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DESIGN OF PYRROLO-1,4-BENZOXAZINE DERIVATIVES AS INHIBITORS OF 5-LIPOXYGENASE AND PAF ANTAGONISTS WITH ANTIHISTAMINIC PROPERTIES

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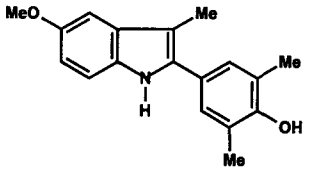
Abstract. A series of 5-hydroxyphenylpyrrolo-1,4-benzoxazine derivatives substituted by a pyridinylpiperazinyl alkyl chain were designed as multimediator compounds with potential antiasthmatic properties. These compounds were prepared and evaluated both *in vitro* against leukotriene synthesis and PAF activity and *in vivo* against histamine. Structure-activity relationships led to compound **11** which displayed the most potent activities in this series.

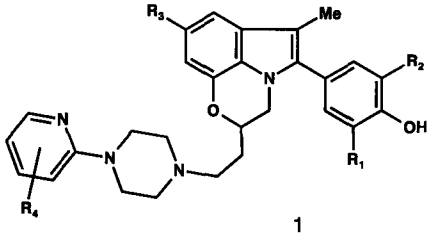
It is now widely accepted that asthma is a chronic inflammatory disease of the conducting airways. The asthmatic inflammation is highly complex and driven by a vast array of mediators and cytokines produced by a mix of inflammatory cells, principally macrophages, lymphocytes and eosinophils[1]. Pro-inflammatory mediators such as histamine, leukotrienes and platelet-activating factor (PAF) released from pulmonary tissues or migrating inflammatory cells possess specific vasoactive, bronchoactive and oedemagenic properties that are thought to be pivotal in the initiation and propagation of the asthmatic response[2,3,4]. However, the relative contributions of these mediators to pathophysiology of asthma are not fully understood. In clinical trials, selective antihistamines including azelastine, ketotifen, cetirizine and terfenadine have demonstrated at least partial efficiency against bronchial asthma[5]. Leukotriene antagonists, such as ICI 204, 219 and leukotriene synthesis inhibitors, such as MK 886, have been reported to significantly reduce allergen-induced bronchoconstriction and airway hyperreactivity in atopic subjects[6,7]. However, neither drug was able to alleviate all the symptoms in asthmatic patients.

Given the complexity of chronic asthma it is unlikely that a single inflammatory mediator could account for all the pathology. Consequently, it can be speculated that only drugs which can simultaneously interfere with at least two mediators are likely to have any profound effect on the inflammation. With this in mind, new original molecules which could simultaneously interfere with histamine, 5-lipoxygenase (5-LO) and PAF were developed.

New compounds **1** described in this paper were designed starting from a series of pyrrolo-1,4-benzoxazines bearing a piperazinyl alkyl chain which were developed [8] as antagonist of PAF and histamine and from the 2-phenol indole derivative TZI 41,127, which is a potent inhibitor of 5-lipoxygenase [9].



TZI 41,127



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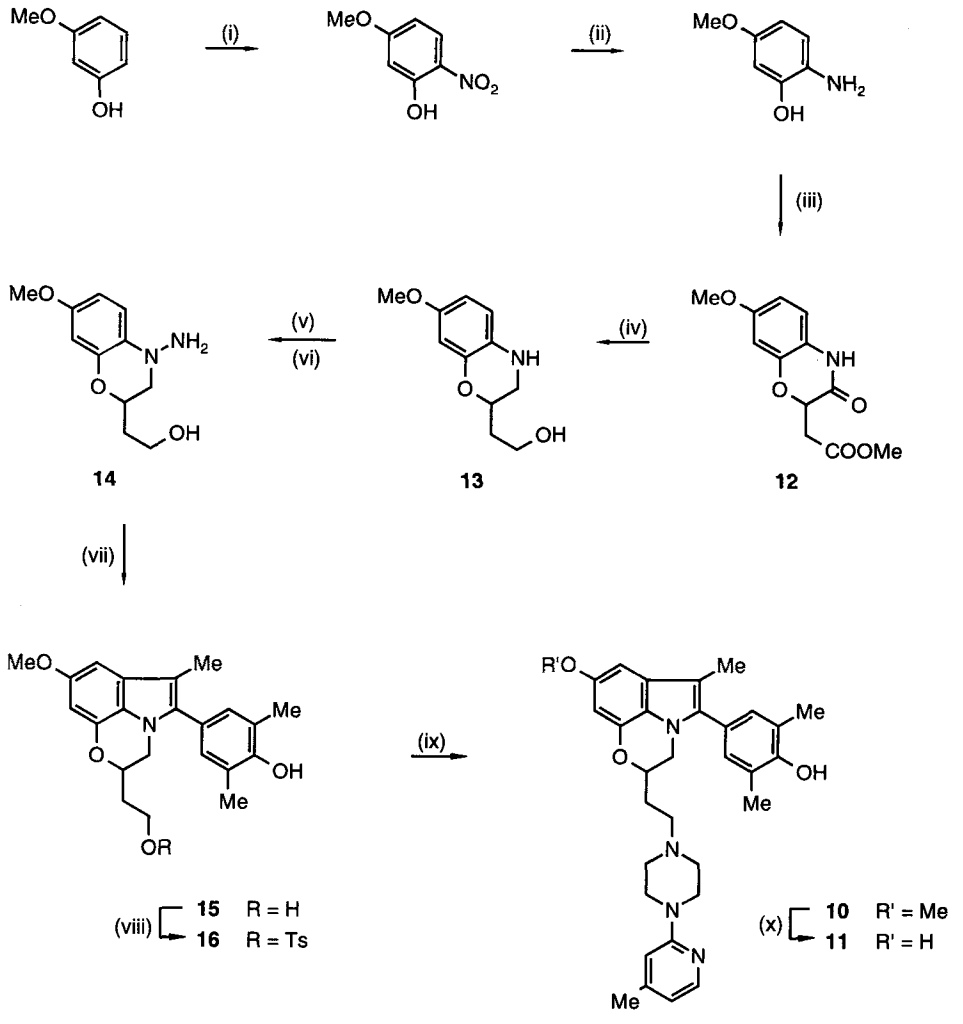
Synthesis

The pyrrolo-1,4-benzoxazine derivatives were prepared as outlined for compound **11** in scheme 1. Synthesis of **11** began with nitrosation of 3-methoxyphenol and subsequent oxidation led to the formation of 5-methoxy-2-nitrophenol in 65% overall yield [10]. Quantitative reduction of the nitro group was achieved by catalytic hydrogenation and led to 2-amino-5-methoxyphenol which was then treated with maleic anhydride to produce the ester **12**, purified by flash chromatography and isolated in 51% yield. Treatment of **12** with $LiAlH_4$ simultaneously reduced the ester and amide functions to the corresponding aminoalcohol **13** in 54% yield. Standard nitrosation of **13**, followed by reduction of the resulting nitroso derivative afforded the hydrazinic derivative **14** in 76 % overall yield. Compound **14** was converted to the indole derivative **15** in 20 % yield using the standard Fischer indole reaction with (3,5-dimethyl-4-hydroxy) propiophenone prepared via a Fries rearrangement [11]. It is worth noting that the modest yield of the Fischer indolization is related to the presence of the methoxy group on the benzo ring and yields are much higher (up to 70%) without this substituent. The alcohol **15** was purified by flash chromatography and then tosylated with one equivalent of tosyl chloride (TsCl) in 50% yield. Subsequent alkylation of 1-(4-methyl-2-pyridinyl) piperazine [12] with the tosylated intermediate **16** gave the methoxyphenol derivative **10** in 55% yield. Standard demethylation with boron tribromide [13] afforded the desired bis-phenol **11** which was crystallized in 47% yield [14].

Structure-activity relationships

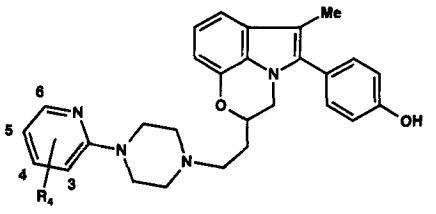
A preliminary series of molecules was designed with $R_1 = R_2 = R_3 = H$ and variation of R_4 (H and CH_3 in the four free positions). Biological results on the three target mediators are shown in Table 1.

Scheme 1[a]



[a]Reagents and conditions : (i) (a) $NaNO_2$, H_2SO_4, H_2O, 0°C, 30 min, (b) HNO_3, RT, 2h; (ii) H_2, 10% Pd/C, MeOH, 60°C, 5h; (iii) maleic anhydride, Et_3N, MeOH, reflux, 6h; (iv) $LiAlH_4$, THF, RT to reflux, 2h; (v) $NaNO_2$, HCl, H_2O, 0°C to RT, 1h; (vi) $LiAlH_4$, THF, RT, 1h; (vii) (3,5-dimethyl-4-hydroxy)propiophenone, AcOH, EtOH, 45°C, 4h; (viii) TsCl, pyridine, 60°C, 5h; (ix) 1-(4-methyl-2-pyridinyl)piperazine, DMF, 80°C, 4h; (x) BBr_3, CH_2Cl_2, - 30°C to RT, 4h.

Table 1

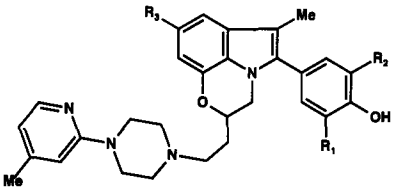


N°	R_4	mp (°C)	IC_{50} (μM)		Histamine in vivo[c] % inhibition (2.10^{-5} mol/kg)
			LTB_4 [a]	PAF [b]	
2	H	199-201	2.2	8.1	47
3	3-Me	189-191	2.3	7.2	57
4	4-Me	213-214	3.0	2.7	10
5	5-Me	217-220	3.1	9.9	58
6	6-Me	176-178	3.3	10.2	39
TZI 41,127			0.11	NA [d]	NA [e]

[a] 5-lipoxygenase inhibition in isolated rat neutrophils was determined based on the inhibition of A 23,187-induced LTB_4 production [15].
[b] inhibition of PAF-induced aggregation was determined on washed rabbit platelets [16].
[c] inhibition of histamine-induced cutaneous reactions was determined after one hour oral pretreatment. Histamine (40μg/site) was injected i.d. [17].
[d] not active at 10^{-5} M
[e] not active at 2.10^{-5} mol/kg

All the phenol derivatives (**2-6**) inhibited the leukotriene synthesis by rat PMNs (IC_{50} about 2-3 μM) but were less potent than TZI 41,127. The nature and position of R_4 had no significant effect on this activity. Interestingly, in comparison with this reference compound, addition of the pyridinylpiperazine moiety led to both *in vitro* PAF antagonism (IC_{50} about 3-10 μM) and *in vivo* inhibition of histamine (up to 58% at 2.10^{-5} mol/kg). As might be expected from results of a previous series[18], both PAF and histamine antagonism was dependent on the nature and position of R_4. It appeared in this series that the *in vivo* anti-histaminic activity was highly unpredictable and thus SAR studies were focused on both PAF antagonism and 5-LO inhibitory activities. Bearing this in mind, new compounds with the 4-methylpyridinylpiperazine moiety were designed to confer the best PAF antagonistic activity and, by analogy with TZI 41,127, substituents were introduced on both the phenol ring (R_1 and R_2) and the indole ring (R_3). Biological results obtained following these modifications are presented in Table 2.

Table 2



N°	R_1	R_2	R_3	mp (°C)	IC_{50} (μM)		Histamine in vivo [c] % inhibition (2.10^{-5} mol/kg)
					LTB_4 [a]	PAF [b]	
7	OMe	H	H	88-89	3.2	5.0	7
8	OH	H	H	253-254	1.9	4.7	NA[e]
9	Me	Me	H	190-192	1.5	1.6	15
10	Me	Me	OMe	100-103	7.6	2.9	32
11	Me	Me	OH	195-197	0.29	0.45	37
TZI 41,127					0.11	NA [d]	NA[e]

[a, b, c, d, e] see Table 1

Substitutions on the phenol ring (R_1 and R_2) slightly modified PAF antagonism and 5-LO inhibition. In particular, the dimethylphenol derivative **9** exhibited approximately a 2-fold increase in potency against both mediators (compare **9** with **4**). In contrast, substituent changes at the 8-position on the indole ring (R_3) significantly influenced the inhibition of 5-LO. Interestingly, comparing the leukotriene synthesis inhibition with the 8-unsubstituted compound **9**, the methoxyphenol derivative **10** was about five times less active whereas the bis-phenol compound **11** conversely showed a 5-fold increase in potency. Moreover, compound **11** displayed potent PAF antagonist activity (IC_{50} of 0.45 μM) and a moderate *in vivo* antihistaminic activity (37% inhibition at 2.10^{-5} mol/kg). In order to confirm the potent 5-LO activity of compound **11** compared to TZI 41,127, both compounds were tested in a semi purified 5-LO enzyme assay (Table 3).

Table 3

IC_{50} (μM)	5-LO [c]	LTB_4 [a]	PAF [b]
11	0.18	0.29	0.45
TZI 41,127	0.24	0.11	NA [d]

[c] Inhibition of semi-purified 5-LO was performed on a 10,000 g supernatant of guinea-pig leukocytes and determined based on the inhibition of 5-HETE generation [19].
[a, b, d] See Table 1

The results in Table 3 showed that both **11** and TZI 41,127 inhibited LTB_4 formation in rat neutrophils by directly acting on the 5-LO enzyme and that both were effective in the same range of potency.

Considering the potential antioxidant activity of **11**, the mechanism by which this compound inhibits 5-LO *in vitro* probably involved reduction of the catalytically active ferric enzyme to the inactive ferrous form.

In conclusion, compound **11** has been shown to be as potent as TZI 41,127 when tested *in vitro* for its ability to inhibit 5-LO. Moreover this prototype possesses marked PAF antagonistic properties *in vitro* and moderate antihistaminic properties following oral administration. The comparison of compound **11** with recently described 2,4-diaryl-1,3-dithiolane derivatives [20] that exhibit dual inhibitory activities of PAF and 5-LO indicated that our compound was slightly more active and was found to show a better balance of antiallergic properties. Efforts to improve the multimediator profile in this series are underway.

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References and notes

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