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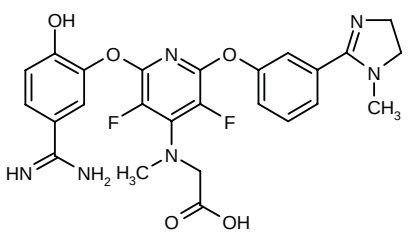
CCC: 0377-8282/2002

ZK-807834

Anticoagulant
Factor Xa Inhibitor

BX-807834
CI-1031
PD-200022
ZK-807191 (as dihydrochloride)

N-[2-(5-Amidino-2-hydroxyphenoxy)-3,5-difluoro-6-[3-(1-methyl-4,5-dihydroimidazol-2-yl)phenoxy]pyridin-4-yl]-*N*-methylglycine



$C_{25}H_{24}F_2N_6O_5$
Mol wt: 526.4976
CAS: 183305-24-0
CAS: 183304-36-1 (as monohydrochloride)
CAS: 213839-64-6 (as dihydrochloride)
EN: 261986

Abstract

Factor Xa (FXa) is a strategic target for antithrombotic therapy due to its central location at the point where the extrinsic and intrinsic coagulation pathways converge. Selective inhibitors of FXa have demonstrated an enhanced efficacy-to-bleeding ratio when compared to other antithrombotic strategies. ZK-807834 is a newly synthesized FXa inhibitor with high affinity for FXa that in animal models has been shown to be a potent and long-lasting antithrombotic agent, with no effect on platelet aggregation.

Synthesis*

ZK-807834 is obtained by condensation of the diaryl ether (I) with the phenol (II) by means of K_2CO_3 to afford the bisaryl ether (III). Reaction of the cyano group of (III), first with HCl in ethanol and then with ammonia, yields the corresponding amidino compound (IV), which is finally demethylated with HBr (1, 2). Scheme 1.

The diaryl ether (I) is synthesized as follows: Reaction of pentafluoropyridine (V) with sarcosinamide (VI) in dichloromethane gives N^2-(2,3,5,6-tetrafluoropyridin-4-yl)sarcosinamide (VII), which is condensed with 3-hydroxy-4-methoxybenzonitrile (VIII) by means of Cs_2CO_3 or K_2CO_3 in acetonitrile (1). Scheme 1.

3-(1-Methyl-4,5-dihydro-1*H*-imidazol-2-yl)phenol (II) can be obtained as follows: Reaction of 3-methoxybenzonitrile (IX) with *N*-methylethylene-1,2-diamine (X) by means of HCl in ethanol gives 2-(3-methoxyphenyl)-1-methyl-4,5-dihydro-1*H*-imidazole (XI), which is demethylated by means of refluxing 48% HBr (1). Scheme 1.

Introduction

The key event initiating blood coagulation after tissue injury is the exposure of blood to tissue factor (TF), a transmembrane receptor that binds activated factor VII (factor VIIa), which is present in trace amounts in circulating blood. This complex catalyzes the activation of factor IX and factor X. Factor IXa then binds to factor VIIIa to form the tenase complex that activates factor X. The principal role of factor Xa after being activated by TF-VIIa is to generate small amounts of thrombin in the proximity of platelets, enhancing their activation. Moreover, factor Xa promotes coagulation by binding to factor Va on membrane surfaces to form the prothrombinase complex. This complex first converts prothrombin into thrombin, which converts fibrinogen into fibrin. Thrombin amplifies its own generation by feedback activation of factors V, VIII and XI. Blood coagulation is controlled by several mechanisms, including the heparin-antithrombin interaction, the tissue factor pathway inhibitor (TFPI), activated protein C and the fibrinolytic systems (3).

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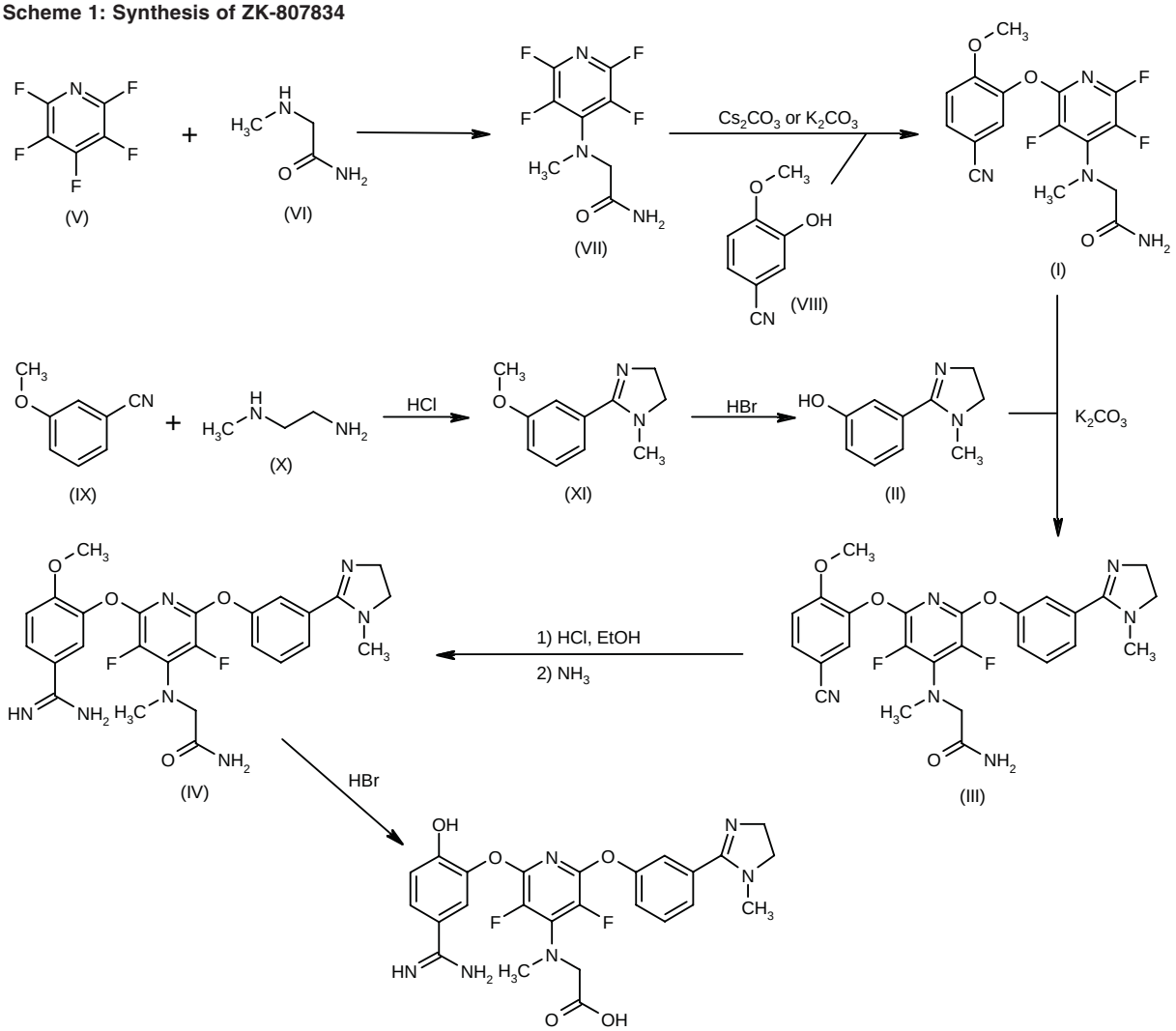
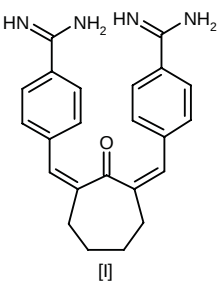
Scheme 1: Synthesis of ZK-807834



Factor Xa (FXa) is a key enzyme in the coagulation cascade because it is positioned at the conversion point of the extrinsic and intrinsic coagulation pathways. It is, therefore, a strategic target for antithrombotic therapy. Unlike thrombin inhibitors, FXa inhibitors do not have a direct effect on platelet aggregation and do not adversely affect the endogenous anticoagulant pathway mediated by thrombomodulin and activated protein C (4). There are selective inhibitors of factor Xa that can be classified according to their indirect or direct effect. Indirect inhibitors, such as pentasaccharide, require antithrombin for their action and only inhibit the activity of free factor Xa. Direct inhibitors bind directly to factor Xa inactivating both free factor Xa and factor Xa in the prothrombinase complex (5). In addition, FXa inhibitors prevent the generation of thrombin, disrupting the thrombin feedback loop that amplifies thrombin production (6), which may result in attenuated platelet activity and clot stabilization.


[I]

ZK-807834 is a novel synthetic inhibitor of the blood coagulation enzyme FXa, derived from the transformation of the photochemically unstable FXa inhibitor (*Z*,*Z*)-2,7-bis(4-amidinobenzylidene)cycloheptan-1-one, (*Z*,*Z*)-BABCH [I] (2). Binding to FXa was increased by

introducing a hydroxyl group on the proximal phenylamidine ring and fluorine atoms at the C-3 and C-5 of the pyridine ring. Pharmacokinetic parameters were improved by balancing the contributions from the substituents on the distal ring and the central pyridine ring. The optimal combination was a methyl-(2*H*)-imidazoline group on the distal ring and a sarcosine at C-4 of the pyridine ring (7).

Inhibition of FXa by selective potent small molecules such as ZK-807834 seems to offer a greater potential benefit/risk ratio as opposed to other antithrombotic approaches.

Pharmacological Actions

ZK-807834 is a newly synthesized, potent and specific FXa inhibitor with a K_i of 0.11 nM against human FXa (2). *In vitro* studies demonstrated that this compound is very selective for FXa compared to other serine proteases (2, 8). Results derived from different studies indicate that ZK-807834 is an effective antithrombotic compound with a favorable efficacy-to-bleeding ratio.

Abendschein *et al.* (9) carried out a study designed to determine whether ZK-807834, administered during and briefly after pharmacologic coronary fibrinolysis, increases 24-h patency. The effect of ZK-807834 (at concentrations ranging from less than or equal to 1.6-13 mg/kg) was compared to a peptide inhibitor of FXa, recombinant tick anticoagulant peptide (rTAP, 13.6 mg/kg), and heparin (150 U/kg bolus and 50 U/kg/h infusion) plus aspirin (5 mg/kg). Administration was performed i.v. over 135 min in dogs after thrombotic occlusion induced by electrical injury to a coronary artery. Fibrinolysis was induced with recombinant human tissue-type plasminogen activator (1.0 mg/kg i.v. over 1 h), and patency was monitored continuously for 24 h with an implanted Doppler probe. Results from this study showed that high-dose ZK-807834 prevented reocclusion in 5 of 6 dogs and delayed reocclusion in 1 dog. Patency after 24 h was 100% in ZK-807834-treated and rTAP-treated dogs compared with 67% in control and 83% in heparin/aspirin-treated dogs. High-dose ZK-807834 resulted in a 3.7-fold increase in prothrombin time (PT), a 4.9-fold increase in activated partial thromboplastin time (aPTT) and a 2.5-fold increase in bleeding time, compared with 1.2-fold, 11.5-fold and 2.3-fold increases, respectively, for heparin/aspirin. It was concluded that inhibition of FXa with ZK-807834 decreases reocclusion and improves patency of recanalized arteries without increasing bleeding compared with heparin/aspirin.

McClanahan *et al.* (9) demonstrated the effectiveness of ZK-807834 as an antithrombotic agent in a canine electrolytic injury model of arterial and venous thrombosis. The study compared the effect of ZK-807834 (at doses of 1.25, 2.5, and 5 μg/kg/min administered as a continuous infusion for 5.5 h) with enoxaparin. After a 90-min infusion, PT increased 1.2-, 1.6- and 2.0-fold at the doses assessed, respectively, over baseline values. Administration of ZK-807834 provided dose-dependent efficacy in increasing the time to thrombus formation (153 ± 22, 137 ± 30 and 214 ± 26 min, respectively, *vs.* 56 ± 11 min in femoral veins, and 127 ± 19, 192 ± 33 and 219 ± 15 min, respectively, *vs.* 69 ± 5 min in femoral arteries), as well as decreasing the thrombus weight (75 ± 16, 51 ± 16 and 25 ± 4 mg, respectively, *vs.* 96 ± 18 mg in femoral veins, and 45 ± 5, 28 ± 10 and 15 ± 3 mg, respectively, *vs.* 51 ± 4 mg in femoral arteries). A plasma concentration of 400 ng/ml produced a 50% reduction in thrombus weight in treated animals as compared to control animals. It was concluded that ZK-807834 may be a safe and effective antithrombotic agent for both arterial and venous indications, providing dose-dependent efficacy with minimal changes in *ex vivo* coagulation parameters.

In another study using the same experimental model (11), the effect of ZK-807834 was evaluated at a maximally effective dose (25 μg/kg i.v. bolus followed by 2.5 μg/kg/min) combined with clinically relevant doses of aspirin (5 mg/kg i.v. bolus) and the GPIIb/IIIa inhibitor, tirofiban (25 μg/kg i.v. bolus followed by 2.5 μg/kg/min). Results were compared with equally effective doses of enoxaparin (1 mg/kg i.v. bolus followed by 0.6 μg/kg/min). Administration of aspirin plus ZK-807834 prolonged PT and aPTT 3.2- and 2.0-fold, respectively, compared to 1.1- and 1.4-fold, respectively, for aspirin plus enoxaparin. Addition of tirofiban to both combinations resulted in no changes in PT and aPTT, but increased the template bleeding time 3.9-fold when combined with aspirin plus ZK-807834 and 4.9-fold when added to aspirin plus enoxaparin. Surgical blood loss was greater with the triple combination of aspirin, enoxaparin and tirofiban. It was concluded that ZK-807834 may provide a better safety margin than enoxaparin when used in combination with aspirin and GPIIb/IIIa antagonists.

In a balloon-induced arterial injury model of thrombosis in hypercholesterolemic rabbits, ZK-807834 was found to be effective in inhibiting thrombus formation (12). At bolus doses of 0.01 and 0.03 mg/kg i.v., ZK-807834 decreased thrombus mass from 20 ± 1 mg (in the control group) to 13 ± 2 and 3 ± 1 mg, respectively. This effect was similar to that observed for enoxaparin and for an active site-blocked factor VIIa (FVIIai). ZK-807834 and FVIIai prolonged PT nearly 2-fold and had less effect on aPTT and bleeding time than enoxaparin. It was suggested that ZK-807834 may be beneficial in patients suffering from acute coronary syndromes.

Using a porcine model of deep carotid arterial injury, Chesebro *et al.* (13) evaluated the ability of ZK-807834 both to inhibit thrombus formation and to promote deaggregation of acutely formed platelet-rich arterial thrombi *in vivo*. Pigs received ZK-807834 at doses of 40 μg by bolus followed by 1.5 μg/min infusion, 20 μg by bolus followed by 1.5 μg/min infusion, 1 μg/kg bolus enoxaparin, or saline. The highest concentration of ZK-807834 prolonged the PT by 2.2-fold, inhibited the formation of carotid thrombi and deaggregated thrombi in all animals. The lower dose of ZK-807834 and enoxaparin were

similar to saline in their effect on thrombus formation. Thrombus deaggregation was achieved in 50% and 17% of the animals treated with the lower dose of ZK-807834 and enoxaparin, respectively. Findings from this study indicate that inhibition of factor Xa with ZK-807834 at a dose sufficient to prolong the PT-ratio by 2.2 effectively inhibits thrombus formation and deaggregates thrombi induced by a full-thickness deep arterial injury.

Recent studies have evaluated the pharmacodynamic markers during inhibition of thrombosis by ZK-807834 in rabbits (14). The *in vivo* antithrombotic effect of ZK-807834 (bolus injection of 60, 240 or 480 μg/kg followed by an infusion of 2, 8 or 16 μg/kg/min for 140 min, respectively) was evaluated in a veno-venous shunt model of thrombosis in anesthetized rabbits. ZK-807834 dose-dependently prolonged time to occlusion (35 ± 21, 62 ± 24 and 120 ± 0 min for the three dose groups, respectively, *vs.* 10 ± 1 min for vehicle), and reduced thrombus mass (42 ± 7, 27 ± 6 and 18 ± 4 mg *vs.* 50 ± 4 mg for vehicle). Maximal thrombus mass reduction was 70% with an IC_{50} of 0.6 μg/ml. Among all the coagulation parameters tested, PT had the best correlation with plasma ZK-807834 concentration (r = 0.97). *Ex vivo* plasma anti-FXa activity also correlated well with plasma concentration of ZK-807834 and with PT (r = 0.96 and 0.98, respectively). From these results it was concluded that ZK-807834 concentration in plasma can be monitored using PT or anti-Xa assays, providing reliable methods to ensure safe and accurate dose titration of ZK-807834.

Post *et al.* (15) have recently reported the human *in vitro* anticoagulant and pharmacodynamic profile of ZK-807834. In the range of 0.3-0.5 μM, ZK-807834 prolonged PT and aPTT 2-fold without affecting thrombin time. The potent prolongation of PT caused by ZK-807834 was independent of gender and lacked intersubject variability. It inhibited FXa in clot-bound prothrombinase with an average IC_{50} of 10 ± 7 nM. ZK-807834 exhibited no direct effect on ADP- or collagen-induced platelet aggregation, indicating selective inhibition of FXa. The *in vitro* permeability and lipophilicity profile of ZK-807834 was also determined. It was concluded that due to the hydrophilic nature of this compound, which may limit the extent of intestinal absorption, additional formulations would be necessary to consider ZK-807834 for oral administration.

Zafar *et al.* (16) evaluated the effect of ZK-807834 on thrombus formation using the Badimon perfusion chamber. ZK-807834 was administered to 18 stable coronary artery disease (CAD) patients blindly randomized to receive the compound at concentrations to achieve PT ratios of 1.125, 1.5 and 2.5, or placebo, as a 24-h infusion. Thrombus formation was measured on the Badimon chamber at 0 and 8 h after the end of infusion and compared with pretreatment values. The greatest thrombus reduction at both times was observed with the higher dose (around 60% and 30% reduction in thrombus growth, respectively), thus exhibiting a long-lasting antithrombotic effect.

ZK-807834 is currently undergoing phase II clinical trials for the treatment of acute coronary disease.

Source

Berlex Laboratories, Inc. (US); codeveloped with Pfizer, Inc. (US).

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