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Nicotinanilides as Inhibitors of Neutrophil Chemotaxis

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Abstract—A series of 4-fluoronicotinanilides was synthesized and shown to be novel, potent, and selective inhibitors against GRO-α-driven human neutrophil chemotaxis. Compounds of this class may be useful for the treatment of inflammatory, autoimmune, and allergic disorders.

Activated neutrophils (polymorphonuclear neutrophils: PMN) have been implicated in the pathogenesis of many inflammatory conditions.[1] Neutrophils are activated and attracted by interleukin-8 (IL-8) and other Glu-Leu-Arg (ELR) containing CXC chemokines, including epithelial cell-derived neutrophil-activating peptide-78 (ENA-78), and growth-related oncogene (GRO)-α, GRO-β, and GRO-γ. In response to traumatic injury or infection, human neutrophils are directed to the site of injury or infection by CXC chemokines that signal via two distinct seven-transmembrane G-protein coupled receptors (GPCRs), CXCR1 and CXCR2. Both CXCR1 and CXCR2 bind IL-8 with high affinity; however, only CXCR2 binds the other neutrophil-activating and ELR motif-bearing chemokines with high affinity. Leukocyte recruitment to these inflammatory sites is multi-faceted involving chemoattractants ranging from formyl-methionyl-leucyl-phenylalanine (FMLP), platelet-activating factor (PAF), leukotriene B_4 (LTB_4) and C5a anaphylotoxin.

Several nonpeptide small molecules have recently been reported to inhibit neutrophil chemotaxis (Fig. 1). Included is SB-265601 (**1**), a compound that has demonstrated potent inhibition of CXCR2 ligand binding and neutrophil chemotaxis ($IC_{50} = 10$ nM) in vitro and was efficacious in vivo in an LPS-induced rabbit model of neutrophilia.[2] PD 0220245 (**2**) was recently claimed as a selective antagonist of IL-8 binding ($IC_{50} = 110$ nM) and potent inhibitor of neutrophil chemotaxis ($IC_{50} = 170$ nM).[3] We recently disclosed that

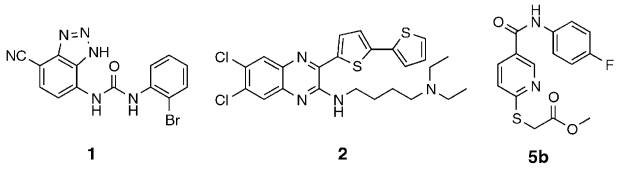

Figure 1. Inhibitors of neutrophil chemotaxis.

a class of nicotinanilide *N*-oxides containing heteroaryl 4-fluoroanilides were antagonists of the CXCR2 receptor and inhibited neutrophil chemotaxis.[4] Further work in this area revealed that the related class of compounds, 6-substituted nicotinanilides, are also potent and selective inhibitors of GRO-α driven human neutrophil chemotaxis. Here we report these results, including the design and synthesis of **5b**, a functionally active and selective inhibitor of chemotaxis ($IC_{50} = 42$ nM).

Two routes were explored toward the synthesis of 6-alkylsulfanyl-nicotinanilides (Scheme 1). In route A, 6-mercaptonicotinic acid **3** was first converted to the corresponding nicotinanilide **4** then *S*-alkylated to provide thioether **5a** in good yield. Because of the limited commercial availability of diverse haloacetates, a second synthetic strategy was developed (route B). Commercially available 6-chloronicotinoyl chloride **6** was converted to anilide **7**.[4] Treatment of **7** with various thioalkoxides led to the thioether analogues **5b–g**. Compound **5h** was readily obtained from a mild saponification of **5b** following neutralization.

Scheme 2 outlines the chemical routes used to prepare the inhibitors shown in Table 2. Treatment of the 6-bromonicotinanilide[5] **8** in the presence of excess of

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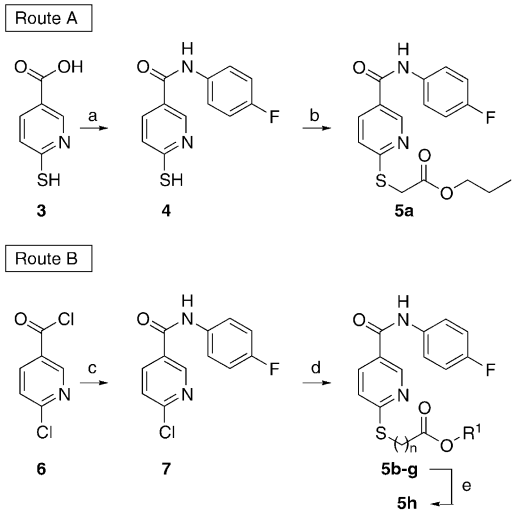
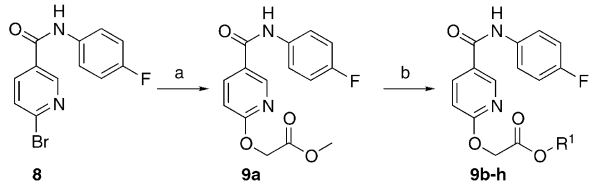
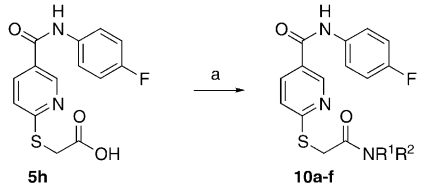


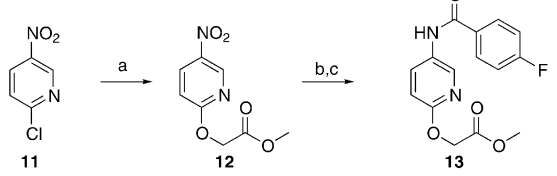
Scheme 1. Reactions and conditions: (a) 4-$FC_6H_4NH_2$, EEDQ, DMF, rt, 90%; (b) propyl bromoacetate, Cs_2CO_3, DMF, rt, 76%; (c) 4-$FC_6H_4NH_2$, *i*-Pr_2NEt, CH_2Cl_2, 0 °C to rt, 92%; (d) $HS(CH_2)_nCO_2R^1$, *t*-BuOK, THF, rt; (e) **5b**, NaOH THF-H_2O, 86%.



Scheme 2. Reactions and conditions: (a) methyl glycolate, *t*-BuOK, THF, 65%; (b) R^1OH, H_2SO_4 (cat.), 80 °C.



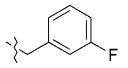
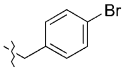
Scheme 3. Reactions and conditions: (a) R^1R^2NH, PyBrOP, DMF, <35%.



Scheme 4. Reactions and conditions: (a) methyl glycolate, *t*-BuOK, THF, 20%; (b) 10% Pd/C, H_2, MeOH; (c) 4-FC_6H_4COCl, *i*-Pr_2NEt, CH_2Cl_2, 55% from **12**.

methyl glycolate with potassium *tert*-butoxide provided the methyl ester **9a**. The less reactive 6-chloro-*N*-(4-fluoro-phenyl)-nicotinamide could also be converted to **9a** under similar conditions but in lower yield. Transesterification of methyl ester **9a** into esters **9b–c** was readily accomplished by heating **9a** with the appropriate alcohol neat in a sealed tube in the presence of a catalytic amount of sulfuric acid or triethylamine. Removal of the excess low molecular weight alcohol was easily accomplished by exposing the crude reaction mixture to reduced pressure. Alternatively, **9a** could be heated to reflux in toluene under acidic conditions with a small excess of the higher molecular weight alcohols to provide analogues **9d–h** after purification by flash chromatography or trituration.

Table 1. Representative activities of nicotinanilides

Compd	*n*	R^1	GRO-α Chemotaxis IC_{50} (μM)[a]	FMLP Chemotaxis IC_{50} (μM)[a]
5a	**1**		0.046	>40
5b	**1**		0.042	nt[b]
5c	**1**		0.043	nt
5d	**1**		0.083	nt
5e	**2**		0.76	>40
5f	**3**	H	5.1	nt
5g	**2**	H	>20	nt
5h	**1**	H	>20	nt

[a]Values were calculated from the geometric mean of at least two experiments.
[b]Not tested.

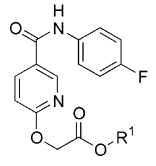
Scheme 3 outlines the route used to prepare 6-substituted-carbamoylmethylsulfanyl-*N*-(4-fluorophenyl)-nicotinanilides. Treatment of the acid **5h** with PyBrOP in the presence of excess amine provided the desired amides **10a–f**. Efforts to improve the chemical yields of this class were limited due to their lower potencies.

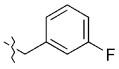
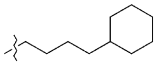
It was of interest to design a synthetic route toward nicotinanilide analogues where SAR diversity could be explored around the 4-fluoroanilide moiety. Scheme 4 describes a route toward the synthesis of analogues where the amide functionality at the 3 position of the nicotinanilide core was reversed. 2-Chloro-5-nitro-pyridine **11** was converted to **12** by treatment with the potassium alkoxide of methyl glycolate. Reduction of the nitro functionality followed by acylation with 4-fluorobenzoyl chloride readily provided **13**.

As part of a lead optimization strategy, nicotinanilides were screened for both their ability to block GRO-α and FMLP driven human neutrophil chemotaxis.[6]

SAR studies initially focused on changes around the 6-position of the nicotinanilide core (Table 1). It was

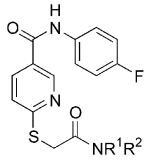
Table 2. Representative activities of nicotinanilides

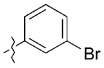


Compd	R^1	GRO-α Chemotaxis IC_{50} (μM)[a]	FMLP Chemotaxis IC_{50} (μM)[a]
9a		0.11	>40
9b		0.16	>40
9c		0.20	>40
9d		0.22	>40
9e		0.41	nt[b]
9f		0.094	>40
9g		0.050	>40
9h		0.68	>40

[a]Values were calculated from the geometric mean of at least two experiments.
[b]Not tested.

Table 3. Representative activities of nicotinanilides



Compd	R^1	R^2	GRO-α Chemotaxis IC_{50} (μM)[a]	FMLP Chemotaxis IC_{50} (μM)[a]
10a	H		>20	nt[b]
10b	H		>20	nt
10c	H		1.0	>40
10d			12.5	>40
10e	H		2.7	>40
10f	H		0.22	>40

[a]Values were calculated from the geometric mean of at least two experiments.
[b]Not tested.

found that the most potent analogues in this series incorporated a thioglycolate ester pharmacophore as illustrated by compounds **5a**–**d**. All had potencies <100 nM against GRO-α driven chemotaxis. Substantial structural diversity about the ester could be tolerated while maintaing acceptable biological activity, however, efforts to extend the methylene unit of the thioglycolate moiety significantly reduced the potency as seen by **5e**. Functional inhibitory activity against chemotaxis was also lost upon conversion of the ester to the corresponding acid as seen by **5f**–**h**. Selectivity was assessed in a neutrophil chemotaxis assay using FMLP as an alternate chemotactic agonist. In all cases the compounds were more potent GRO-α antagonists and displayed little or no antagonist activity toward FMLP. The restricted antagonism toward GRO-α infers interference is proximal to CXCR2 since these two receptors extensively share their signal transduction cascade. To further validate the selectivity of this class of compounds for CXCR2, **5b** and **5h** were tested as representative compounds in additional assays. Preliminary tests indicated that **5b** selectively interfered with CXCR2 binding as it had an $IC_{50}=32$ nM against GRO-α induced calcium flux against human neutrophils but was inactive at antagonizing CCR2 and CCR3 in transfected CHO cells.[7] Interestingly, the ester **5b** showed an inability to block ligand binding in a filter mat assay using CXCR2 membranes (data not shown).[8] **5b** and **5h** were subsequently tested for GRO-α-dependent GTP-γ-S exchange with CXCR2 membranes.[9] The carboxylate **5h** showed selective antagonism of GRO-α mediated exchange ($IC_{50}=614$ nm vs inactive against CCR1 and CCR9) while ester **5b** was inactive. Lastly, based on this observation, the free acid **5h** was reevaluated and determined to have an $IC_{50}=1.2$ μM in a CXCR2 filter mat binding assay.[8] A possible explanation for these results is that the nicotinanilide esters are membrane permeable and able to pass into the interior of neutrophils. The esters are then hydrolyzed by endogenous esterases to liberate the correspondingly less permeable carboxylate salt, which accumulates intracellularly and interferes with signaling of the CXCR2 receptor. Additional studies to validate the cellular targets of these compounds are ongoing.

Because of concerns regarding the potential oxidation of the thioether moiety at the 6-position of the nicotinanilide core to the corresponding sulfoxide and sulfone, oxygen-based analogues were investigated (Table 2). As was observed with the thioglycolate-derived analogues, a considerable amount of diversity was allowed about the ester moiety while maintaining submicromolar potencies. In general, the benzyl ester analogues exemplified by **9f** and **9g** displayed the highest functional potencies. This class retained the earlier

selectivity against GRO-α versus FMLP but removed potential oxidative liabilities of the thioglycolate-based analogues.

Potential replacements for the ester functional group were investigated (Table 3). In general, aliphatic amides displayed limited potency against GRO-α driven chemotaxis while the aniline-based derivatives showed modest potencies. Compound **10f** was the only analogue tested that showed activity $<1\,\mu M$. Again, these analogues have a similar selectivity profile as the series previously described. Several explanations of the attenuated overall potencies of this series is possible. The compounds may display a reduced ability to pass through the cell membrane or have increased enzymatic stability toward hydrolases in the cell which could result in lower intracellular concentrations of **5h**. Alternatively, this series may simply have a reduced affinity for the CXCR2 receptor.

Lastly, a limited amount of SAR was explored where the amide functionality at the 3 position of the nicotinanilide core was reversed (Scheme 4). Compound **13**, representative of this class of antagonists, showed an $IC_{50}=360\,nM$ against GRO-α driven neutrophil chemotaxis. Since significant potency and selectivity of this series has been retained it is expected that additional changes to this portion of the molecule may be tolerated.

In conclusion, the synthesis and evaluation of a novel series of potent and selective GRO-α driven neutrophil chemotaxis inhibitors have been described. Initial data indicates that this class of compounds may function as ester prodrugs by which they penetrate the cell and are then converted to the bioactive species via enzymatic hydrolysis to the corresponding carboxylate salt.[10] Additional studies are planned to further explore the cellular signaling pathway by which these compounds function.

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