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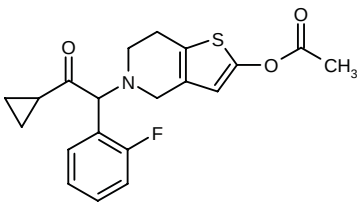
CCC: 0377-8282/2001

CS-747 and R-99224

Platelet Antiaggregatory
P2T Antagonist

CS-747

Acetic acid 5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-2-yl ester



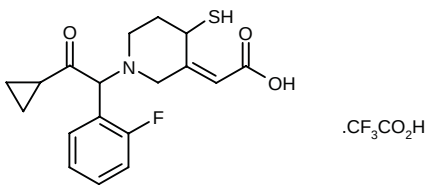
$C_{20}H_{20}FNO_3S$ Mol wt: 373.4460

CAS: 150322-43-3

EN: 273686

R-99224 (active metabolite of CS-747)

(*Z*)-2-[1-[2-Cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4-sulfanylpiperidin-3-ylidene]ethanoic acid trifluoroacetate



$.CF_3CO_2H$

$C_{18}H_{20}FNO_3S.C_2HF_3O_2$ Mol wt: 463.4459

CAS: 239466-75-2
CAS: 239466-74-1 (as free base)
CAS: 204204-72-8 (as hydrochloride)

En: 299858

Synthesis*

Synthesis of CS-747

CS-747 can be prepared by two related ways:

1) Reaction of the Grignard reagent prepared from 2-fluorobenzyl bromide (I) and Mg in ether with cyclopropyl cyanide (II) in the same solvent gives 1-cyclopropyl-2-(2-fluorophenyl)ethanone (III), which is brominated with Br_2 in CCl_4 to yield the α-bromoketone (IV). Condensation of ketone (IV) with 2,3,4,5,6,7-hexahydrothieno[3,2-*c*]pyridin-2-one hydrochloride (V) by means of K_2CO_3 in DMF affords the adduct (VI), which is finally treated with acetic anhydride and NaH in DMF (1). Scheme 1.

2) Reaction of 2,3,4,5,6,7-hexahydrothieno[3,2-*c*]-pyridin-2-one *p*-toluenesulfonate (VII) with TBDMS-Cl and TEA in dichloromethane gives the silylated enol ether (VIII), which is condensed with 1-cyclopropyl-2-chloro-2-(2-fluorophenyl)ethanone (IX) by means of TEA in the same solvent to provide the adduct (X). Finally, this compound is treated with TEA and DMAP and acetylated with Ac_2O (2). Scheme 2.

Synthesis of R-99224

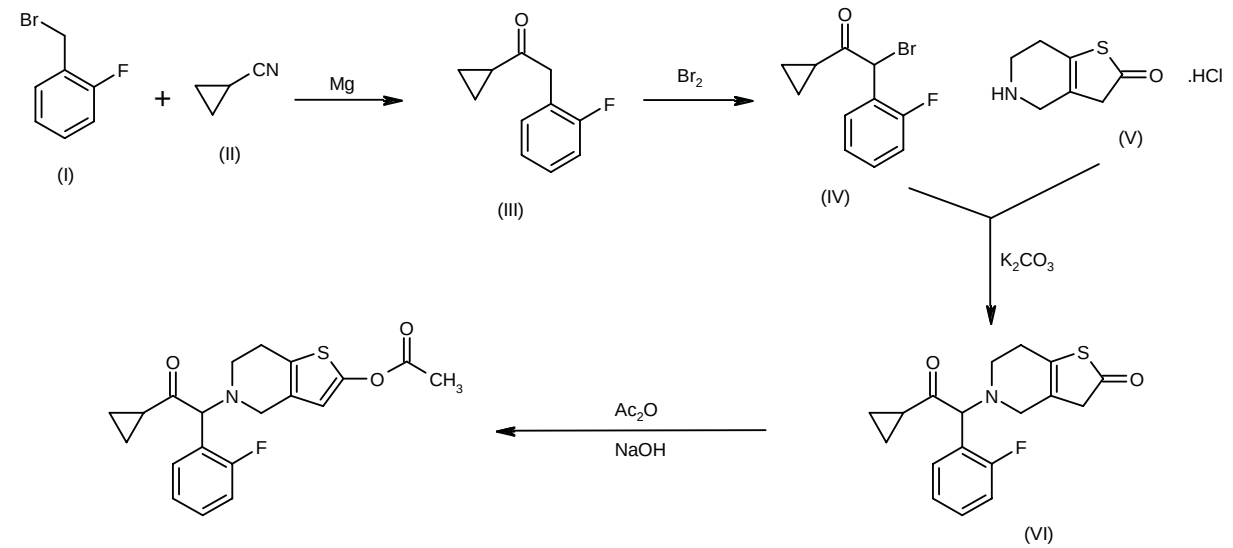
Protection of 4-piperidone (I) with trityl chloride and TEA gives 1-tritylpiperidin-4-one (II), which is condensed with ethyl 2-oxoacetate (III) by means of pyrrolidine in refluxing benzene to yield 3-(ethoxycarbonylmethylene)-1-tritylpiperidin-4-one (IV). The deprotection and simultaneous reduction of compound (IV) with $NaBH_4$ in methanol affords the 4-hydroxypiperidine derivative (V), which is condensed with 2-bromo-1-cyclopropyl-2-(2-fluorophenyl)ethanone (VI) by means of K_2CO_3 in DMF to provide the adduct (VII). Reaction of the OH group of (VII) with CBr_4 and PPh_3 in dichloromethane gives the 4-bromopiperidine derivative (VIII), which by reaction with potassium thioacetate (IX) in ethanol provides the 4-(acetylthio)piperidine derivative (X). Selective hydrolysis of compound (X) with HCl in ethanol yields the 4-sulfanylpiperidine derivative (XI), which is finally submitted to a new hydrolysis with HCl in acetic acid (3, 4). Scheme 3.

Introduction

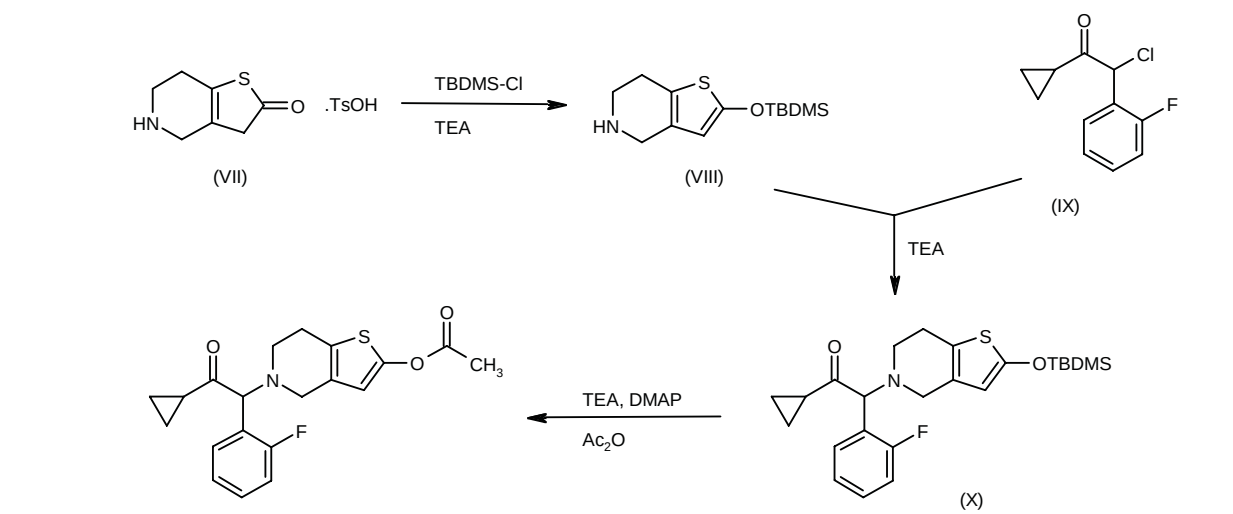
Adenosine 5'-diphosphate (ADP) is an important physiological and pathological platelet agonist. Upon vascular injury, ADP is released into the bloodstream from damaged cells and activated platelets, and in turn acts on

S.A. Doggrell[1], N.E. Mealy[2], J. Castañer[2]*. [1]Department of Physiology and Pharmacology, The University of Queensland, Brisbane, Qld. 4072, Australia; [2]Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

Scheme 1: Synthesis of CS-747



Scheme 2: Synthesis of CS-747

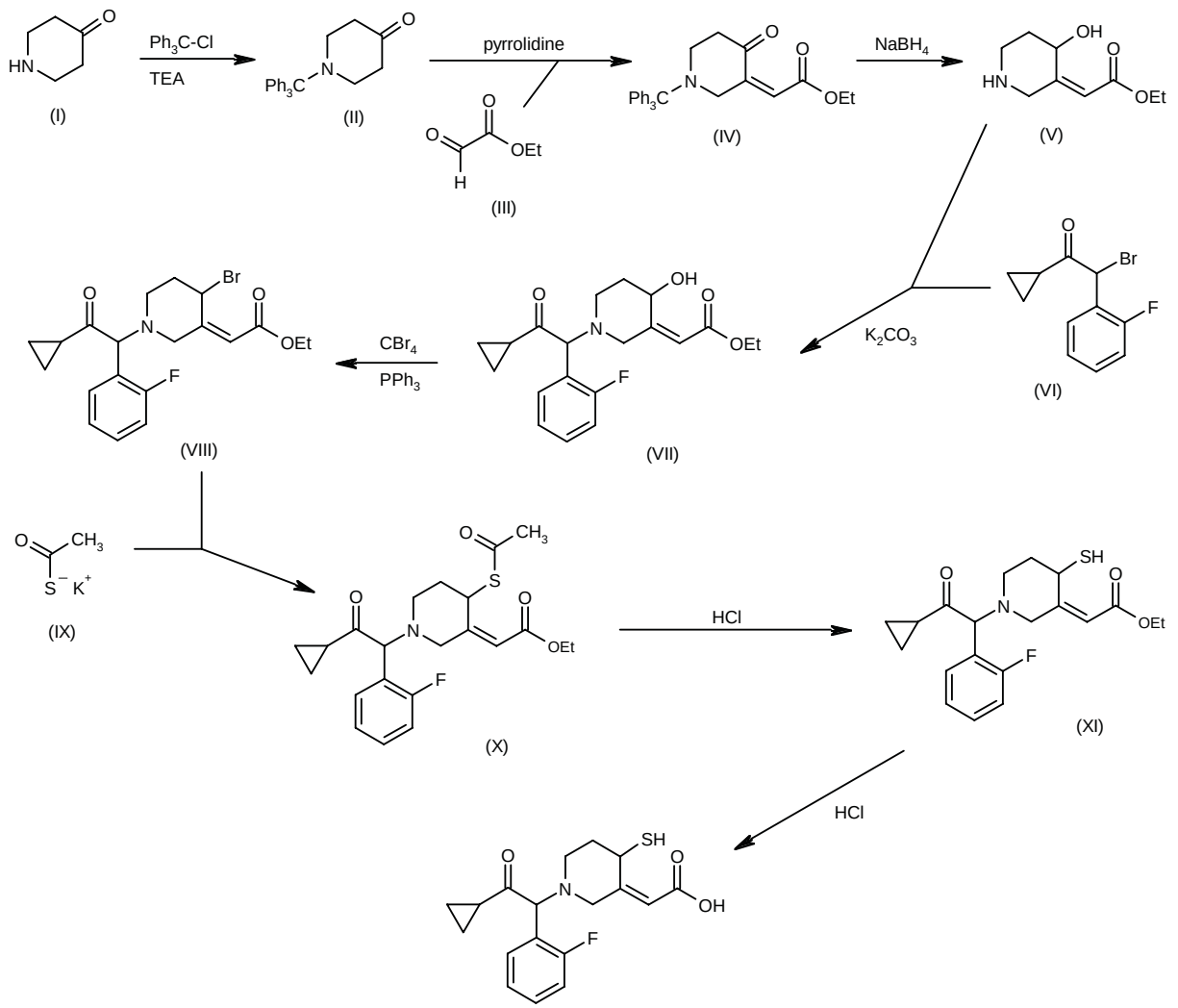


other platelets. ADP induces a number of platelet responses, including shape change from disc to sphere, aggregation and secretion of granule contents. These ADP-induced platelet responses contribute to hemostasis, pathological thrombus formation and vascular occlusion. These responses are considered to be mediated by the interaction of ADP with specific binding sites on the platelet membrane that have been tentatively designated as P2T receptors (5, 6). Interest in the development of P2T receptors as inhibitors of platelet aggregation has grown during the last 3 years, as seen in Table I. CS-747 is a new prodrug-type antiplatelet agent that acts as a $P2T_{AC}$ receptor antagonist via its active metabolite R-99224.

Pharmacological Actions

The effects of CS-747 have been compared to clopidogrel and ticlopidine, which are established inhibitors of

Scheme 3: Synthesis of R-99224



ADP-induced platelet aggregation. CS-747 at 300 μM had no effect on the binding of [^{3}H]-2-MeS-ADP to rat platelets (5), establishing that CS-747 itself has no effect on platelet aggregation. However, when CS-747 (0.3-10 mg/kg) was orally administered to rats, it partially decreased the binding of [^{3}H]-2-MeS-ADP to rat platelets *ex vivo* with a maximum effect of 43% (5, 7, 8). Clopidogrel was also a partial inhibitor of the binding, but it was 10 times less potent than CS-747. Ticlopidine has previously been shown to be a partial inhibitor of [^{3}H]-2-MeS-ADP binding. CS-747 (3 mg/kg p.o.) treatment neutralized ADP-induced decreases in platelet cAMP induced by PGE_1, suggesting that metabolites of CS-747 interfere with the G_i-linked P2T receptor (5).

CS-747 is selective for ADP-induced platelet aggregation. A single oral dose of CS-747 (0.3 and 3 mg/kg) markedly and dose-dependently inhibited *ex vivo* aggregation of washed platelets in response to ADP and collagen, but not to thrombin (5, 7). Platelet-derived ADP plays a major role in collagen-induced aggregation of rat platelets, and this probably explains the inhibitory effect of CS-747. The lack of effect on thrombin-induced aggregation indicates that the antiplatelet action of CS-747 is not due to interference with fibrinogen receptors (5).

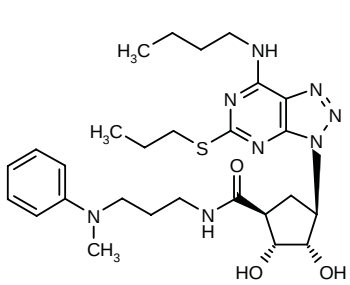
In platelet-rich plasma (PRP), CS-747 was more potent in inhibiting ADP-induced aggregation than clopidogrel or ticlopidine, with respective ED_{50} values at 4 h after single oral doses of 1.2 mg/kg, 16 mg/kg and > 300 mg/kg. The antiaggregatory effect of CS-747 was rapid in onset (< 0.5 h) and long lasting (> 3 days), which suggests that the inhibition may be irreversible (5, 8). Multiple oral doses of CS-747 also produced sustained inhibition

Table I: Companies with interest in P2T antagonists (patents from 1999 to 2001) [Prous Science Integrity database].

AstraZeneca
1. WO 9905142
2. WO 9905144
3. WO 9941254
4. WO 0004021
5. WO 0034283
6. WO 0119826
7. WO 0136421
8. WO 0136438

COR Therapeutics
9. WO 9936425

Sankyo/Ube Industries
10. WO 9943648
11. JP 1999315020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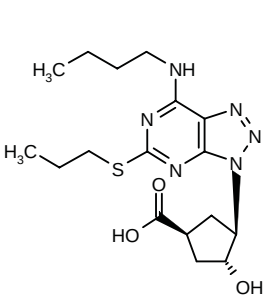
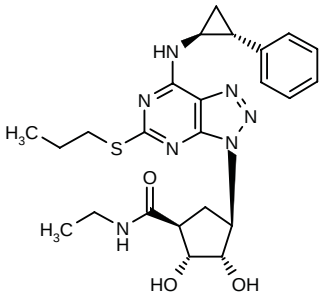

(2)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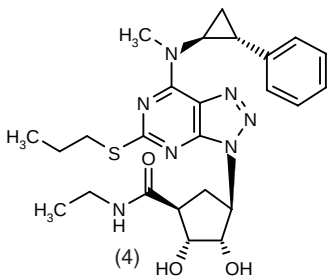
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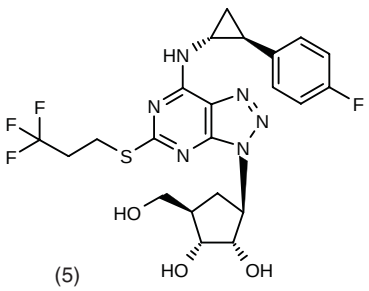
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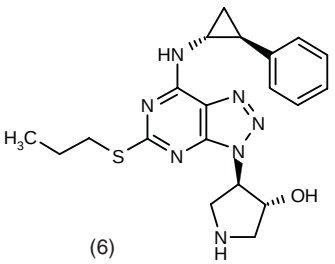
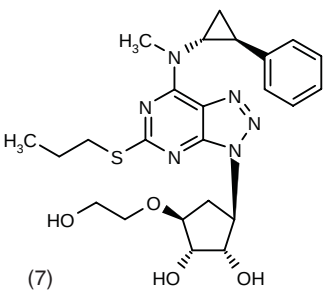
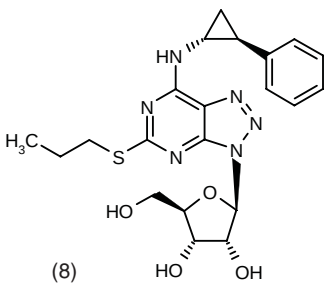
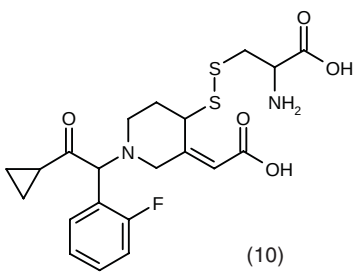

(6)


(7)


(8)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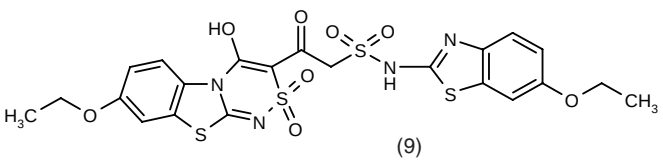
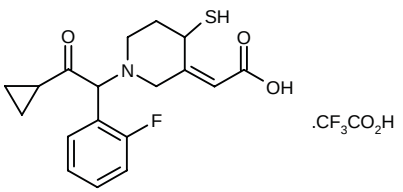

(9)


$.CF_3CO_2H$
(11)

of platelet aggregation in rats (8). These *ex vivo* antiplatelet effects of CS-747 were mimicked by the active metabolite R-99224 *in vitro* (5, 7).

A rat arteriovenous shunt model was used to examine the antithrombotic effects of the drugs. CS-747 (0.1-3 mg/kg p.o.) inhibited thrombus formation with an ED_{50} of 0.68 mg/kg. Clopidogrel was less potent, with an ED_{50} of 6.2 mg/kg, and the maximum effect of ticlopidine (10-300 mg/kg p.o.) was less than 40% (5, 8).

Rat tail transection bleeding times were measured to determine the antihemostatic effects of the drugs. The BT_2 (dose that doubles the bleeding time) value for CS-747 was 0.50 mg/kg p.o. compared to values of 4.6 and 130 mg/kg p.o. for clopidogrel and ticlopidine, respectively. The order of potency for antihemostatic effects was the same as for antiplatelet/antithrombotic efficacy, and CS-747 showed a similar benefit/bleeding risk ratio, *i.e.*, 1.4-2.4 *versus* 1.3-3.5 for clopidogrel and > 2.3 for ticlopidine (5, 8). Similar results were obtained in a rat carotid arterial thrombosis model using multiple doses of CS-747 (0.1-1 mg/kg/day x 3), clopidogrel (1-10 mg/kg/day x 3) and ticlopidine (30-300 mg/kg/day x 3) as regards both antithrombotic efficacy and bleeding risk (9).

R-99224 has been identified as the hepatic metabolite of CS-747, and further studies have been undertaken with this compound. R-99224 inhibits *in vitro* platelet

aggregation in washed human platelets, and was relatively selective for ADP (IC_{50} = 0.11 μg/ml) compared to collagen and thrombin. Similar effects were seen in washed rat platelets (IC_{50} = 0.42 μg/ml against ADP-induced aggregation). R-99224 (0.1-100 μM) decreased the binding of [^{3}H]-2-MeS-ADP to washed human platelets, an effect mediated by G_i-linked P2T receptors. Thus, R-99224 (10 μM) in combination with ARL-66096, an ATP analogue-type G_i-linked P2T antagonist, produced no additional inhibition of [^{3}H]-2-MeS-ADP binding. In contrast, inhibition of [^{3}H]-2-MeS-ADP binding was completely abolished by R-99224 in combination with A3P5PS, a selective $P2Y_1$ antagonist. *Ex vivo*, R-99224 gave an ED_{50} value of approximately 0.48 mg/kg i.v. for inhibition of ADP-induced platelet aggregation in rats (6).

Fibrinogen binding to platelets is the final common step of platelet aggregation. ADP-induced [^{125}I]-fibrinogen binding to platelets was inhibited by R-99224 (0.01-3 μg/ml) (6).

$P2Y_1$ receptors are linked to heterodimeric G-proteins that stimulate phospholipase C, which leads to mobilization of Ca^{2+} ions. Activation of $P2X_1$ receptors linked to Ca^{2+} channels also leads to elevation of intracellular Ca^{2+}. ADP acts at these receptors on platelets to increase intracellular Ca^{2+}, and this response is not altered by R-99224, providing further evidence that R-99224 does not act at $P2Y_1$ or $P2X_1$ receptors. R-99224 treatment neutralized ADP-induced decreases in cAMP induced by PGE_1, confirming that R-99224 antagonizes the G_i-linked P2T receptor (6).

Pharmacokinetics

The pharmacokinetics and metabolism of [^{14}C]-CS-747 have been evaluated in rats and dogs given a dose of 5 mg/kg p.o. Comparison of i.v. and p.o. data in rats demonstrated the good oral absorption of the drug (55-68%). Radioactivity was mainly eliminated in the feces within 72 h (73.2% in rats, 60.3% in dogs), and the rest was excreted in the urine. Tissue distribution studies in rats demonstrated the highest radioactivity in liver, followed by the kidney, adrenals, lung, blood and heart. No unchanged compound was detected in plasma in rats or dogs and the compound was extensively metabolized (10). The active metabolites were found to have free sulfhydryl groups, both in vitro in rat hepatocytes and following oral administration to rats and dogs (10, 11). The disulfide-type cysteine conjugates of the active metabolites were the major components in plasma. Like the parent compound, these conjugates were inactive *in vitro* but demonstrated pharmacological activity *in vivo* after i.v. dosing to rats (11).

Clinical Studies

The antiplatelet effects and pharmacokinetics of the major metabolites of CS-747 were also investigated in healthy male volunteers administered single and multiple oral doses of CS-747 or placebo. At single doses above 30 mg, ADP-induced platelet aggregation was inhibited by more than 50%, and this effect was rapid in onset (1 h) and sustained (48 h). Food had no significant effect on the pharmacokinetics of CS-747 metabolites. Following multiple doses (2.5 or 10 mg once daily for 10 days), antiplatelet effects were seen for the higher dose at 2 days after the first administration. No serious adverse events were observed and bleeding time was temporarily prolonged. Pharmacokinetics of the metabolites were proportional to dose in all cases. Thus, CS-747 is a potent and long-acting platelet aggregation inhibitor in man and is well tolerated. It is considered to have potential in the treatment of patients with unstable angina or at risk of myocardial infarction or stroke (12).

Lilly and Sankyo have signed a letter of intent to collaborate on CS-747, which is being tested in phase I human clinical trials for its ability to prevent blood clotting in the coronary artery. The novel oral compound was discovered by Sankyo and Ube and will be developed for the secondary prevention of thrombotic cardiovascular complications in patients with a recent ischemic stroke or with acute coronary syndromes. CS-747 will also be developed for reducing secondary complications, including death, recurrent myocardial infarction, recurrent stroke and rehospitalization for severe angina (13).

Manufacturer

Sankyo Co., Ltd. (JP) and Ube Industries, Ltd. (JP); licensed to Eli Lilly and Company (US).

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13. *Lilly and Sankyo to collaborate on CS-747 development.* DailyDrugNews.com (Daily Essentials) December 28, 2000.