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Design, Synthesis and Pharmacological Evaluation of Novel Pyrazolo[3,4-*b*]thieno[2,3-*d*]pyridine Acid Derivatives: A New Class of Anti-inflammatory and Anti-platelet Agents

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Abstract—A series of pyrazolo[3,4-*b*]thieno[2,3-*d*]pyridine alkanoic acid derivatives has been synthesized and evaluated as thromboxane synthetase inhibitors and leukotriene D_4 receptor antagonists. The glutaric acid derivative LASSBio341 (**6**) was shown to be active in arachidonic acid-induced platelet aggregation ($IC_{50} = 0.14\,\mu M$) and inhibition of the contraction of guinea pig tracheal strip induced with LTD_4 ($IC_{50} = 43.7\,\mu M$), displaying still in vivo anti-inflammatory profile.

Introduction

Asthma is a complex, chronic inflammatory disease of airways, which affects more than 100 million people worldwide, making it a serious global health problem.[1] This disorder involves multiple inflammatory mediators that are released from mast cells as a consequence of immunological response in the airways.[2] Both leukotriene D_4 (LTD_4) and thromboxane A_2 (TXA_2) are autacoids derived from arachidonic acid (AA) metabolism implicated in the pathogenesis of asthma.[3–5] LTD_4 is a potent constrictor of airway smooth muscle[6] and act on the vasculature to produce vasodilatation and increase vascular permeability,[7] processes that are likely relevant to the recruitment of leukocytes to an inflammatory response. These changes promoted by LTD_4 and other *cys*-LTs induces mucous secretion and decrease of the airway caliber, inherent to asthma.[8] On the other hand, TXA_2 is a potent vasoconstrictor, broncoconstrictor, and platelet-aggregating agent.[9] Therefore, considerable effort has been devoted to modulate the actions of these eicosanoids through the design of TXS-inhibitors (TXSi)[10] or alternatively, block the effects of LTD_4 on the *cys*-LT receptor (*cys*-LTant.).[11] The former approach, in fact, has found application in therapeutics, as illustrated by ozagrel (**1**), an important anti-thrombotic, anti-asthmatic agent that acts as a TXSi (Fig. 1).[10,12] On the other hand, the development of potent *cys*-LT antagonists, for example, zafirlukast[13,14] (**2**) and montelukast[15] (**3**), represents an important therapeutical innovation for the treatment of the inflammatory symptoms of bronchial asthma.

As part of a research program for the design, synthesis, and pharmacological evaluation of new lead candidates useful for asthma treatment, the preparation of a new series of pyrazolo[3,4-*b*]thieno[2,3-*d*]pyridine alkanoic acid derivatives **4**–**7** has been carried out.[16] The synthesis of these new compounds representing a structural hybridization of ozagrel (**1**) and the quinolinic derivative **3** (Fig. 1) and envisioned as new anti-asthmatic candidates possessing dual action, *cys*-LT receptor antagonists and TXA_2 synthetase inhibitors, is the subject of this paper.

Chemistry

These new derivatives **4**–**7** were synthesized by the route illustrated in Scheme 1. The approach used for the

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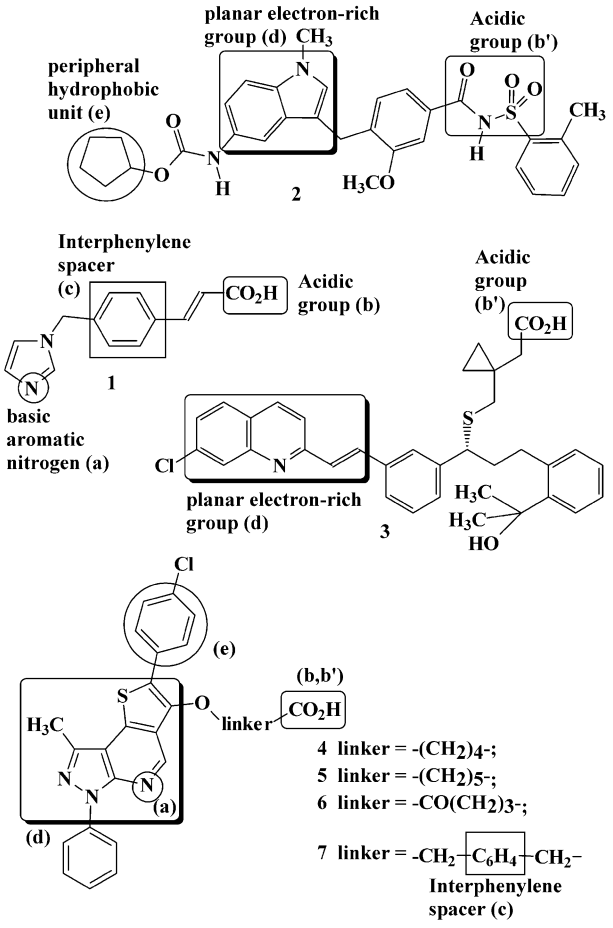
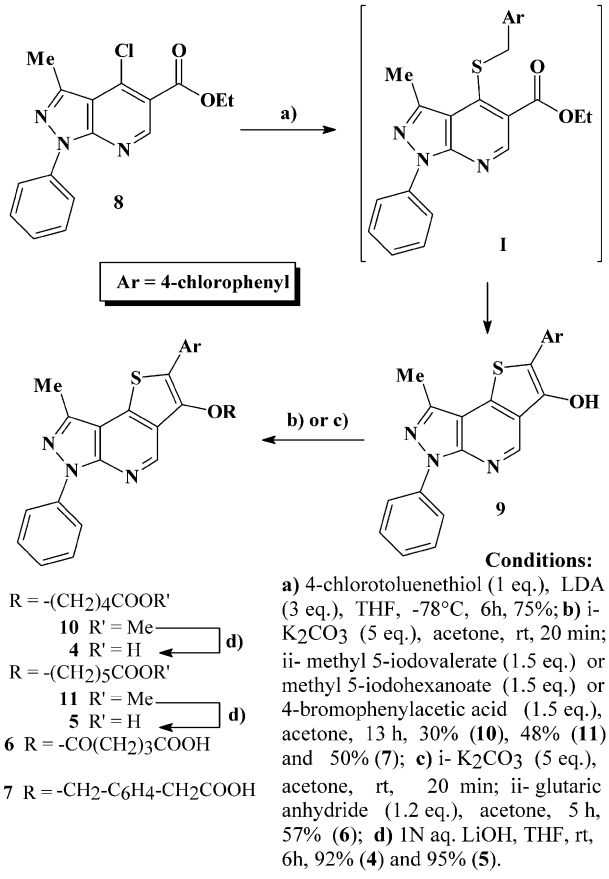


Figure 1. Design concept of new tricyclic TXSi/LTant.



Scheme 1. Synthesis of new pyrazolo[3,4-*b*]yhieno[2,3-*d*]pyridine derivatives **4**–**7**.

construction of the functionalized pyrazolo[3,4-*b*]thieno[2,3-*d*]pyridine nucleus employed the previously described one-pot transformation,[17,18] starting from the chloroester **8**.[19] Thus, this material was subjected to heteroatomic nucleophilic displacement of chlorine atom at C-4 of **8** with the lithium salt of 4-chlorotoluenethiol, to give the thioether intermediate I, which underwent intramolecular Claisen condensation, promoted by excess of LDA present in the reaction medium, to afford in 75% yield the

Table 2. Effect of zafirlukast (**2**) and LASSBio341 (**6**) on LTD_4 (100 nM) induced contraction of isolated guinea pig tracheal strip

Compd	IC_{50} (M)[a]
Zafirlukast[b] (**2**)	$1.03\ (\pm 0.06)\times 10^{-9}$
LASSBio341[c] (**6**)	$43.7\ (\pm\ 1.9)\times 10^{-6}$

[a]Values are means of five independent experiments, standard deviation is given in parentheses; Concentration range.
[b]0.1 nM–100 μM.
[c]10 M–300 μM.

Table 1. Anti-inflammatory effects of pyrazolo[3,4-*b*]thieno[2,3-*d*]pyridine acid derivatives **4**–**7** and indomethacin in the carrageenan-induced rat pleurisy[24]

Compd	Dose[a] (μmol/Kg)	*n*[b]	Cell number ($\times 10^6$ cells/cavity)	% Inhibition[c]	Exsudate volume (mL)	Δ Exsudate volume (mL)
Vehicle control	—	10	30.7±1.1	—	3.25±0.04	—
Indomethacin	100	10	23.3±2.2	24.1*	3.21±0.05	−0.04 n.s.[d]
4	100	4	21.5±1.8	30.0*	3.22±0.02	−0.03 n.s.[d]
5	100	7	17.8±1.8	42.0*	3.16±0.04	−0.09 n.s.[d]
6	100	4	19.6±1.4	36.2*	3.05±0.03	−0.20*
7	100	7	21.9±2.0	28.7*	3.10±0.06	−0.15 n.s.[d]

*$p < 0.05$ (Student's *t*-test). Results are expressed as mean±SEM.
[a]All compounds were administered po.
[b]*n* = number of animals.
[c]Percentage of inhibition obtained by comparison with control group.
[d]n.s. = not significant.

Table 3. Effects of pyrazolo[3,4-*b*]thieno[2,3-*d*]pyridine acid derivatives **4**–**7** and indomethacin on in vitro platelet aggregation of citrated rabbit platelet-rich plasma induced by arachidonic acid and collagen[2]

Compd	C[a] (μM)	Arachidonic acid (200 μM)			Collagen (5 μg/mL)		
		n[b]	Aggregation (%)	Inhibition[c] (%)	n[b]	Aggregation (slope)	Inhibition[c] (%)
Control	—	5	68.3±3.3	—	4	8.8±0.6	—
Indomethacin	10	4	0.0±0.0	100*	4	0.4±0.1	95.4*
4	100	3	58.9±3.8	13.8 n.s.[d]	4	7.5±1.3	14.8 n.s.[d]
5	100	4	56.6±4.7	17.1 n.s.[d]	4	5.8±0.9	34.1*
LASSBio341 (**6**)	100	5	0.0±0.0	100*	5	0.0±0.0	100*
7	100	5	60.5±3.4	11.4 n.s.[d]	4	4.7±1.3	46.6*

*$p < 0.05$ compared to appropriate control (Student's t-test). Results are expressed as mean ± SEM.
[a]C = final concentration.
[b]n = number of independent experiments carried out in triplicate.
[c]Percentage of inhibition obtained by comparison with control group.
[d]n.s. = not significant.

key-intermediate, 2-(4′-chlorophenyl)-3-hydroxy-6-phenyl-8-methylpyrazolo[3,4-*b*]thieno[2,3-*d*]pyridine (**9**) (Scheme 1).

The next step in the synthesis of the new derivatives **4**–**7**, consisted in *O*-alkylation or *O*-acylation of the pseudophenolic hydroxyl group of compound **9**, which was effected by treatment with 5 equiv of potassium carbonate in acetone, followed by the addition of the appropriate electrophilic species.[20] Employing this procedure, methyl esters **10** and **11** were obtained with methyl 5-iodovalerate and methyl 6-iodohexanoate, which was followed by mild hydrolysis with lithium hydroxyde[21] in THF to give acids **3** and **4** in 27% and 45% yield, respectively[22] (Scheme 1). Compounds **6** and **7** were obtained in 50 and 57% yield, respectively, with glutaric anhydride and 4-bromomethylphenylacetic acid.[22]

Results and Discussion

The dual *cys*-LTant/TXSi profile of the new pyrazolo[3,4-*b*]thieno[2,3-*d*]pyridine acid derivatives **4**–**7** was initially evaluated by using carrageenan-induced pleurisy[23] and arachidonic acid-induced platelet aggregation[24] models.

The results obtained for the anti-inflammatory activity of the pyrazolo[3,4-*b*]thieno[2,3-*d*]pyridine acid derivatives **4**–**7** are compiled in Table 1.

All compounds, when administered orally at a dose of 100 μmol/kg, were able to inhibit significantly cellular migration. Compounds **5** and **6** were the most active, with inhibitions of 42.0 and 36.2%, respectively. Only compound **6** was able to inhibit significantly exsudation and it showed no activity on the carrageenan-induced rat paw edema (data not given). Otherwise, all compounds were more active than indomethacin and they did not induce any gastric toxicity when orally administered.

In order to correlate the in vivo anti-chemoatractant and anti-edematogenic profile of the compound **6** observed in rat pleurisy model with an anti-leukotriene action,[25,26] we performed its in vitro evaluation in LTD_4-induced contraction of guinea-pig tracheal strip,[27] using zafirlukast (**2**) as standard (Table 2).

Zafirlukast (**2**) and compound **6**, named LASSBio341, inhibited in a concentration-dependent manner, the contraction of tracheal strips induced by 100 nM of LTD_4, with IC_{50} values of 1.03 nM and 43.7 μM, respectively, confirming its actions as *cys*-LT receptor antagonists.

Neither of the two substances was effective against tracheal contraction induced by histamine and U-46619.

To evaluate the expected dual profile of these new derivatives **4**–**7** they were assayed for in vitro platelet aggregation induced by AA, collagen, and U-46619 (Table 3). Compounds **5** and **7** inhibited significantly the collagen-induced aggregation, with 34.1 and 46.6% inhibition, respectively. On the other hand, LASSBio341 (**6**), at 100 μM concentration, strongly inhibited the platelet aggregation induced by both AA and collagen by 100%. DMSO, used as vehicle, had neither pro- nor anti-platelet aggregation activity.

Therefore these results lead us to study the concentration–response of LASSBio341 (**6**), which inhibited the platelet aggregation induced by 200 μM of AA, with an IC_{50} of 0.14 μM. When compound **6** was tested, at 100 μM, against increasing concentrations of AA-induced aggregation it presents a competitive inhibition behavior. At 500 μM AA-induced aggregation, the new lead-compound **6** presented an inhibition at the same magnitude of ozagrel[27] (**1**) (61×50%, respectively) (data not shown). None of the compounds inhibited significantly U-46619-induced aggregation, suggesting that they did not act at the TXA_2 receptor level.

Concluding Remarks

In summary, we are able to design the new aza-heterocyclic glutaric acid derivative (**6**, LASSBio341), as a hybrid of ozagrel (**1**) and montelukast (**2**), possessing the adequate structural requirements to inhibit the contraction of guinea-pig tracheal strip induced by LTD_4 and promote efficient inhibition of platelet aggregation induced by AA, strongly suggesting that the dual profile observed is due to *cys*-LT receptor blockage and TXS inhibition, representing an useful profile for asthma treatment.[28]

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