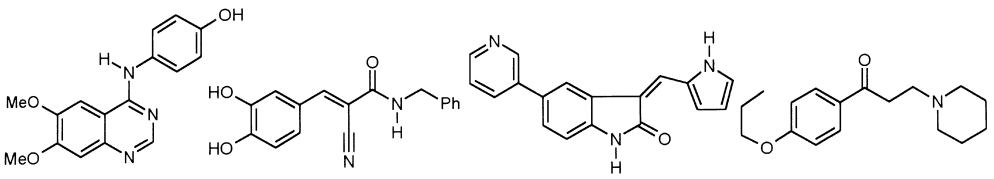
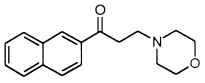
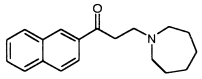
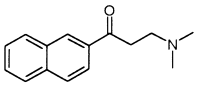
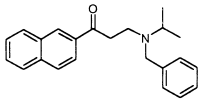
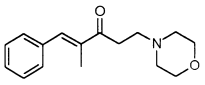
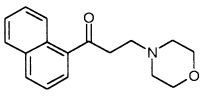
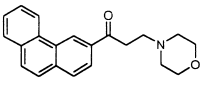
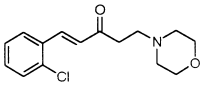


1 2 3 12

Table 1. 2-Aminoethylketone inhibitors of Jak3 kinase

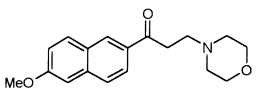
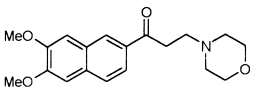
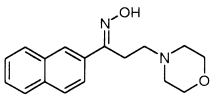
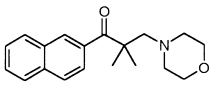
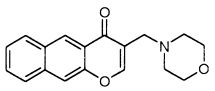
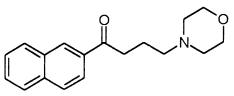
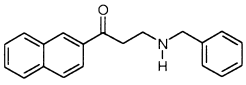
Compound	Structure	Formula	Mp (°C)	pIC_{50} (±0.3, $n=2$)
4		$C_{17}H_{19}NO_2 \cdot HCl$	190–3	5.7
5		$C_{19}H_{23}NO \cdot HCl$	156–8	6.1
6		$C_{15}H_{17}NO \cdot HCl$	155–7	6.2
7		$C_{23}H_{25}NO \cdot HCl$	145–8	7.1
8		$C_{16}H_{21}NO_2 \cdot HCl$	166–8	4.9
9		$C_{17}H_{19}NO_2 \cdot HCl$	160–3	5.5
10		$C_{21}H_{21}NO_2 \cdot HCl$	212–5	6.3
11		$C_{15}H_{18}ClNO_2 \cdot HCl$	172–4	4.9
2	AG 490	—	—	4.9

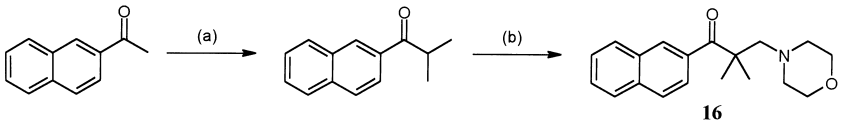
Reaction of the morpholine amide **19** with pyrrolidine and subsequent reduction with $LiAlH_4$ afforded **20** after acidic hydrolysis (Scheme 3). The vinyl ketone **22** (Table 3) was obtained by filtration of **4** through a silica gel column in 1% $MeOH/CH_2Cl_2$, and the corresponding alcohol **23** by reaction of vinylmagnesium bromide with 2-naphthaldehyde.

Results and Discussion

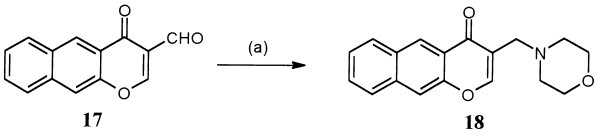
The results in Table 1 indicated that the 2-naphthyl derivatives **4**–**7**, were particularly potent Jak3 inhibitors, whereas compounds **8** and **11**, which contain only part of the naphthyl ring, were significantly less potent inhibitors. Variation of the amino-group alkyl substituents showed that compound **7** with the most lipophilic *N*-benzyl-*N*-isopropyl substitution gave superior Jak3 inhibition. Despite the clinical use[13] of some AEKs (e.g. falicain **12**, an anaesthetic), there are publications[15–17] reporting that these compounds could breakdown, by either retro-Michael or retro-Mannich reactions. Detailed studies[17] indicate that fragmentation via retro-Michael reaction is the significant breakdown pathway, so the synthesis of potentially more stable AEK analogues was carried out (Table 2). Introduction of a methoxy group **13**, in order to increase stability, resulted in a considerable fall in inhibitory potency. Moreover significant inhibitory activity was lost when an additional stabilizing methoxy group **14**, was incorporated.

Table 2. Jak3 kinase inhibition of more stable 2-aminoethylketones

Compound	Structure	Formula	Mp (°C)	pIC_{50} (±0.3, $n=2$)
13		$C_{18}H_{12}NO_3 \cdot HCl$	198–201	4.8
14		$C_{19}H_{23}NO_4 \cdot HCl$	200–3	3.7
15		$C_{17}H_{29}N_2O_2 \cdot HCl$	139–41	< 3.0
16		$C_{19}H_{23}NO_2 \cdot HCl$	—	< 3.0
18		$C_{18}H_{17}NO_3 \cdot HCl$	130–3	< 3.0
20		$C_{18}H_{21}NO_2$	77–80	< 3.0
21		$C_{20}H_{19}NO \cdot HCl$	175–8	< 3.0



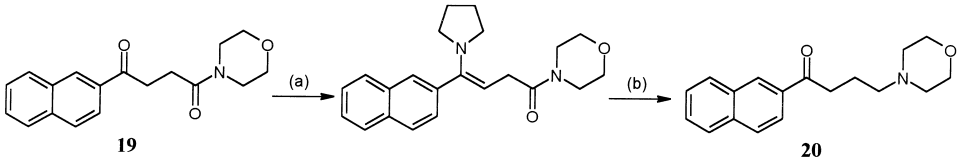
Scheme 1. Reagents and conditions: (a) KHMDS (2 equiv), MeI (2 equiv); (b) HCHO, morpholine HCl.



Scheme 2. Reagents and conditions: (a) morpholine, $NaBH(OAc)_3$.

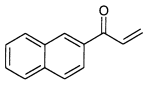
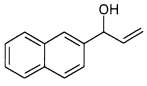
It is known[17] that the ketoxime derivatives of AEKs are more stable than the corresponding ketones, and with the oxime **15**, inhibitory activity was also lost. Placing substituents at the α-position of the ethyl group adjacent to the carbonyl group (as in **16**, **18**) in an attempt to avoid retro-Michael elimination of the amino function, also gave compounds without useful Jak3 inhibitory activity. It is possible that the structural changes employed (Table 2) to increase compound stability had also resulted in a poor fit at the Jak3 enzyme inhibitory site, and had thereby accounted for the much reduced inhibitory potency. However, due to the diversity of the structural variation of the AEKs in Table 2, it was postulated that the inhibitory potency of the compounds in Table 1 was attributable to decomposition during the ELISA assay for Jak3 inhibition. Examination of compound **7**, at a concentration of 20 μM in a similar phosphate buffer (pH 7.43) to that used in the Jak3 ELISA assay, indicated that the half life for decomposition was 36 min, at 25 °C. The only breakdown product was identical by HPLC (Hi-RPB 250×2.1 mm i.d., 40% MeCN/0.2% HCO_2H, 0.5 mL/min, UV detection at 244 nm) to a synthetic sample of vinyl ketone **22** (Table 3), the putative retro-Michael breakdown product.

The structure–activity relationship for the compounds in Table 1 indicated the need for a lipophilic aryl ring system, substituted with a carbonyl group, which itself has a β-aminoethyl substituent. Results in Table 2 showed that if the carbon atom where the retro-Michael reaction would be triggered had its hydrogen substituents replaced by carbons, inhibitory activity was lost. In addition, when the compounds were more stable, inhibitory potency declined. The breakdown product **22** (Table 3) gave similar inhibitory potency to



Scheme 3. Reagents and conditions: (a) pyrrolidine, PhMe, *p*-toluenesulfonic acid; (b) $LiAlH_4/Et_2O$, HCl.

Table 3. Jak3 inhibition of vinyl derivatives

Compound	Structure	Formula	Mp (°C)	pIC_{50} (± 0.3, $n=2$)
22		$C_{13}H_{10}O$	68–70	7.1
23		$C_{13}H_{12}O$	Oil	3.3

the corresponding AEK **7**, and the analogous alcohol **23** (where carbonyl group activation of the vinyl group was removed) had considerably less inhibitory potency. Thus **7** and related structures were acting as prodrugs.

Phenyl-substituted (as opposed to naphthyl-substituted) AEKs have been reported[18] as potent inhibitors of the tyrosine kinase EGF-R, but **7**, and **22**, were only very weak inhibitors of EGF-R compared to Jak3 (Table 4). Inhibitory activity against the Janus kinase family member Jak1 (pIC_{50} **7** = 4.3; **22** = 4.7), was also weak, and there was insignificant inhibition of the tyrosine kinases Lck and CDK4 ($pIC_{50} < 5.0$). Thus high selectivity has been shown against other tyrosine kinases, but this statement is tempered by knowledge that there are many such kinases involved in cell signalling pathways.

Although the naphthyl ketone **22**, was shown to be a potent inhibitor of Jak3 kinase, it may not be suitable for clinical development because of the known[19] mutagenicity of the vinyl ketone chemical class. The new Jak3 inhibitor **22**, gave competitive binding at the Jak3 ATP site, inhibition of STAT-5 phosphorylation and inhibition of T-cell proliferation.[20]

In summary, **7** decomposed in neutral buffer to afford potent inhibition of the Janus kinase 3, and could be used as a standard Jak3 inhibitor in assays where breakdown could occur. The retro-Michael breakdown product of **7**, i.e. 2-naphthylvinyl ketone **22**, will also be a useful pharmacological tool for further investigation of the role of the Janus tyrosine kinase Jak3.

Table 4. Selectivity comparisons ($pIC_{50} \pm 0.3$, $n=2$) of the Jak3 inhibitors **7** and **22**

Compound	Jak3 pIC_{50}	EGF-R pIC_{50}	Jak1 pIC_{50}
7	7.1	5.6	4.4
22	6.8	5.0	4.7

Acknowledgements

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References and Notes

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11. Jak3 inhibition assay: plates (Maxisorb flat bottom) were coated overnight at 4 °C with Poly (Glu, Ala, Tyr) 6:3:1 (−20 μg/mL) and stored at −20 °C. The plates were washed twice with phosphate buffered saline 0.05% Tween (PBST), and blotted dry. ATP: $MgCl_2$ soln (25 μL) was added to the plate to give final concentrations of 2 μM ATP, 5 mM $MgCl_2$, and 10% DMSO (25 μL) or test compound was added. The reaction was started with Jak3 enzyme 1:1000 dilution (50 μL), and the plates incubated for 60 min at 30 °C. The plate was washed three times with PBST and blotted dry. HRP-Conjugated 4G10 antibody (100 μL) was added and the plate incubated for 90 min at 30 °C. The plate was washed three times with PBST and blotted dry, before TMB soln (100 μL) was added and further incubated for 30 min. 1M H2SO4 (100 μL) was added to stop the reaction, and the plate was read at 450 nm. The optical densities obtained were analysed using the Origin 5.0 computer programme, and from the resulting graphs IC_{50} values were read. These values were converted to nM concentrations, and the pIC_{50} derived.
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