New Thienylacylhydrazone Derivatives as Potent Platelet Aggregation Inhibitors

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INTRODUCTION AND GOALS

Antiplatelet drugs have an established place in the prevention of vascular events in a variety of clinical conditions, such as myocardial infarction, stroke and cardiovascular death. Both European and American guidelines recommend the use of antiplatelet drugs in patients with established coronary heart disease and other atherosclerotic disease (LAUER, 2002).

In high-risk patients, such as those with post-acute myocardial infarction, ischaemic stroke or transient ischaemic attack, and in patients with stable or unstable angina, peripheral arterial occlusive disease or atrial fibrillation, antiplatelet treatment may reduce the risk of a serious cardiovascular event by approximately 25%,including reduction of non-fatal myocardial infarction by 1/6, non-fatal stroke by 1/4 and cardiovascular death by 1/6 (TENDERA & WOJAKOVSKI, 2003).

The development of new antiplatelet drugs is relevant since the available therapeutic arsenal is restricted and sometimes it is not useful. The present study has been conducted in order to investigate the antiplatelet activity of a new series of thienylacylhydrazone compounds (Figure 1), analogous to the lead compound LASSBio 294, and to contribute for the comprehension of its mechanism of action.

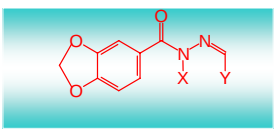
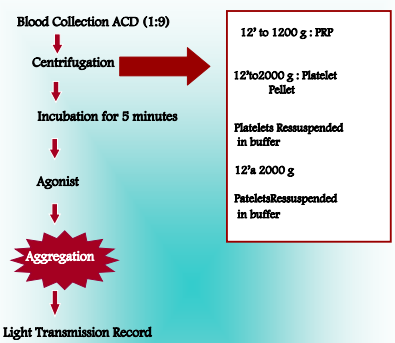


Figure 1 – Thienylacylhydrazone Compounds

METHODS

PLATELET AGGREGATION ASSAY
(Turbidimetric Method, Born & Cross, 1963):



MEASUREMENT OF PLATELET CYCLIC AMP AND GMP LEVELS BY EIA

✓ For the experiments 0.4 mL aliquots of platelet suspension in siliconized Eppendorf caps were incubated for 5 minutes under agitation at 37°C. The reaction was stopped with EDTA 10mM and imediately boiled for 2 minutes and kept on ice.

✓ The aliquots were centrifuged and the supernatant was transferred to assay.

✓ AMPc and GMPc determination was performed using the Amershan Biotrack cAMP and GMPc EIA kit.

RESULTS

Table 1-Thienylacylhydrazone Compounds Anti-Platelet Potency in Rabbit PRP

COMPOUNDS	AA	Collagen
	IC_{50} (µM)	IC_{50} (µM)
LASSBio 294	15.3 ± 0.2	18.3 ± 8.7
LASSBio 785	0.3 ± 0.1	0.9 ± 0.6
LASSBio 786	0.02 ± 0.6	7.9 ± 2.4
LASSBio 787	20.9 ± 0.4	21.1 ± 0.2
LASSBio 788	0.2 ± 0.3	1.5 ± 0.2
LASSBio 789	3.1 ± 0.3	3.4 ± 0.2

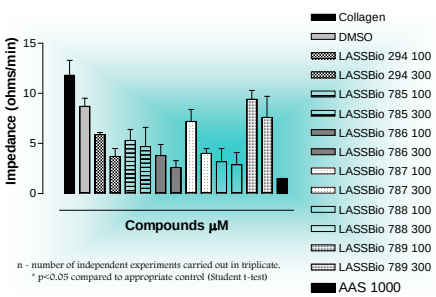
n - number of independent experiments carried out in triplicate.
* p<0.05 compared to appropriate control (Student t-test)

Table 2- Thienylacylhydrazone Compounds Effects in ATP Secretion in Human PRP

COMPOUNDS (100 µM)	n	ATP (nM)	INHIBITION %
Collagen	4	2.0 ± 0.0	-
DMSO	4	2.0 ± 0.0	-
LASSBio 294	4	0.06 ± 0.01	97.1*
LASSBio 785	4	0.07 ± 0.01	96.5*
LASSBio 786	4	0.08 ± 0.03	95.9*
LASSBio 787	4	0.09 ± 0.02	95.2*
LASSBio 788	4	0.1 ± 0.02	93.7*
LASSBio 789	4	0.2 ± 0.01	89.6*

n - number of independent experiments carried out in triplicate.
* p<0.05 compared to appropriate control (Student t-test)

Figure 2- Thienylacylhydrazone Compounds Effects in Human Whole Blood



n - number of independent experiments carried out in triplicate.
* p<0.05 compared to appropriate control (Student t-test)

Table 3- Thienylacylhydrazone Compounds Inhibitory Potency in TXB_2 Formation in Human Platelets

Compounds	IC_{50} (µM)
LASSBio 294	63.0 ± 3.7
LASSBio 785	30.4 ± 3.4
LASSBio 786	34.1 ± 3.7
LASSBio 787	211.7 ± 1.2
LASSBio 788	2.6 ± 1.6
LASSBio 789	257.8 ± 1.9

Table 4- Thienylacylhydrazone Compounds Effects on Cyclic Nucleotides Production in Human PRP

Compounds (µM)	n	AMPc Production (fmol/well)	GMPc Production (fmol/well)
Control	3	5.4 ± 0.4	82.9 ± 7.6
DMSO	3	5.4 ± 0.3	88.9 ± 8.0
Milrinone (100)	3	8.1 ± 0.8*	193.3 ± 12.1*
EHNA (100)	3	5.5 ± 0.6 n.s.	171.3 ± 2.2*
PGE_1 (10)	3	16.0 ± 1.8*	96.7 ± 9.6*
Zaprinast (100)	3	5.5 ± 0.6 n.s.	178.8 ± 1.7*
AAS (100)	3	4.3 ± 1.0 n.s.	129.4 ± 12.7*
SNP (10)	3	4.3 ± 0.7 n.s.	251.5 ± 8.6*
SNP (100)	3	7.1 ± 0.6*	-
LASSBio 294 (100)	3	4.3 ± 0.7 n.s.	207.7 ± 16.9*
LASSBio 785 (100)	3	4.3 ± 0.0 n.s.	182.4 ± 9.4*
LASSBio 786 (100)	3	4.3 ± 0.7 n.s.	156.3 ± 12.3*
LASSBio 787 (100)	3	4.3 ± 0.0 n.s.	163.1 ± 9.6*
LASSBio 788 (100)	3	4.7 ± 0.4 n.s.	177.6 ± 14.8*
LASSBio 789 (100)	3	4.9 ± 0.4 n.s.	189.7 ± 8.9*

n - number of independent experiments carried out in triplicate.
* p<0.05 compared to appropriate control (Student t-test)

DISCUSSION AND CONCLUSION

✓ The new thienylacylhydrazone compounds have been able to interfere in platelet aggregation stimulated by different agonists, been more potent in collagen and AA induced platelet aggregation (PA). Among the compounds studied, LASSBio 785, 788 and 789 are the most potent presenting an IC50 for AA-PA of 0.3, 0.2 and 3.1 µM, for COL-PA of 0.9, 1.5 and 3.4 µM, respectively. They were 20-70 folds more potent than LASSBio 294 (Table 1).

✓ They inhibit the release reaction by 95% (Table 2) and the whole blood TXB2 formation (Table 3). In addition such derivatives have presented an in vivo effect increasing the bleeding time in mice and were able to inhibit the whole blood platelet aggregation by 35%-45% (Figure 2).

✓ Previous platelet functional studies in the presence of SNP suggested a PDE2-like effect for LASSBio 785, 788 and 789 (Brito *et al*, FESBE 2005). In non-stimulated platelets the AMPc levels were not modified by the compounds but they reversed the increase observed in PGE1-stimulated as EHNA, a PDE2 inhibitor (Table 4).

✓ On the other hand, they were able to elevate the GMPc levels in non-stimulated platelets and reversed the inhibition observed for ODQ (Table 4). They also elevated the GMPc levels in both SNP (SNP = 112.0±2.2; L785 = 197.6±20*; L788 = 217.3±6.8*; EHNA = 205.2±12.4* fmol/well) and SNP+PGE1 (SNP+PGE1 = 125.8±2.4; L785 = 216.4±16.7*; L788 = 255.7±16.6*; L789 = 297.9±15.2*) - stimulated platelets with a similar behavior of PDEs inhibitors.

The results suggested that the antiplatelet aggregation activity exert by the thienylacylhydrazones derivatives is through the regulation of cyclic nucleotides, mainly GMPc, and TXB2 inhibition. Taken together, these results shown that the structural modifications introduced in the compound LASSBio 294 led to an optimization of its pharmacological properties, indicating a potent antiplatelet activity and an antithrombotic potential for this new series of compounds.

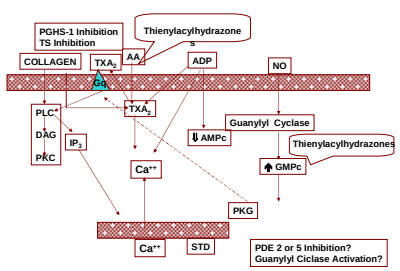


Figure 3- Antiplatelet Mechanism of Action of Thienylacylhydrazone Compounds

ACKNOWLEDGEMENTS

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