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N-HYDROXYUREA AND HYDROXAMIC ACID INHIBITORS OF CYCLOOXYGENASE AND 5-LIPOXYGENASE

Peter J. Connolly,* Steven K. Wetter, Kimberly N. Beers, Stephanie C. Hamel, Robert H. K. Chen, Michael P. Wachter, Justin Ansell, Monica M. Singer, Michele Steber, David M. Ritchie, and Dennis C. Argentieri

The R. W. Johnson Pharmaceutical Research Institute, 1000 Route 202, Raritan, NJ 08869, U.S.A.

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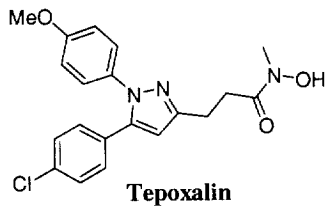
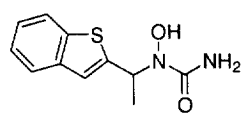
Abstract: Two series of compounds (**1** and **2**) having structural features of the dual COX/5-LO inhibitor tepoxalin and the 5-LO inhibitor ABT-761 were prepared. Many of these hybrid compounds are potent COX and 5-LO inhibitors; two compounds (**1a** and **2t**) inhibit eicosanoid biosynthesis in an ex vivo assay.

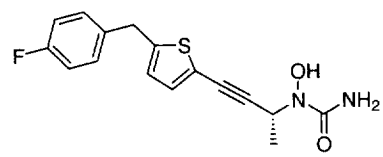
Modern approaches to the treatment of rheumatoid arthritis, asthma, and other inflammatory diseases are based on intervention in the biosynthesis of inflammatory mediators derived from arachidonic acid (AA).[1] For example, nonsteroidal antiinflammatory drugs (NSAIDs) act primarily as inhibitors of cyclooxygenase (COX), the enzyme that catalyzes the first step in the transformation of AA into prostaglandins. Many well known antiarthritic drugs, including aspirin, indomethacin, and naproxen, are COX inhibitors. Recently, the identification of two isoforms of COX, the constitutive COX-1 present in healthy tissue and the inducible COX-2 associated with inflammatory conditions, has resulted in the development of selective COX-2 inhibitors with potentially fewer side effects than traditional NSAIDS.[2] Another important enzyme in the inflammatory process is 5-lipoxygenase (5-LO), the enzyme responsible for the conversion of AA into 5-hydroperoxyeicosatetraenoic acid (5-HPETE), precursor to the leukotrienes. The observation that arachidonoyl hydroxamate[3] inhibits 5-LO led to the discovery of clinically useful selective 5-LO inhibitors[4,5] including the N-hydroxyurea derivatives zileuton[6] and ABT-761.[7]



Tepoxalin



Zileuton



ABT-761

Our interest in improved therapies for the treatment of inflammatory diseases focused on the discovery of agents that inhibit both COX and 5-LO. One result of our work is the dual COX/5-LO inhibitor tepoxalin, a pyrazole-containing hydroxamic acid that has undergone clinical evaluation for psoriasis and RA.[8] In spite of tepoxalin's in vitro efficacy, its in vivo 5-LO inhibitory activity is short-lived due rapid metabolism of the hydroxamate functionality into the corresponding carboxylate.[9] In an effort to find metabolically stable dual

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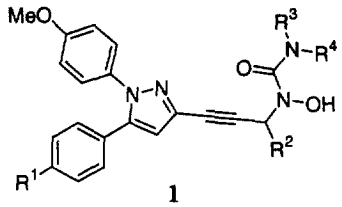
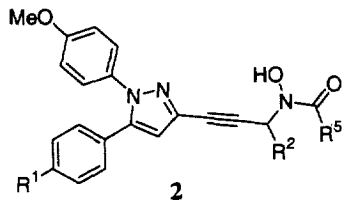
inhibitors, we prepared compounds incorporating structural features of both tepoxalin and the in vivo-active 5-LO inhibitor ABT-761. Toward that end, two series of hybrid compounds having the generic structures **1** and **2** were synthesized and evaluated for their ability to inhibit COX and 5-LO.



1



2

Chemistry

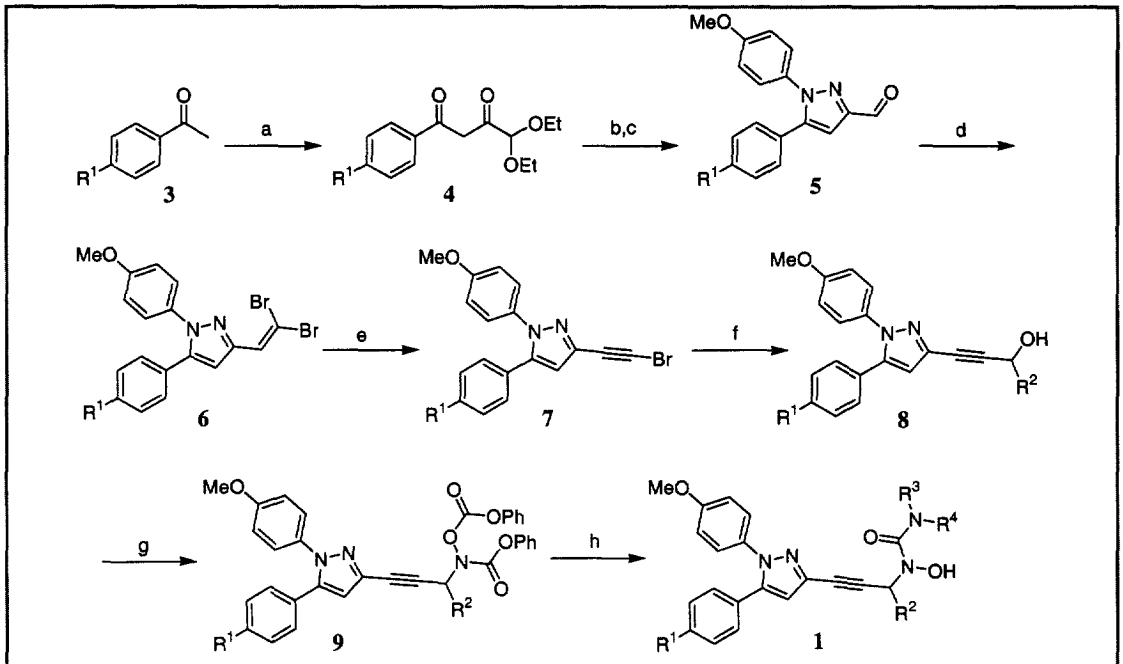
N-Hydroxyureas (**1**) and hydroxamic acids (**2**) were synthesized as shown in the accompanying reaction schemes.[10] The appropriately substituted acetophenone **3** (1 equiv) was treated with lithium hexamethyldisilazide (2 equiv) and ethyl diethoxyacetate (1 equiv) to give diketo acetal **4** (Scheme 1). Pyrazole formation was accomplished by treatment with 4-methoxyphenylhydrazine followed by deprotection with aqueous hydrochloric acid, affording pyrazole-3-carboxaldehyde **5**. Using a modified Corey–Fuchs procedure, **5** was converted first to dibromoethylene **6** with carbon tetrabromide and triphenylphosphine, and then to bromoacetylene **7** with tetra-*n*-butylammonium fluoride.[11] After purification, halogen-metal exchange with *n*-butyllithium or *s*-butyllithium and subsequent reaction with the appropriate aldehyde yielded the acetylenic alcohol **8,** which served as a common intermediate in the synthesis of both **1** and **2**.

N-Hydroxyureas (**1**, Table 1) were synthesized by a literature method involving Mitsunobu coupling of **8** and *N,O*-bis(phenoxycarbonyl)hydroxylamine[12] to give *N,O*-bis-protected *N*-alkyl hydroxylamine **9** (Scheme 1). Conversion to the desired *N*-hydroxyurea **1** was accomplished by stirring **9** in a methanolic solution of the appropriate amine. Hydroxamic acids (**2**, Table 2) were prepared by a modification of the *N*-hydroxyurea procedure (Scheme 2). Mitsunobu coupling of **8** and *N,O*-bis(*t*-butoxycarbonyl)hydroxylamine provided protected *N*-alkyl hydroxylamine **10**, which was deprotected by treatment with trifluoroacetic acid or a saturated solution of HCl in ethyl acetate to yield **11**. When trifluoroacetic acid was used, the trifluoroacetate salt was neutralized to give the free base; when HCl/EtOAc was used, the resulting hydrochloride salt was isolated as a solid and used directly. Treatment of **11** with an excess of the appropriate acid chloride and pyridine provided the *O*-acyl hydroxamic acid **12**, and conversion to the target hydroxamic acid **2** was completed by cleavage of the *O*-acyl group in methanolic NaOH. Alternatively, **11** could be converted directly to **2** by reaction with the acid chloride under Schotten–Bauman conditions.

Results and Discussion

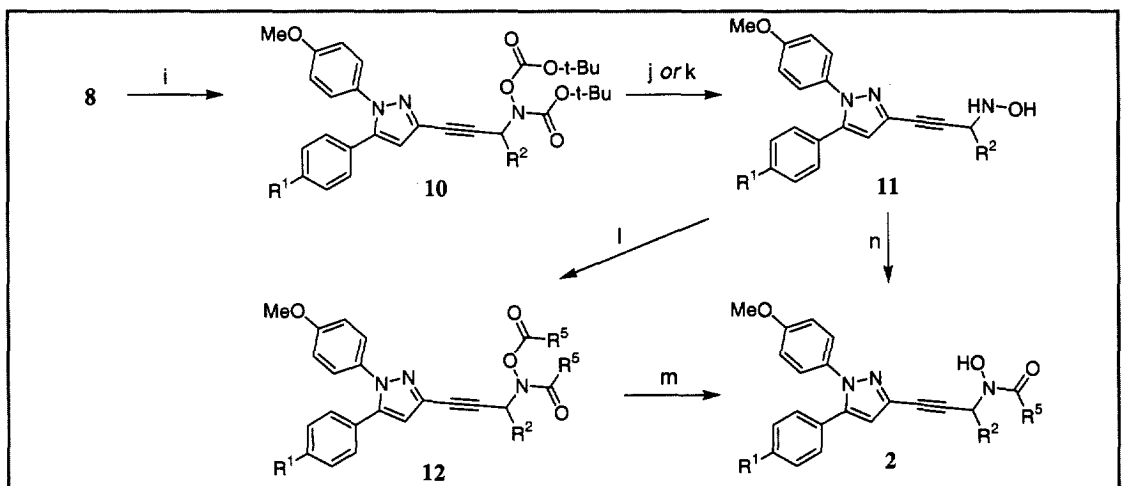
Target compounds were tested in three primary assays for their ability to inhibit the biosynthesis of inflammatory mediators. COX activity was evaluated using sheep seminal vesicle (SSV) enzyme (COX-1).[13]

Scheme 1



(a) 2 equiv $LiN(SiMe_3)_2$, $(EtO)_2CHCO_2Et$, THF, -78 °C; (b) MeOH, 4-$MeOC_6H_4NHNH_2$; (c) aq HCl, THF; (d) Ph_3P, CBr_4, THF; (e) *n*-Bu_4NF, THF; (f) *n*-BuLi or *s*-BuLi, R^2CHO or $(CH_2O)_n$, THF or Et_2O; (g) Ph_3P, $EtO_2CN{=}NCO_2Et$, $PhO_2CNH(OCO_2Ph)$, THF; (h) R^3R^4NH or NH_3, MeOH.

Scheme 2



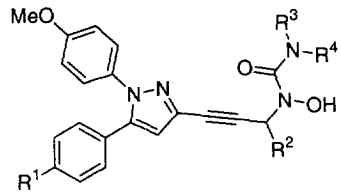
(i) Ph_3P, $EtO_2CN{=}NCO_2Et$, *t*-$BuO_2CNH(OCO_2$-*t*-Bu), THF; (j) F_3CCO_2H, then base; (k) saturated HCl/EtOAc; (l) R^5COCl or $(R^5CO)_2O$, pyridine, CH_2Cl_2; (m) aq NaOH, MeOH; (n) R^5COCl or $(R^5CO)_2O$, aq NaOH, ether.

5-LO activity was measured in both intact and broken rat basophilic leukemia cells (RBL-1) as the ability of test compound to inhibit production of 5-hydroxyeicosatetraenoic acid (5-HETE). While primarily a 5-LO assay, this procedure also provided a readout for COX activity via inhibition of prostaglandin D_2 synthesis.[8]

In general, the N-hydroxyureas (**1**) are potent, selective 5-LO inhibitors in the broken cell RBL-1 assay, with the most potent analog (**1f**) having an IC_{50} value of 0.01 μM (Table 1). The presence of alkyl groups at R^2, R^3, and/or R^4 has no predictable effect on 5-LO potency. In the intact RBL-1 cell assay, however, 5-LO inhibition is consistently weaker, with only one order of magnitude difference between the most potent (**1e**, IC_{50} = 0.9 μM) and least potent (**1c**, IC_{50} = 21.3 μM) derivatives tested. Although SSV-COX inhibition for the series is minimal (with the exception of compound **1a**), several analogs were found to be reasonably potent COX inhibitors in the RBL-1 cell assays.

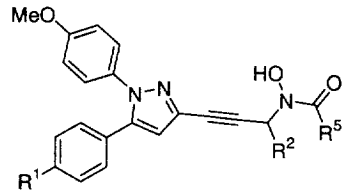
By contrast, the hydroxamic acids (**2**) are much more effective dual COX/5-LO inhibitors in vitro (Table 2). COX inhibition across all three assays is dependent on the nature of the R^5 substituent, with small alkyl groups preferred over aromatic, heterocyclic, or polar substituents. 5-LO inhibition in the broken RBL-1 cell assay is relatively unaffected by the R^5 substituent, as revealed by high 5-LO potency (or activity at 10 μM) for many analogs. As can be observed in the N-hydroxyurea series, 5-LO inhibition is attenuated

Table 1



Cpd	R^1	R^2	R^3	R^4	SSV-COX IC_{50}, μM (% inh @ 10 μM)	Broken RBL-1 Cell IC_{50}, μM (% inh @ 10 μM) COX	5-LO	Intact RBL-1 Cell IC_{50}, μM (% inh @ 10 μM) COX	5-LO
Tepoxalin[8]					4.6	4.2	1.7	2.85	0.15
1a	Me	H	H	H	1.02	0.31	0.07	12.1	4.7
1b	Me	Me	H	H	(10%)	9.4	0.37	1.4	7.7
1c	Me	Et	H	H	(22%)	15.9	0.07	2.1	21.3
1d	Me	*i*-Pr	H	H	(29%)	4.6	0.24	1.2	8.4
1e	Cl	H	H	H	(9%)	(0%)	0.08		0.9
1f	Cl	Me	H	H	32	(0%)	0.01	6.2	3.5
1g	Cl	Me	H	Me	(5%)	(0%)	(79%)		
1h	Cl	Me	H	*n*-Pr	(14%)	(32%)	(82%)		2.8
1i	Cl	Me	$(CH_2)_5$		(44%)	(82%)	(76%)	0.69	8
1j	Cl	Me	$(CH_2)_2O(CH_2)_2$		(14%)	(58%)	(81%)	1.6	12
1k	Cl	Et	H	H	(0%)	(30%)	(91%)	70.4	2.17
1l	Cl	*i*-Pr	H	H	(38%)	(0%)	(90%)	14.5	3.9

Table 2



Cpd	R^1	R^2	R^5	SSV-COX IC_{50}, μM (% inh @ 10 μM)	Broken RBL-1 Cell IC_{50}, μM (% inh @ 10 μM) COX	5-LO	Intact RBL-1 Cell IC_{50}, μM (% inh @ 10 μM) COX	5-LO
2a	Me	H	Me	(74%)	(100%)	(100%)	0.03	7.2
2b	Me	H	Et	(79%)	(100%)	(100%)	0.06	3
2c	Me	H	Ph	(52%)	(83%)	(100%)	0.93	8
2d	Me	H	4-pyridyl	(17%)	(66%)	(89%)	5.5	26.6
2e	Me	H	OMe	(74%)	(100%)	(100%)	0.28	8.8
2f	Me	Me	Me	0.31	0.01	0.08	0.11	13.1
2g	Me	Me	i-Pr	0.39	0.04	0.1	0.09	5.6
2h	Me	Me	$CH_2CH_2CO_2Et$	2.93	(100%)	(97%)	10	10.1
2i	Me	Me	CF_3	0.42	0.05	1.66	0.19	23.5
2j	Me	Me	CO_2Et	(66%)	(50%)	(71%)	3.4	8.5
2k	Me	Me	2-furyl	1.3	(61%)	(70%)	2.9	2.9
2l	Me	Me	3-pyridyl	6	(54%)	(88%)	11.5	25.4
2m	Me	Me	OMe	0.53	0.14	0.28	0.49	7.1
2n	Me	Et	Me	0.43	0.01	0.07	0.22	10.7
2o	Et	Me	Me	2.51	0.06	0.27	0.34	11.9
2p	Et	Me	i-Pr	0.59	(73%)	(93%)	0.18	13.1
2q	Et	Me	CH_2Cl	6.1	(86%)	(100%)	1.7	36.8
2r	Et	Me	$CH_2CH_2CO_2Et$	(3%)	(52%)	(97%)	14	5.1
2s	Et	Me	OMe	(21%)	(72%)	(78%)	0.75	57.4
2t	Cl	Me	Me	2.54	0.03	0.11	0.16	8.1
2u	Cl	Me	Ph	(5%)	14.9	0.92	5.5	1.8
2v	Cl	Me	OMe	1.62	(84%)	(77%)	0.29	8.7

and variable in the intact RBL-1 cell assay, possibly indicating that the compounds are exhibit poor cell membrane permeability.

Two compounds (**1a** and **2t**) were evaluated in a canine blood ex vivo assay for COX and 5-LO inhibitory activity via measurement of thromboxane B_2 (TxB_2) and leukotriene B_4 (LTB_4) synthesis, respectively.[14] As shown in Table 3, N-hydroxyurea **1a** shows strong COX inhibition and reasonable, if short-lived, 5-LO activity. Hydroxamic acid **2t** exhibits similarly robust and longer-acting COX inhibition but weak, short-lasting 5-LO inhibition. Unfortunately, neither candidate proved to be as efficacious as tepoxalin (COX ED_{50} = 0.015 mg/kg, 5-LO ED_{50} = 2.37 mg/kg).[8] From these data, it is apparent that neither **1a** nor **2t** achieved the desired goal of longer duration of 5-LO inhibitory activity.

Table 3

Cpd	Number of animals	dose, mg/kg	Time, h	COX % inh	5-LO % inh
1a	3	5	2	82	53
			4	76	32
			8	46	15
			24	11	10
2t	2	5	2	92	34
			4	75	20
			8	62	31
			24	68	12

In summary, we have demonstrated potent COX and/or 5-LO inhibitory activity in two chemical series based on tepoxalin and ABT-761. Like ABT-761, compounds in the N-hydroxyurea series (**1a–1l**) are primarily 5-LO inhibitors. Conversely, most compounds in the hydroxamic acid series (**2a–2v**) are potent dual inhibitors in the broken RBL-1 cell assay, with activity dropping off significantly in the intact RBL-1 cell assay. Although **1a** and **2t** were found to be orally active dual inhibitors in the canine ex vivo assay, neither improved on the main deficiency of tepoxalin, duration of 5-LO inhibitory activity.

References and Notes

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10. All target compounds (**1** and **2**) and intermediates were purified by recrystallization or MPLC on silica gel and were characterized by ^{1}H NMR, IR, and mass spectroscopy. Analytical purity of targets was determined by combustion analysis (C, H, N).
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13. Tepoxalin is a much more potent inhibitor of COX-1 than COX-2 in intact cells: COX-1 IC_{50} = 0.02 μM (human platelets, n = 16); COX-2 IC_{50} = 6.6 μM (ECV-304 cells, n = 6).
14. Compounds were administered orally and blood samples were drawn at various times after dosing. Following stimulation with calcium ionophore A-23187, the samples were analyzed for the presence of TxB_2 and LTB_4.[8]