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3-Substituted pyrazoles and 4-substituted triazoles as inhibitors of human 15-lipoxygenase-1

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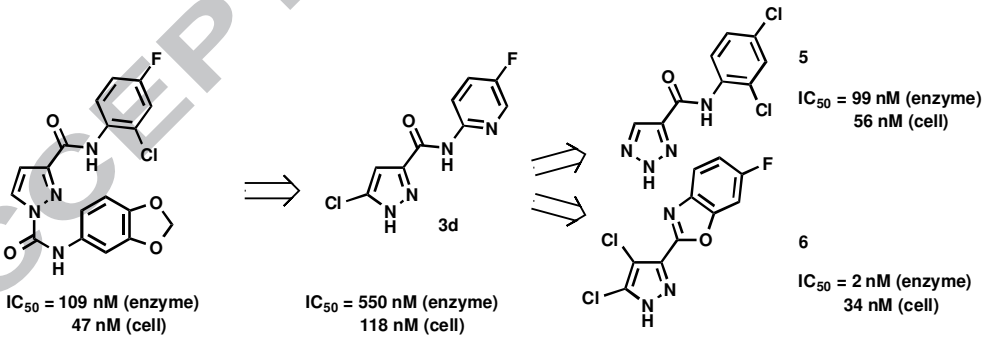
Abstract: Investigation of 1*N*-substituted pyrazole-3-carboxanilides as 15-lipoxygenase-1 (15-LOX-1) inhibitors demonstrated that the 1*N*-substituent was not essential for activity or selectivity. Additional halogen substituents on the pyrazole ring, however, increased activity. Further development led to triazole-4-carboxanilides and 2-(3-pyrazolyl) benzoxazoles, which are potent and selective 15-LOX-1 inhibitors.

Keywords: 15-lipoxygenase, inhibitors, arachidonic acid, pyrazole, triazole, benzoxazole.

Graphical abstract:



Lipoxygenases are a well-studied class of non-heme, iron containing oxidative enzymes that insert molecular oxygen into long-chain unsaturated fatty acids and derivatives thereof having a *cis-cis*-1,4-pentadiene motif. The human 15-lipoxygenase enzyme has its name due to the insertion of oxygen at position 15 of arachidonic acid ((5*Z*,8*Z*,11*Z*,13*E*)- 5,8,11,14-eicosatetraenoic acid) to give 15*(S)*-hydroperoxy-5*Z*,8*Z*,11*Z*,13*E*)- 5,8,11,14-eicosatetraenoic acid (15-HPETE) and

exists in two variants, 15-LOX-1 and 15-LOX-2.[1] Several lines of evidence indicate that inhibition of 15-LOX-1 can be effective in the treatment of respiratory inflammatory diseases, in particular asthma.[2,3]

In the preceding paper in this series we described the discovery and synthesis of 1*N*-substituted pyrazole-3-carboxanilides as potent and selective inhibitors of 15-LOX-1 (Figure 1).[3] Unfortunately, but not unexpectedly, the propensity of the pyrazole acting as a leaving group made compounds with an alkyl or an aryl group linked to the 1-nitrogen of the pyrazole, via a carbonyl or a sulfonyl group, were chemically unstable. Initial studies demonstrated that the intermediates in the syntheses, i.e. pyrazoles lacking 1-substituents (**3**, Scheme 1) where at least as active as the desired products. Due to a complex patentability landscape, they were at that point not further investigated, but when we realized that the chemical stability issue could not be solved, all efforts in the development program were redirected to 1-H-pyrazoles. This paper will describe how the synthetic program evolved into the development of clinical candidates based on pyrazol-3-carboxanilides and triazole-4-carboxanilides.

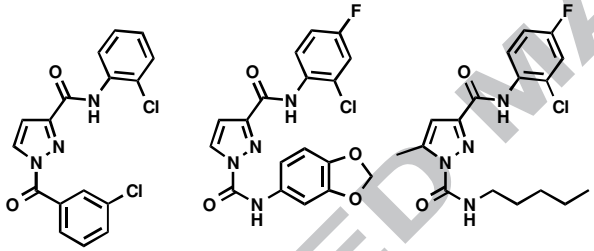
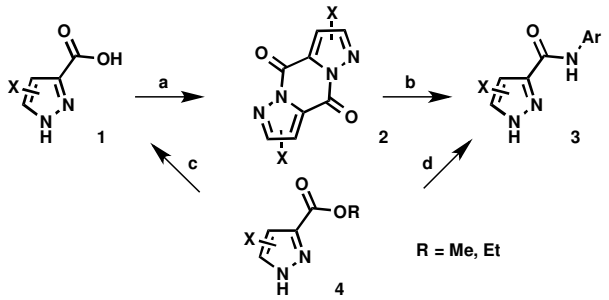


Figure 1. Examples of 1*N*-substituted pyrazole-3-carboxamide 15-LOX-1 inhibitors.

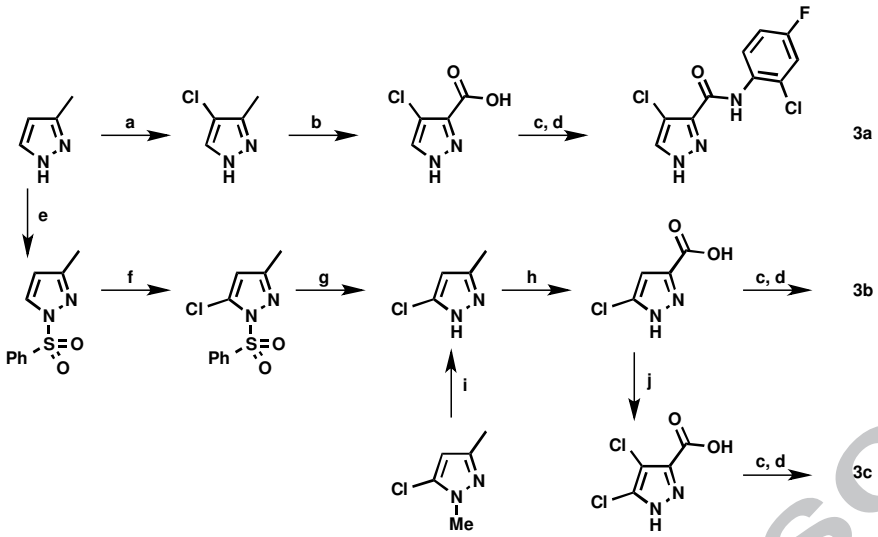
The synthesis of the carboxanilides **3** from pyrazole-3-carboxylic acids **1** is described in the preceding paper[3] and in the patent literature, which also contains experimental details.[4-9] When pyrazole-3-carboxylic acids (**1**) are readily available, the preferred route for the synthesis of **3** is the one depicted in Scheme 1, steps a and b. Simply heating **1** in $SOCl_2$ with a catalytic amount of DMAP (4-dimethylaminopyridine) gives the diketopiperazines **2**. After evaporation of the $SOCl_2$, crude **2** is obtained which is used as such in the following step, where it is treated with the appropriate aniline. This reaction is preferably performed by heating **2** with the aniline in the presence of DMAP in CH_2Cl_2, or neat with an excess of the aniline. Compounds **3** can also be obtained directly from **1** and an aniline using a coupling reagent, but although not optimized, in a lower total yield. In some cases pyrazole carboxylic acids esters (**4**) are more readily obtainable than the acids **1** themselves. Although the esters were readily hydrolyzed, we later developed a more direct route to (**3**) involving a trimethylaluminium-mediated amidation (Scheme 1, step d).[6]



Scheme 1. General route to pyrazole-3-carboxamides. (a) $SOCl_2$ (or the like); (b) $ArNH_2$; (c) NaOH; (d) $ArNH_2/Me_3Al/CH_2Cl_2$, rt, 20 °C.

We wanted to investigate the influence of substituents on the pyrazole ring on the activity, but only a few substituted pyrazole-3-carboxylic acids/esters were commercially available in useful amounts at a reasonable price, e.g. 4- and 5-nitro-pyrazole-3-carboxylic acid. The preparation of 4- and 5-alkyl substituted pyrazole-3-carboxanilides are described in the preceding paper in this issue.[3]

Many substituted 3-methylpyrazoles are readily available, either commercially or by synthesis, and are excellent starting materials for the syntheses of pyrazole-3-carboxylic acids as $KMnO_4$ expediently oxidizes the methyl group to a carboxylic acid in acceptable yields.[10] This approach is used e.g. for the chloro substituted pyrazoles **3a**, **3b** and **3c** (Scheme 2).[6] Chlorination of 3-methylpyrazole using Cl_2 in CCl_4 introduces a chlorine into the 4-position of the pyrazole. A chloro substituent can also be introduced in the same starting material in the 5-position using a lithiation approach. This however requires protection/deprotection steps, and a more convenient method to synthesize 5-chloro-3-methylpyrazole is to *N*-demethylate 5-chloro-1,3-dimethylpyrazole by heating it in pyridinium chloride. Chlorination of 5-chloropyrazole-3-carboxylic acid in the 4-position proceeds in high yield with Cl_2 in water. 4-Bromo and 4-iodo substituted pyrazoles are readily prepared using similar tactics (not shown). As usual, the acids are converted to the corresponding carboxanilides using a $SOCl_2$ mediated dimerization to a diketopiperazine followed by treatment of an appropriately substituted aniline.

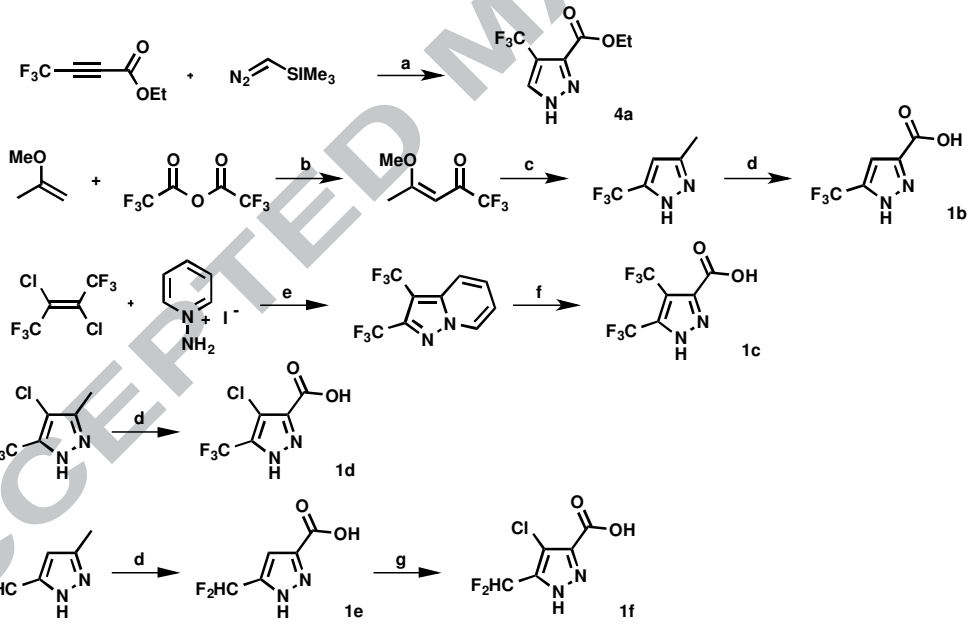


Scheme 2. Reagents and conditions: (a) Cl_2, CCl_4, -78 °C then rt overnight (98 %); (b) $KMnO_4$, H_2O, rt, 3 d, 70 °C, 5 h (22 %); (c) $SOCl_2$, rx, 3 d; (d) 2-chloro-4-fluoroaniline, 120 °C, 1 h (77 % for **3a**, 39% for **3b**, over steps (c) and (d); (e) $PhSO_2Cl/Et_3N$/MeCN, rx, 30 min (86 %); (f) BuLi/THF then Cl_3CCCl_3, -78 °C, 10 min; (g) NaOMe/MeOH, rt, 45 min (21 % over steps (f) and (g); (h) $KMnO_4$, H_2O, *t*-BuOH, 70 °C (64 %); (i) pyridine-HCl, 200 °C, 2 h (67 %); (j) Cl_2, H_2O, rt, 3h, then rt 18 h (86 %)

The synthesis of fluoro substituted pyrazoles pose a real challenge. Fluorine has been introduced in the 4-postion of pyrazole-3-carboxylic acid derivatives using F_2 in anhydrous HF[11] or AcOH[12] but as we did not have the equipment to handle hazardous and corrosive F_2 we had 4-fluoropyrazole-3-carboxylic acid ethyl ester ordered. We did not receive any details of the experimental procedure, but the small amounts of the compound we obtained consisted of a 2:1 mixture of 4-fluoropyrazole-3-carboxylic acid ethyl ester and the unsubstituted starting material, pyrazole-3-carboxylic acid ethyl ester. Hydrolysis, dimerization with $SOCl_2$ and treatment with 2-chloro-4-fluoroaniline as described above, gives the desired carboxanilide (Entry 11, Table 2) in a 10:1 mixture with corresponding 4-unsubstituted compound (Entry 6, Table 1). We were also able to fluorinate pyrazole-3-carboxylic acid ethyl ester in the 4-position by using XeF_2 / CF_3SO_3Ag as the fluorinating agent, but the yield was only 7 %. Attempts to synthesize 5-fluoropyrazoles all failed. Interestingly, there are several suppliers that nowadays catalog 5-fluoropyrazole-3-carboxylic acid ethyl ester, but its synthesis has to our knowledge neither been described in patents nor in the scientific literature.

Routes to trifluoromethyl substituted pyrazole-3-carboxylic acid derivatives are delineated in Scheme 3.[6] 4-Trifluoromethylpyrazole-3-carboxylic acid ethyl ester (**4a**) was prepared by the [2,3]-dipolar cycloaddition of trimethylsilyldiazomethane to ethyl 4,4,4-trifluoro-2-butynate. The reaction is crafted after the analogous reaction

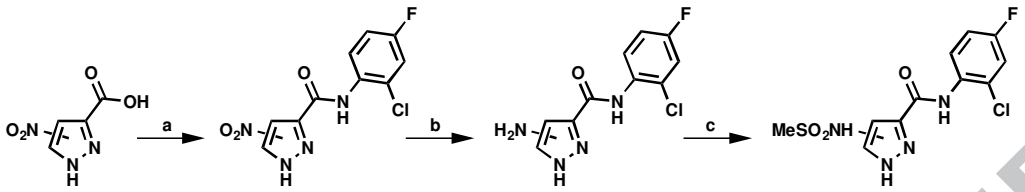
between diazomethane and 4,4,4-trifluoro-2-butynoic acid, which however gives the desired compound (as the methyl ester) together with the corresponding 1*N*-methyl- and 2*N*-methylpyrazoles.[13] Our synthetic route toward the 5-trifluoromethyl analogue begins with the reaction of 2-methoxypropene and trifluoroacetic anhydride. The masked 1,3-diketone formed is heated with hydrazine hydrate in ethanol to give 3-methyl-5-trifluoromethylpyrazole, which as described before, is oxidized to **1b** using $KMnO_4$. The synthesis of 4,5-bis(trifluoromethyl)pyrazoles employs the reaction between 1-aminopyridinium iodide and a cis/trans mixture of 2,3-dichloro-1,1,1,4,4,4-hexafluorobut-2-ene. The pyridine ring of the formed pyrazolo[1,5-*a*]pyridine is oxidatively ruptured with $KMnO_4$ to give **1c**.[14] 4-Chloro-5-trifluoromethylpyrazole-3-carboxylic acid (**1d**) and the two 5-difluoromethyl substituted pyrazoles **1e** and **1f** are obtained using the oxidation and chlorination approach described in Scheme 2. The ester **4a** is converted to the corresponding 4-fluoro-2-chlorocarboxanilide (Entry 16, Table 2) using the trimethylaluminium-mediated amidation mentioned above. For the acids **1b** to **1f**, the standard $SOCl_2$/aniline protocol was used to obtain the corresponding carboxanilides (Entries 17-21, Table 2).



Scheme 3. Reagents and conditions: (a) Et_2O, 0 °C, then rt 2 h (75 %); (b) 1. pyridine, -30 °C; 2. Et_2O, rt, 18 h; (c) $N_2H_4xH_2O$/EtOH, rx, 2 h, 79 % over steps (b) and (c); (d) $KMnO_4$/*t*-BuOH/H_2O, 70 °C; (e) K_2CO_3/THF, rt, 24 h (86 %); (f) $KMnO_4$/*t*-BuOH/H_2O, rt, 24 h (67 %); (g) Cl_2/H_2O, rt, 3 h (87 %).

Starting with commercially available 4- or 5-nitropyrazole-3-carboxylic acid, anilides containing nitro, amino or methylsulfonamido were synthesized. Here, manipulation of the substituents on the pyrazole ring was conducted after the anilide formation (Scheme 4). The reduction of the nitro group was performed with PtO_2 with $NaBH_4$ as

the hydrogen donor.[15] If H_2 was used, the chlorine on the phenyl group was replaced by hydrogen.



Scheme 4. Reagents and conditions: (a) 1. $SOCl_2$, 80 °C, 18 h; 2. $ArNH_2/CH_2Cl_2$, 60 °C, 8 h; (b) $PtO_2/NaBH_4$/MeOH, rt, 18 h; (c) $MeSO_2Cl$/pyridine, 50 °C, 18 h.

Other heterocyclic cores were explored in parallel to the investigation of the pyrazoles (Figure 2). Only 1,2,3-triazoles show any appreciable inhibitory activity and these compounds were investigated further (Table 3). They were prepared using the same methodology as for the pyrazoles, i.e. dimerization followed by addition of an aniline.[16]

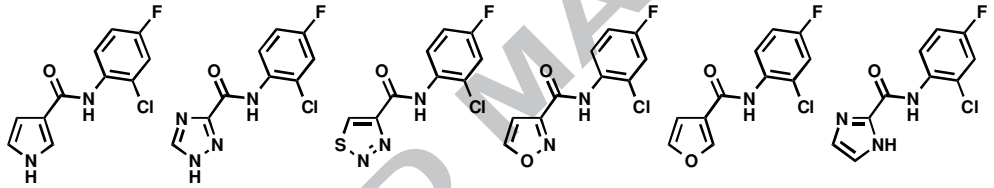
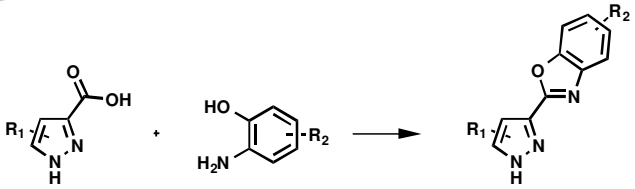


Figure 2. Investigated pyrazole bioisosters

Having an anilide moiety in a drug may be problematic as anilines, present as minute impurities, or formed by hydrolysis in the stomach or in the intestines, are potentially mutagenic. We therefore prepared a series of benzoxazolyl-substituted pyrazoles, as a benzoxazole can be considered to be an anilide bioisoster. The compounds are readily prepared by condensing pyrazole-3-carboxylic acids and 2-aminophenols using polyphosphoric acids (Scheme 5).[17] The yields were at best modest, but we did not spend time optimizing the reaction.



Scheme 5. Reagents and conditions: polyphosphoric acid, 160 °C, 18 h.

In Table 1 a selection of amides of pyrazole-3-carboxylic acid, unsubstituted on the pyrazole core, are shown. The comparison of the anilide (Entry 1) with the benzylamide (Entry 2) clearly shows that moving the phenyl away from the pyrazole skeleton essentially eliminates inhibitory activity. On the anilide portion fluoro and/or chloro substituents, particularly in the 2 and 4-position, are favorable (Entries 3-6). Replacing fluoro or chloro with trifluoromethyl decreases activity, but not by much (Entries 3 and 6 *vs.* 7 and 8). The unsubstituted 2-pyridyl amide (Entry 9) is significantly more potent than the 3- and 4-pyridyls (Entries 10 and 11), and also here addition of halogen substituents increases activity (Entries 12-13). Some 5-membered heterocylic as well as some bicyclic heterocyclic amides also show good activity (Entries 14-18). The lack of activity for *N*-alkylated carboxanilides (Entry 19) suggests that an amide NH is needed for activity.

Table 2 shows *N*-(2-chloro-4-fluorophenyl)pyrazole-3-carboxamides with various substituents in the 4- and/or 5-positions. Compared to the unsubstituted compound (Entry 1), alkyl substituents decrease activity (Entries 2-6). A 4-phenyl substituent abolishes activity. Introduction of one halogen generally does not have much influence on the inhibitory activity whereas two halogens gave stronger inhibition (Entries 8-15). One or two trifluoromethyl groups decrease the activity, which is restored by the addition of a chlorine (Entries 16-19). Compounds with difluoromethyl substituents behaved similarly (Entries 20-21). 4-Nitro, and particularly, 4-amino substitution gave decent activity, whereas the 5-substituted ones only showed low activity (Entries 22-25). The sulfonamido-substituted compounds (Entries 26-27) were practically inactive.

In analogy with the pyrazole SAR, the activity of triazole-3-carboxanilides is increased by a fluoro or chloro substituents in the 2-*,* and particular in the 4-position, which was further increased by 2,3-dihalo substitution (Entries 1-6, Table 3). In contrast to the pyrazole series, the pyridyl and quinoline amides were not particularly active (cf. Entries 7-9, Table 3 with Entries 11,12 and 16, Table 1).

Judging by the lack of activity for *N*-alkylated carboxanilides, Entry 18, Table 1, one would tend to believe that the amide NH is essential for activity. However, this notion is not correct as evident from the activity of the 2-(pyrazol-3-yl)benzoxazoles in Table 4. A chloro substituent, particularly in the 6-positon (Entry 2) of the benzoxazole increases activity. As before, chloro substituents on the pyrazole core are beneficial for activity and compound **6** (Entry 7) was the most active of all tested compounds.

Unfortunately, all benzoxazoles of Table 4 suffered from very low metabolic stability ($t_{1/2}$ a few minutes) and were deemed unsuitable for further development.

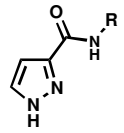
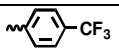
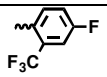
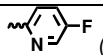
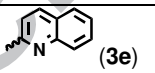
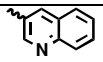
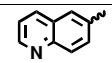
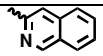
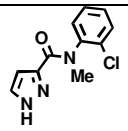
Table 5 exemplifies three of the compounds investigated in the selection for a candidate drug (CD). Our first criteria was an $IC_{50} < 100$ nM in both the cell and enzyme assays, and the chloropyrazole BLX-2481 (**3d**) did not fulfil that. Although relatively potent, the bicyclic arylamide BLX-1873 (**3e)**, showed relatively low solubility and metabolic stability and poor pharmacokinetic properties. The triazole BLX-2477 (**5**) which had a decent IC_{50} in the cell assay (56 nM) was more extensively investigated. Although it is a strong inhibitor of several human cytochrome P450's, particularly 2C9 and 2C19, this was not considered a showstopper. Although the high metabolic stability may indicate that it inhibits its own metabolism by being a substrate for the CYP's, this was not investigated at this point. The compound as such did not show any genotoxicity in the SOS/umu assay, but this does not say anything about any possible genotoxicity of its metabolites or degradation products. As BLX-2477, despite its high plasma protein binding, had favourable pharmacokinetic properties: a long i.v. mean residence time (MRT) of about 1.8 h, and high exposure in the 10-day toxicology study (150 mg/kg p.o.) i.e. a C_{max} up to 589 µM, it was selected as a CD.

As already discussed,[3] compounds may inhibit 15-LOX-1 and its orthologues from other species to a different degree. It is of course important that a CD exhibits good activity in the species that will be used in advanced *in vivo* studies and the activity of some of our compounds on 15-LOX-1 orthologues were thus investigated. From Table 5 it is evident that compound **5** does not inhibit the rat or the dog enzymes to any large extent, but shows good activity towards the pig orthologue. Consequently, preclinical *in vivo* studies needs to be conducted using a mini-pig model. In their search for 15-LOX-1 inhibitors for use in anti-stroke therapy, Maloney and Holman used a mouse model, but they identified their HTS hits by simultaneously screening both 15-LOX-1 and the mouse ortholog.[18] Obviously, the compounds described in our paper would not have been considered interesting in their study. Nevertheless, the importance of using 15-LOX-1 in (one of) the primary screen(s) must be emphasized.

In conclusion we have developed pyrazole-3-carboxanilides, triazole-4-carboxanilides and 2-(3-pyrazolyl)benzoxazoles that are good inhibitors of 15-LOX-1. BLX-2477, *N*-(2-Chloro-4-fluorophenyl) triazole-4-carboxamide (**5**) was selected as a clinical candidate, but regrettably, adverse non-target associated effects were found

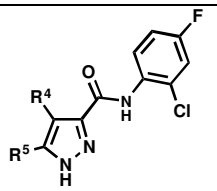
during the toxicological studies using mini-pigs, which forced us to terminate the program.

Table 1. 15-LOX-1 inhibitory activity of pyrazoly-3-carboxamides[19,20]

		$IC_{50}/(I_{max})$ enzyme	$IC_{50}/(I_{max})$ cell
Entry	R	nM (%)	nM (%)
1		45*	47*
2		NI	NT
3		NT	270 (80)
4		645	618
5		180	1320
6		81	24
7		448	519 (69)
8		168 (79)	94 (70)
9		1207 (71)	3410 (53)
10		NT	NI
11		NT	NI
12	 (**3d**)	550 (86)	118 (84)
13		26 (75)	43 (71)
14		384	130 (74)
15	 (**3e**)	334 (80)	3740 (68)
16		180 (89)	92 (47)
17		970 (82)	837 (74)
18		843 (88)	406 (64)
19		NI	NI

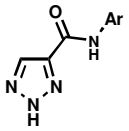
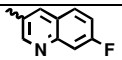
NI = no inhibition; NT = not tested; When no I_{max} is given it is >90%;
* % inhibition @10 μM compound concentration instead of IC_{50};
Arachidonic acid is used as a substrate.

Table 2. 15-LOX-1 inhibitory activity of 4- and/or 5-substituted pyrazoly-3-carboxanilides[19,20]

			$IC_{50}/(I_{max})$ enzyme	$IC_{50}/(I_{max})$ cell
Entry	R^4	R^5	nM (%)	nM (%)
1	H	H	81	24
2	Me	$SiMe_3$	672 (65)	1330 (60)
3	Me	H	453	183
4	H	Me	376 (71)	757 (76)
5	*n*-Bu	H	NT	855 (69)
6	H	*t*-Bu	NT	42 (37)
7	Ph	H	NT	NI
8 (**3a**)	Cl	H	85	33
9 (**3b**)	H	Cl	71 (70)	22 (88)
10 (**3c**)	Cl	Cl	32	14
11	F	H	NT	42 (80)
12	Br	H	290 (75)	39 (87)
13	Br	Cl	1	10
14	I	H	184 (78)	551 (76)
15	H	I	150 (89)	27 (86)
16	CF_3	H	NT	117 (87)
17	H	CF_3	1000	112 (82)
18	CF_3	CF_3	NT	279
19	Cl	CF_3	NT	37 (80)
20	H	CHF_2	NT	216 (72)
21	Cl	CHF_2	NT	39
22	NO_2	H	5900	616
23	H	NO_2	NI	3290 (81)
24	NH_2	H	641	69
25	H	NH_2	5980	334 (58)
26	$NHSO_2Me$	H	NT	NI
27	H	$NHSO_2Me$	NT	NI

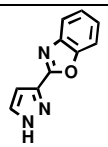
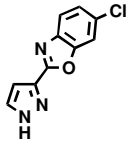
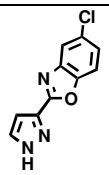
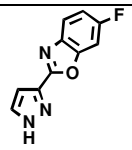
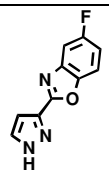
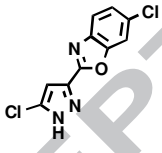
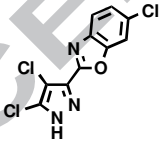
NI = no inhibition; NT = not tested; When no I_{max} is given it is >90%; Arachidonic acid is used as a substrate.

Table 3. 15-LOX-1 inhibitory activity of triazolyl-4-carboxanilides[19,20]

		$IC_{50}/(I_{max})$ enzyme	$IC_{50}/(I_{max})$ cell
Entry	Ar	nM (%)	nM (%)
1		NI	97 (42)
2		947	2426 (77)
3		6976	392 (69)
4		571	68 (81)
5	 (**5**)	99	56
6		22530 (82)	79 (54)
7		3965	3110 (80)
8		1400	1020 (47)
9		NT	596 (50)

NI = no inhibition; NT = not tested; When no I_{max} is given it is >90%;
Arachidonic acid is used as a substrate.

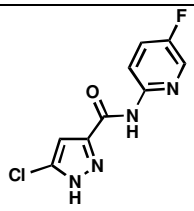
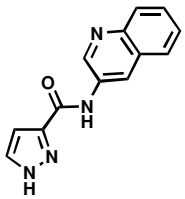
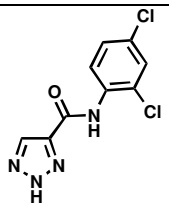
Table 4. 15-LOX-1 inhibitory activity of 2-(pyrazol-3-yl) benzoxazoles[19,20]

Entry		$IC_{50}/(I_{max})$ enzyme nM (%)	$IC_{50}/(I_{max})$ cell nM (%)
1		3273	638 (76)
2		20	13 (75)
3		443	124 (60)
4		NT	716
5		NT	22240
6		4	131
7	 (**6**)	2	34

NT = not tested; When no I_{max} is given it is >90%;
Arachidonic acid is used as a substrate.

Table 5.

Experimental data used in the evaluation of possible clinical candidates.[19,20]

		 BLX-2481 (**3d**)	 BLX-1873 (**3e**)	 BLX-2477 (**5**)
15-LOX-1 enzyme (IC_{50}, nM)		550 (I_{max} 86 %)	180 (I_{max} 89 %)	99
15-LOX-1 cell (IC_{50}, nM)		118 (I_{max} 84 %)	56 (I_{max} 68 %)	56
15-LOX-2 enzyme (IC_{50}, nM)		NT	NT	NI
5-LOX cell		<50 %	NT	<50 %
12-LOX enzyme		<50 %	NT	42 %
COX-1 enzyme		NI	9	4
COX-2 enzyme		NI	NI	10
15-LOX-1 orthologs enzyme (IC_{50})	rat	NT	NT	24 µM (I_{max} 79 %)
	dog	NT	NT	2.4 µM
	pig	NT	NT	78 nM
Aqeous solubility (µg/mL)	pH 2	NT	573.1	24.3
	pH 7.4	3.4	10.1	83.6
	pH 10	NT	10.1	>286.6
Metab. stab. human microsomes	$t_{1/2}$ (min)	36	>100	>100
	CL_{int} (µl/min/mg)	17	7	<1
Permeability (CACO-2)		high, no efflux	high, no efflux	high, no efflux
P450 inhibition (IC_{50} or % at 10 µM)	1A2	45 %	75 %	21.6 µM
	2B6	NT	NT	93 %
	2C8	NT	NT	NI
	2C9	26 %	NI	0.5 µM
	2C19	6 %	NI	1.4 µM
	2D6	9 %	NT	>100 µM
	2E1	NT	NT	10 %
	3A4	NI	NT	58.1 µM
Genotoxicity (SOS/umu)		negative	negative	negative
Plasma protein binding		92.9	92.8	>99 %
hERG safety assay		NI	NI	NI

NI = no inhibition; NT = not tested; When no I_{max} is given it is >90%;
The values are given as either IC_{50} or as %-inhibition at 10 µM of the indicated inhibitor.
Arachidonic acid is used as a substrate. Unless stated otherwise, all enzymes/cells are human.

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19. **Enzyme assays.** For the enzyme activity assay of human 15-LOX-1, the enzyme was expressed in Sf9 cells. The enzyme was purified with ion exchange chromatography. The purity of the enzyme was determined by SD-PAGE analysis. The human 15-LOX-2 and rat and dog ortologues of 15-LOX-1 was cloned into a Sf9-cell expression vector and subsequently purified.[20] The porcine orthologue was purchased from Cayman company (USA). Human 12-LOX was partially purified from platelets. In all experiments, the enzyme and compound was preincubated for 5 min prior at room temperature followed by incubation with arachidonic acid for another period of 10 min. The LOX activity was determined by the conversion of arachidonic acid to 15-HETE for the human 15-LOX enzymes, or to 12-HETE for human platelet 12-LOX and the orthologues of 15-LOX-1.[20] COX-1 and COX-2 assays were purchased from Cayman company (USA). The inhibition of 12-LOX and COX-1 and 2 was determined as percent inhibition at 10 μM concentration of the indicated compound. The formation of 15-HETE/12-HETE was followed by HPLC-UV-absorbance at 235 nm. Data analysis was performed using GraphPad Prism software.
Cell assay. The human Hodgkin cell line L1236 has high 15-LOX-1 activity and was used in the cell assay.[21] The cells were suspended in phosphate buffer (PBS) and preincubated 5 min in room temperature with the compound prior to addition of arachidonic acid for 5 min. The formation of 15-HETE was analyzed as described above in the enzyme assay. The inhibition of the 5-LOX pathway

was investigated in purified human granulocytes. The cells were incubated with calcium ionophore plus arachidonic acid and the amounts of formed LTB4 was detected by HPLC-UV-absorbance at 270 nm.

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