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Natural Product Derived Inhibitors of Lipoprotein Associated Phospholipase A_2, Synthesis and Activity of Analogues of SB-253514

Ivan L. Pinto,* Helen F. Boyd and Deirdre M. B. Hickey

SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, UK

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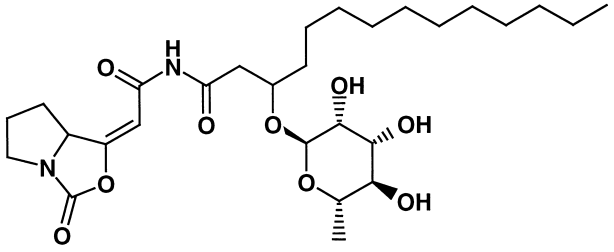
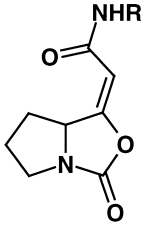
Abstract—The synthesis of analogues of SB-253514, a novel natural product derived inhibitor of lipoprotein associated phospholipase A_2 (Lp-PLA_2), is described together with their ability to inhibit Lp-PLA_2.

Lipoprotein associated phospholipase A_2 (Lp-PLA_2), also known as platelet-activating factor acetylhydrolyase, is a novel serine phospholipase that is responsible for hydrolysing PAF and oxidatively modified phosphatidylcholine.[1] The hydrolysis products, lysophosphatidylcholine and oxidised free fatty acids, have been shown to be pro-inflammatory causing macrophage proliferation and endolithial dysfunction.[2–4] It would be expected therefore that inhibitors of Lp-PLA_2 would have a beneficial role in the treatment of inflammatory diseases such as atherosclerosis.[5] As a result of a natural product screening exercise for inhibitors of Lp-PLA_2, SB-253514 was identified as one of a novel family of cyclic enol-carbamates isolated from *Pseudomonas fluorescens* which were found to be potent inhibitors of the enzyme.[6] The novel structure and potency of this natural product provided an attractive starting point for a programme directed at developing inhibitors of Lp-PLA_2 for the treatment of atherosclerosis.



SB-253514

It has already been shown that the sugar moiety, an α-rhamnose, did not contribute to the in vitro activity of SB-253514 against the isolated Lp-PLA_2 enzyme,[6] and it was speculated that the enol-carbamate moiety rather than the imide function was acting as an acylating agent to inactivate the lipase. As such we targetted the amide analogue **1** as a suitable analogue for investigating the scope of the enol-carbamate bicycle as a novel serine lipase inhibitor.



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Bicyclic enol-carbamates of this nature have not been exemplified in the literature to our knowledge and retrosynthetic analysis suggested that proline would be a appropriate starting material for the synthesis. Since the stereochemistry of the ring junction in the natural product SB-253514 was not established at the outset of this work we chose to use the natural (*S*) enantiomer of proline (Scheme 1). Borane reduction of (*S*)-Boc-proline followed by oxidation with pyridium chlorochromate provided the chiral aldehyde **4**, which was treated with the anion of diazomethane to yield the acetylene **5** in moderate yield.[7] Cyclisation to the enol-carbamate was effected by treatment with iodine and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (DEC)

*Corresponding author. Fax: +44-1279-627841; e-mail: ivan_pinto-1@sbphrd.com

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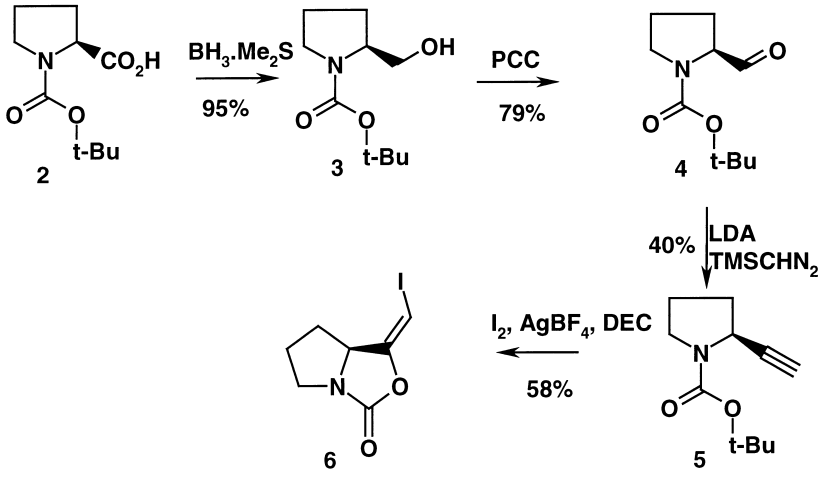
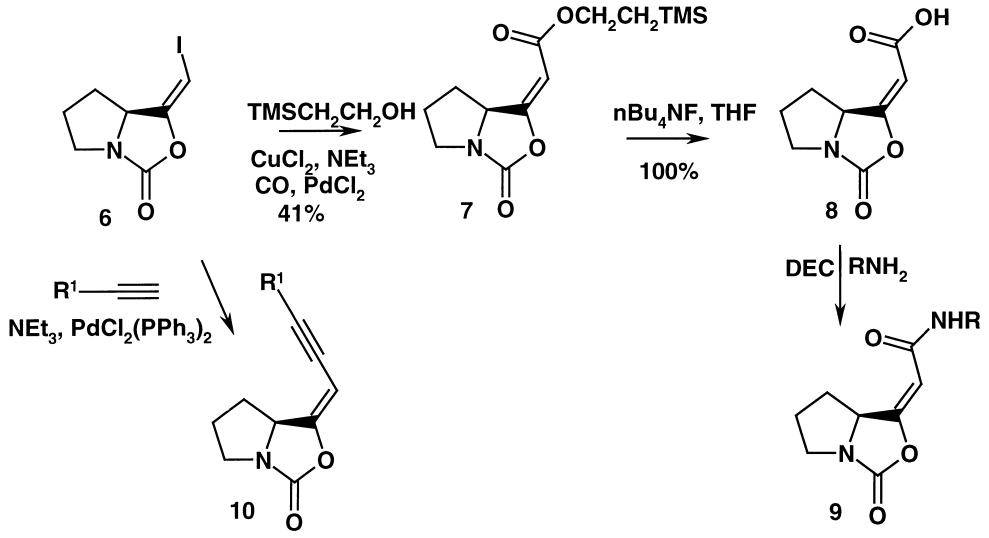


Scheme 1.

in the presence of silver tetrafluoroborate to yield a 9:1 *E:Z* mixture of vinyl iodides.[8]

Conversion of the *E*-vinyl iodide **6** to the amide **9** was achieved by first carbonylation of **6** with carbon monoxide in the presence of 2-trimethylsilylethanol and palladium(0) followed by deprotection with fluoride affording the acid **8** (Scheme 2).[9] The acid **8** was a key intermediate in the preparation of amide analogues such as **9** through coupling with suitable amines using DEC, while the iodide could also be converted to alkynyl derivatives such as **10** in good yield (Scheme 2).[9]

The enol-carbamates were assayed against Lp-PLA_2 using 1-decanoyl-2-(4-nitrophenylglutaryl)phosphatidylcholine as a substrate.[5] In the case of the amide analogues **9**, a time dependant inhibition of the enzyme was observed, such that without pre-incubation the IC_{50} varied from 2 to >10 μM. However a 10 minute pre-incubation was sufficient to observe maximum inhibition of the enzyme, and the amide analogues **9a–c** proved to be potent inhibitors of Lp-PLA_2 (Table 1). A similar time dependancy was observed for the natural product inhibitor SB-253514 (Table 1).[3] Potency for the amide series **9** improved with increasing chain length and lipophilicity, with the C-12 and C-16 chains **9a** and **9c** demonstrating IC_{50}s of 170 and 25 nm respectively. The inhibitory activity of **9c** was about 2-fold more potent that the natural product SB-253514. However lipophilicity does not appear to be the sole determinant of potency since the alkynyl derivatives **10a–c** were devoid of activity. It may be possible that the amidic carbonyl, which is conjugated into the enol ether, activates the carbamate towards the enzyme. It is unlikely that the inhibition of Lp-PLA_2 by **9** is simply a function of a highly reactive acylating system as **7** was devoid of any activity. It therefore appears that not only the activating capacity of the amidic carbonyl but also a long fatty chain is required for potency against the lipase. Stability studies were also carried out on **9a** at pH 7 (aqueous media) which demonstrated a half-life of 62 h, suggesting this series is not overtly hydrolytically unstable.



Scheme 2.

Table 1.

R	IC_{50} nM (10 min pre-incubation)	R^1	IC_{50} nM (10 min pre-incubation)
9a = $C_{12}H_{25}$	170	**10a** = C_4H_9OH	Inactive
9b = $C_{14}H_{29}$	28	**10b** = $C_{14}H_{29}$	Inactive
9c = $C_{16}H_{31}$ (*S* isomer)	25	**10c** = $C_4H_9O(C{=}O)C_7H_{15}$	Inactive
9d = $C_{16}H_{31}$ (*R* isomer)	12	SB-253514	51

All the above compounds were prepared from natural (*S*)-proline, however the absolute stereochemistry of SB-253514 was unknown at the inception of this programme of work. We therefore prepared the (*R*) isomer **9d** of the C_{16} compound **9c** to determine the influence of the ring stereochemistry on potency. The (*R*) isomer **9d** was only two fold more potent than the (*S*) isomer **9c** and it suggests that inhibition of the enzyme is not critically dependant on the ring junction stereochemistry. It was subsequently found that the stereochemistry of the natural product ring junction was in fact (*R*).[6] Inhibitory tests of **9c** against a range of serine proteases including elastase, trypsin and chymotrypsin as well as the metallo-protease thermolysin demonstrated no inhibition of these enzymes, demonstrating this novel series of serine lipase inhibitor is specific to Lp-PLA_2.

In conclusion, a novel and potent series of lipase inhibitor which is selective for Lp-PLA_2 has been disclosed. The inhibition of Lp-PLA_2 could have therapeutic potential for the treatment of atherosclerosis.

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References and Notes

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