New highly selective 5-HT$_{2B}$ receptor antagonist for the treatment of fibrosis

L. Pettersson, C. Wenglén, H. Arozenius, G. Ekström
AnaMar AB, Lund, Sweden

INTRODUCTION

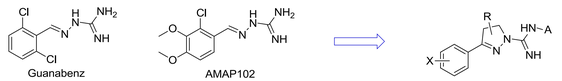
Serotonin (5-HT) is known to be associated with fibrosis and recent studies support that 5-HT$_{2B}$ receptors have an important role in fibrotic disease by regulating production of pro-fibrotic mediators and modifying cell differentiation and activation.

Most 5-HT$_{2B}$ receptor antagonists developed so far are associated with certain liabilities, such as poor selectivity and inadequate pharmacokinetic properties.

Starting from the clinical compounds Guanabenz and AMAP102, which are weak 5-HT$_{2B}$ receptor antagonists, we have developed new, highly potent and selective antagonists. The lead compound AM1125 (K_i 0.87 nM, Mw 218), prepared in two synthetic steps followed by chiral separation, has been shown effective in *in vivo* models of fibrosis.

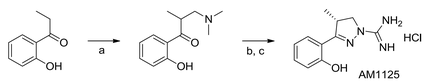
Here we report on the preparation and the very robust SAR of the 5-HT$_{2B}$ receptor binding for this compound class.

Lead Finding Strategy



Strip off substituents - Constrain structure by ring-closure - Redecorate with X, R, and A.

Lead Compound AM1125



a: $HNMe_2$·HCl / 1,3-dioxolan, cat. HCl (conc.), 90°C, 4h (77%). b: $H_2NNHC(NH)NH_2$·HCl / EtOH, reflux, 2 h (30%). c: Chiral separation by SFC (36%).

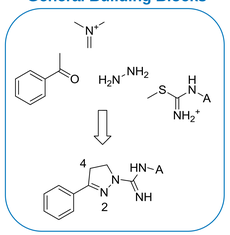
The racemate of AM1125 was identified among the first 15 compounds prepared as a low nM 5-HT$_{2B}$ receptor ligand. Separation of the enantiomers and X-ray crystallography followed by *in vitro* characterization demonstrated that the R-enantiomer AM1125 was a selective 5-HT$_{2B}$ receptor pure antagonist.

5-HT$_{2A/2B/2C}$ binding (K_i/nM):	310 / 0.87 / 53	Mw 218
5-HT$_{2A/2B/2C}$ antag. (IC_{50}/nM):	5080 / 2.4 / 1230	LogD -1.1
Off-targets (>50), binding and activity (<50%):		Aq. sol. >10 mg/mL
5-HT$_{other}$	approx. 1-10 uM	CLint (h hepatocytes) 3.2
GPCR	approx. >10 uM	(uL/min/million cells)
NR, Enz., Ion-ch., Transp.	approx. >10 uM	
hERG	>10 uM	

PREPARATIVE CHEMISTRY of 1-Amidino-3-Aryl-2-Pyrazolines[1]

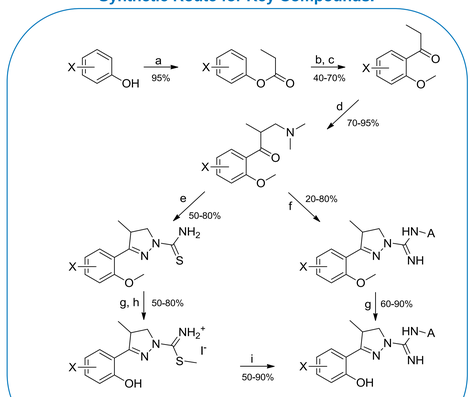
The 1-amidino-3-aryl-2-pyrazolines have been described since the 1950s and can be prepared by conventional methods. The order of assembling the different structural moieties can be varied.

General Building Blocks



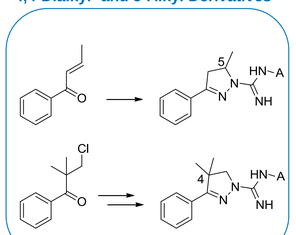
- Mannich base formation from an alkyl-arylketone.
- Amino-guanidine formation from hydrazine and an S-methylated thiourea.
- Pyrazoline formation from the Mannich base and amino-guanidine.
- Alternatively, the Mannich base can first be reacted with hydrazine.
- (Thio)Semicarbazides can also be used to form the pyrazoline ring.

Synthetic Route for Key Compounds.



a: CH_3CH_2COCl, pyridine, CH_2Cl_2. b: $AlCl_3$, 1,2-dichlorobenzene. c: MeI, K_2CO_3, DMF.
d: 1,3-dioxolane, H_2NNMe_2Cl, HCl (or Eschenmoser's salt). e: $H_2NNHC(S)NH_2$, NaOH (aq.), EtOH.
f: $H_2NNHC(NH)NHA$, NaOH (aq.), EtOH. g: BBr_3, CH_2Cl_2. h: MeI, MeOH. i: A-NH_2, MeOH.

4,4-Dialkyl- and 5-Alkyl Derivatives

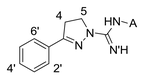


- β-Substituted Michael acceptors are suitable for the preparation of 5-substituted pyrazoline derivatives.
- 4,4-Dimethyl derivatives can be prepared from 1,1-dimethyl-2-chloro-ethylketones. Hydrazine was used for the pyrazoline ring formation.

STRUCTURE-ACTIVITY RELATIONSHIPS (SAR) of 5-HT$_{2B}$ Receptor Binding[2]

Eff. of pyrazoline subst. on binding:
(4R)-Me > 4-Me > 4-Et > **H ≈** 5-Me > 4,5-$(CH_2)_3$- > 5-Et > 4,4-di-Me **≈ (4S)-Me** > 5-Ph

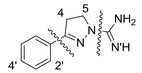
Decreased binding:
- 4'-subst.
- 3',4'-disubst.
- 3',4'-annulated rings.



Eff. of guanidine structure on binding:
- A: Bn, n-Bu, c-Hex, Ph > heteroaryl > Me ≈ H
- A: H > Ac > OH ≈ OMe > CN
- "Non-actives": N,N-disubst., N,N'-disubst.
- (Thio)Ureas: N'H > O > S

Increased binding:
- 2'-OH, -OMe, -Me, -iso-Pr, -Cl, -Br
- 2',3'-disubst.
- 2',3'-annulated rings.

5-HT$_{2B}$ Receptor Binding[2]



Data is presented as % displacement of radioligand at indicated compound concentrations (representative examples).

	Test conc. (nM)				Aryl	Pyrazoline	Guanidine
	1	10	100	1000			
				38	-	-	-
Aromatic				90	2'-OH	-	-
				76	2'-OMe	-	-
				95	2'-Cl	-	-
			81	99	2',3'-$(CH)_4$-	-	-
				6	3',4'-di-$(OMe)_2$	-	-
			23	52	2'-OH, 6'-Cl	-	-
				13	4'-OMe	-	-
			29	77	4'-SMe	4-Me	-
			3	-12	4'-SO_2Me	4-Me	-
Pyrazoline				68	-	4-Me	-
		54	91	100	2'-OH	4-Me	-
	45	83	95	99	2'-OH	(4R)-Me	-
		-1	35	72	2'-OH	(4S)-Me	-
			28	79	2'-OH	4,4-di-Me_2	-
			10	63	2'-OH	5-Ph	-
Guanidine	1	42	92	99	2'-OH	4-Me	-C(NH)NH-Me
	79	91	94		2'-OH, 3'-Cl	4-Me	-C(NH)NH-Bu
	49	97	98	99	2'-OH	4-Me	-C(NH)NH-Bn
	91	101	103		2'-OH, 3'-Cl	(4R)-Me	-C(NH)NH-Bn
	88	100	97		2'-OH	4-Me	-C(NH)NH-Ph
	12	4	27		2'-OH, 3'-Cl	4-Me	-C(NH)N$(Me)_2$
			-3	30	2'-OH	-	-C(NMe)NH-Me
	-15	27	74		2'-OH, 3'-Cl	4-Me	-C(NH)NH-CN
	48	97	104		2'-OH, 3'-Cl	4-Me	-C(NH)NH-thiophen-3-yl
	55	93	99		2'-OH, 3'-Cl	4-Me	-C(NH)NH-pyrazin-2-yl
			21	68	2'-OH	4-Me	-C(S)NH_2
		27	70	93	2'-OH, 3'-Cl	4-Me	-C(O)NH_2
	32	81	97		2'-OH, 3'-Cl	4-Me	-C(O)NH-Bn

- Optimized binding (K_i 0.1-1 nM) is obtained with aromatic 2'- (or 2', 3'-) subst., (4R)-Me, and lipophilic mono-N-subst.
- (4R)-Enantiomers show considerably higher binding (x100-200) than the corresponding (4S)-enantiomers.
- A basic guanidine moiety is not a requisite for binding.

1. See PCT application WO2016/207231A1 for a detailed description of the preparative chemistry and 5-HT$_{2B}$ receptor binding data.
2. 5-HT$_{2B}$ receptor (human) binding was performed at Eurofins Panlabs Taiwan, Ltd., in a ligand displacement assay using ^{3}H-LSD as radioligand (assay no. 271700).