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Synthesis and structure–activity relationships of 3,5-diarylisoxazoles and 3,5-diaryl-1,2,4-oxadiazoles, novel classes of small molecule interleukin-8 (IL-8) receptor antagonists

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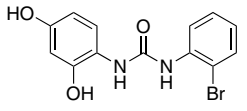
Abstract—A novel series of 3,5-diarylisoxazole and 3,5-diaryl-1,2,4-oxadiazole IL-8 antagonists has been identified. These compounds exhibit activity in an IL-8 binding assay as well as in a functional assay of IL-8 induced elastase release from neutrophils. In addition, one of the compounds exhibits oral activity in a rat adjuvant arthritis model.

Interleukin-8 (IL-8), a 72-amino acid peptide, is a member of the C–X–C family of chemokines. It is produced primarily by monocytes and macrophages.[1] Its production can be triggered by inflammatory stimuli such as IL-1, TNF-α, LPS, and IL-4.[2] IL-8 is a potent chemotactic agent for neutrophils, and can stimulate neutrophil degranulation and induce basophil histamine release.[1] Neutrophils are prime players in the inflammatory response and are responsible for extensive tissue damage due to the release of lysosomal enzymes such as elastase, myeloperoxidase, cathepsins, etc. High levels of IL-8 have been detected in sites of artherosclerosis and ischemic reperfusion injury, in the synovial fluid and cells from rheumatoid arthritis patients and also in psoriatic plaques.[1] The IL-8 receptor is a seven transmembrane domain G-protein coupled receptor (GPCR) that is found in abundant quantities on neutrophils.[3] Inhibition of the actions of IL-8 on neutrophils should limit their migration to the sites of inflammation, prevent activation, and thus inhibit the subsequent release of lysosomal enzymes. Therefore, IL-8 receptor antagonists may lead to unique anti-inflammatory agents.

Relatively few classes of small molecule IL-8 receptor antagonists have been reported, making the identification of new classes of antagonists an important objective. Widdowson and co-workers described the first potent nonpeptide antagonist of IL-8.[4,5] This compound, a substituted diphenyl urea **1**, was shown to inhibit IL-8 induced neutrophil migration with an $IC_{50} = 22$ nM. Recently, Li et al. has disclosed a series of 2-amino-3-heteroarylquinoxalines **2** that inhibited IL-8 binding with potencies in the range from 0.11 to 33 μM.[6] Baxter et al. reported on a series of orally bioavailable triazolethiol inhibitors, such as compound **3**.[7]



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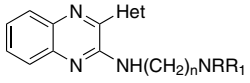

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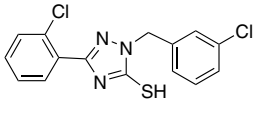

3

We have identified a series of regioisomeric 3,5-diarylisoxazoles **4** and **5** along with a series of 3,5-diaryl-1,2,4-oxadiazoles **6**, which act as IL-8 receptor antagonists.[8,9]

Keywords: IL-8; Inflammation; Diarylisoxazoles.
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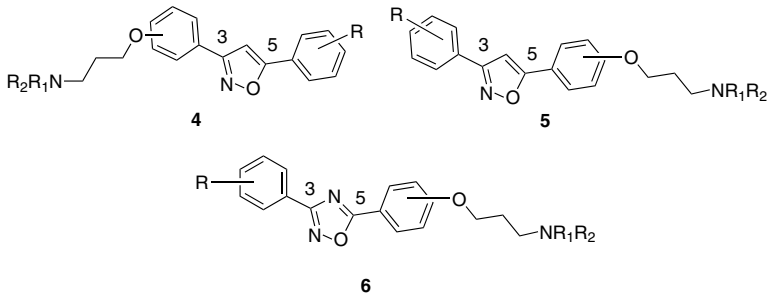


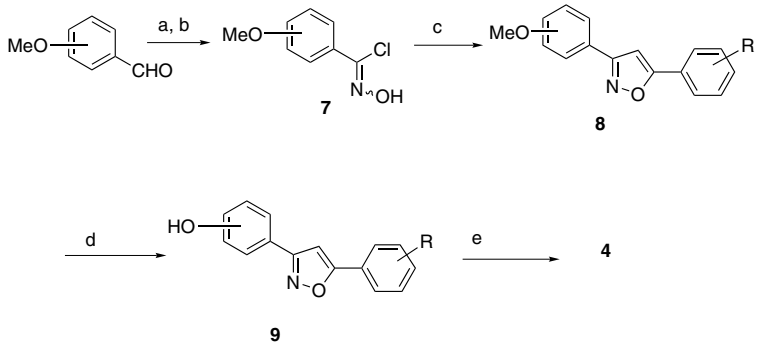
Herein we describe the synthesis and structure–activity relationships of these novel series.

The key reaction for the preparation of the isoxazoles **4** and **5** is a nitrile oxide [3+2] dipolar cycloaddition reaction between an appropriately substituted chloro oxime and a phenyl acetylene derivative. Scheme 1 exemplifies the synthesis of isoxazoles **4**. Anisaldehyde was converted in two steps into the chloro oxime **7** utilizing *N*-chlorosuccinimide as the chlorinating agent.[10] This was reacted with the appropriately substituted phenylacetylene derivative to afford isoxazole **8**. Deprotection of the aryl methyl ether with boron tribromide afforded phenol **9** in good overall yield. Alkylation with the appropriately substituted chloro alkylamine produced isoxazole targets **4**. Isoxazoles **5** were generated in an analogous manner utilizing the appropriately substituted chloro oxime and methoxyphenylacetylene in the cycloaddition reaction.

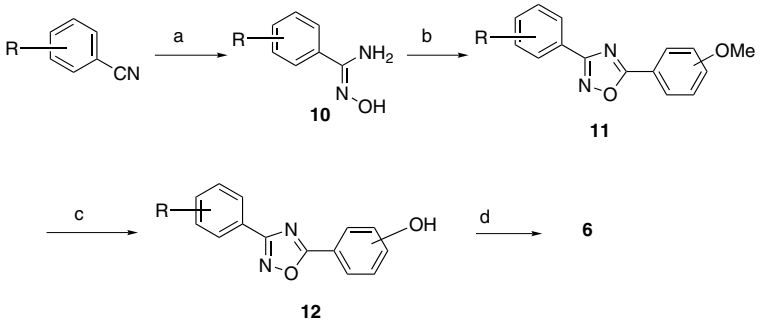
1,2,4-Oxadiazoles **6** were synthesized according to Scheme 2. Amide oxime **10**, prepared from the desired benzonitrile derivative, was condensed with 4-methoxy benzoyl chloride in pyridine to afford oxadiazole nucleus **11**.[11] Phenol **12**, generated by deprotection of **11** with boron tribromide, was alkylated with the appropriately substituted chloro alkyl amine to yield ether **6**.

The compounds were screened in a receptor-binding assay in human neutrophils using ^{125}I-IL-8 as ligand with the results being reported as an IC_{50}.[12]

Early on it was clear that a basic side chain was required for activity since the methoxy intermediates **8** and **11** and phenol intermediates **9** and **12** were inactive in the assay. In addition, several analogs of **4** in which the amine was replaced with a carboxylic acid were inactive (data not shown). The 5-aryl group of the isoxazole **4** was essential for activity since replacement with either a

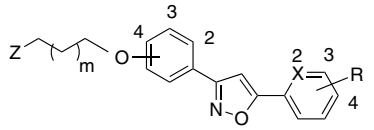


Scheme 1. Reagents and conditions: (a) hydroxylamine hydrochloride, pyridine, methylene chloride, 95%; (b) *N*-chlorosuccinimide, DMF, 0 °C, 93%; (c) phenylacetylene, triethylamine, ethyl acetate, 80–90%; (d) BBr_3, methylene chloride, 0 °C, 75–90%; (e) chloroalkyl amine, potassium carbonate, DMF, 90 °C, 60–85%.

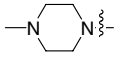
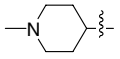
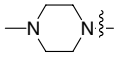
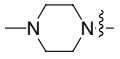
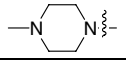


Scheme 2. Reagents and conditions: (a) aq hydroxylamine, ethanol, reflux, 78–89%; (b) methoxy benzoyl chloride, pyridine, reflux, 70–85%; (c) BBr_3, methylene chloride, 0 °C, 75–90%; (e) chloroalkyl amine, potassium carbonate, DMF, 90 °C, 60–85%.

Table 1. IL-8 receptor antagonist activity of isoxazoles **4**



4

Compound	Z	*m*	Position	X	R	IC_{50} (μM)
4a	NMe_2	1	4	CH	H	10.6
4b	NMe_2	1	4	CH	4-Cl	2.7
4c	NMe_2	1	4	CH	4-F	3.1
4d	NMe_2	1	4	CH	4-NO_2	12.1
4e	NMe_2	1	3	CH	4-Cl	8.1
4f	NMe_2	1	2	CH	4-Cl	4.4
4g		1	4	CH	H	5.4
4h		1	4	CH	H	6.2
4i		1	4	CH	H	7.6
4j		1	4	CH	4-Cl	1.6
4k		2	4	CH	4-Cl	8.9
4l		1	4	N	H	11.5

benzyl or *n*-butyl group led to complete loss of activity. In addition, replacement of the ether linkage of isoxazole **4** or oxadiazole **6** with an amide gave compounds 2–5-fold less potent (data not shown).

The relationship between substitution of the 5-phenyl ring of **4** and binding activity was investigated by varying the dipolarophile utilized in the cycloaddition reaction. From the data in Table 1, a variety of functional groups were tolerated, though halogens seem to be optimal with **4b** and **4c** being more potent than **4a** and **4d**.

The effect of regiochemistry of the aminoalkoxy chain on activity was examined. The *ortho* and *para* substituted analogs, **4f** and **4b**, respectively, were slightly more potent than *meta* analog **4e**.

The aminoalkoxy side chain was modified in two ways: by varying the nature of the amine and by changing the length of the chain. Comparison of amines **4a**,**g**,**h**, and **4i** indicates that there is not a significant difference in activity as the amine is varied; therefore, we decided to utilize the *N*-methylpiperazinyl group to flesh out the structure–activity relationships for this series of isoxazoles. From the limited number of chain lengths examined, the three-carbon length **4j** was approximately 5-fold more potent than the four-carbon chain **4k**. The two-carbon analog with a dimethylamino group was less potent than the three carbon analog **4a** (data not shown) possibly due to the lower pK_a of the dimethylamino group of the former. In addition, the 5-(4-chlorophenyl) group was replaced with a 2-pyridyl ring leading to a 10-fold reduction in potency (**4j** vs **4l**).

The structure–activity relationships of the regioisomeric isoxazoles **5** were also determined. As is evident from the data in Table 2, the *N*-methylpiperazinyl group is slightly better than the dimethylamino group (**5a** vs **5b** and **5c** vs **5d**).

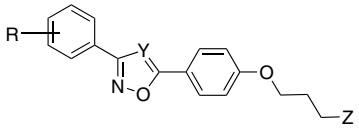
A wide variety of substituents on the 3-phenyl ring were investigated since numerous benzaldehyde derivatives were commercially available. The 4-phenoxy compound **5g** as well as the 3-chloro analog **5e** showed decreased potency. The 4-chloro analog **5d** and the 4-fluoro analog **5f** are equipotent, similar to the trend found with isoxazoles **4**.

With respect to the central heteroaryl nucleus, generally, isoxazoles **5** were equipotent to isoxazoles **4**. The 1,2,4-oxadiazoles **6** exhibit the same trends as isoxazoles **5** (some data not shown for the oxadiazoles), except that now there is a difference in activity between the 4-fluoro (**6a**) and 4-chloro (**6b**) analogs with the 4-chloro analog **6b** being more potent. Replacement of the *N*-methylpiperazinyl group with a morpholine (**6c**) resulted in a complete loss of activity, most likely due to the reduced basicity of the ring nitrogen.

Several of the more potent compounds were tested in a functional assay to determine the inhibition of IL-8 induced elastase release from neutrophils[13] (Table 3). These compounds displayed inhibitory activity in the functional assay with similar potency as in the IL-8 receptor binding assay.

Compound **5f** was tested in vivo in a rat adjuvant arthritis model.[14] In this assay, *Mycobacteria butyricum*

Table 2. IL-8 receptor antagonist activity of isoxazoles **5** and oxadiazoles **6**



5, 6

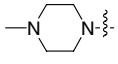
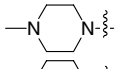
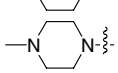
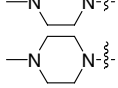
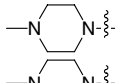
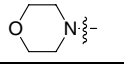
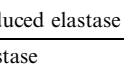
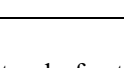
Compound	R	Z	Y	IC_{50} (μM)
5a	H	NMe_2	CH	6.7
5b	H		CH	3.0
5c	4-Cl	NMe_2	CH	3.9
5d	4-Cl		CH	2.1
5e	3-Cl		CH	9.6
5f	4-F		CH	2.0
5g	4-PhO		CH	22.7
5h	4-CF_3		CH	6.0
6a	4-F		N	10.5
6b	4-Cl		N	2.5
6c	4-Cl		N	>25

Table 3. Inhibition of IL-8 induced elastase release from neutrophils

Compound	Elastase IC_{50} (μM)	IL-8 IC_{50} (μM)
4c	4.0	3.1
5d	3.6	2.1
5f	3.8	2.0
6b	4.5	2.5

was injected into the footpad of rats. Adjuvant-induced arthritis was allowed to develop for 10 days. At this time, a baseline paw size was determined. Indomethacin was used as a positive control and vehicle as a negative control. Compound **5f** was administered orally once a day from day 10 through day 14. The amount of swelling in the paw on day 14 was compared to that of day 10. A reduction in paw swelling indicates that the compound is exhibiting therapeutic anti-inflammatory effects. Isoxazole **5f** afforded a 30% reduction in paw volume at 10 mg/kg. Minimal effect was seen at lower doses of **5f** (Fig. 1).

We have identified a series of 3,5-diarylisoxazole and 3,5-diaryl-1,2,4-oxadiazole IL-8 receptor antagonists with low micromolar potency. Several of these compounds demonstrate activity in a functional assay involving the inhibition of IL-8 induced release of elastase from neutrophils. Oral activity was observed for compound **5f** in a rat adjuvant arthritis model.

Although the desired nanomolar potency was not achieved with this series of inhibitors, these compounds

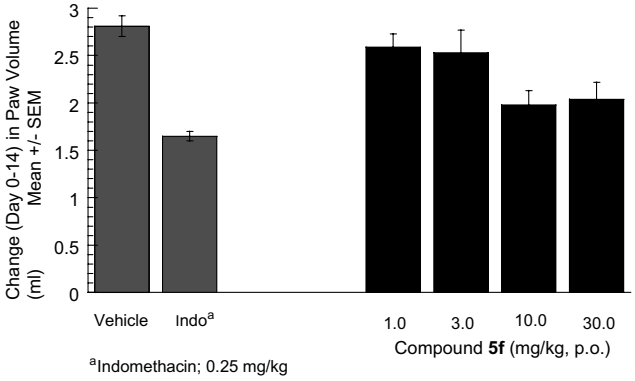


Figure 1. Rat adjuvant arthritis model.

may serve as a starting point for the design of more potent analogs.

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12. IL-8 receptor binding assay: This is a radiolabeled receptor binding assay conducted in human neutrophils which have been purified from fresh human blood by dextran sedimentation. The experiment is conducted by adding reaction buffer to each well, followed by the addition of drug, 20 μL I^{125}-IL-8 (final concentration = 0.125 nM) ligand and cell suspension. After the

plates are incubated at 37 °C for 90 min they are aspirated and counted for 1 min in a Packard TopCount. Drug treated wells are compared to vehicle wells for inhibition of binding. Unlabeled IL-8 serves as the standard antagonist and has an IC_{50} of approximately 1 nM.

13. Elastase assay: Neutrophils are purified from human blood by dextran sedimentation. The procedure is conducted by adding 100 μL IL-8 and 47 μL PBS to each well of a 96-well plate, followed by the addition of 3 μL drug (2.5 mM in 25% methanol) or vehicle. Neutrophils stimulated with cytochalasin B (30 μg/mL in 3% DMSO) are then added and the plate is incubated for 30 min at 37 °C. The plate is then centrifuged and a fraction of the supernatant is transferred to a 96-well black plate to which 50 μL elastase substrate is added. The fluorescence of the wells is read at 0 time and at 90 min to determine the change over time (velocity) in a spectrofluorometer with an excitation @ 360 and emission @ 460. Velocity of the reaction is proportional to the amount of elastase released by IL-8 challenge since the substrate is in molar excess. Inhibition of the reaction by drug treatment is determined by comparing the drug treated wells with those treated with vehicle.
14. Adjuvant-induced Arthritis model: Male Lewis rats are injected in the footpad with 0.1 mL of *Mycobacteria butyricum* suspended in light mineral oil at a concentration of 7.5 mg/mL. Adjuvant-induced arthritis is allowed to develop and on day 10 a baseline paw size reading is made on the uninjected paw with a mercury displacement edema computer. Dosing with test compound begins on day 10 and continues through day 14, with the paws again dipped on day 14 to measure paw swelling. Compound is administered by oral gavage and the effects of test compounds are determined by comparing the paw swelling in drug treated animals with that of vehicle treated controls.