Drugs of the Future 2007, 32(4): 310-314

CCC: 0377-8282/2007
DOI: 10.1358/dof.2007.032.04.1091152

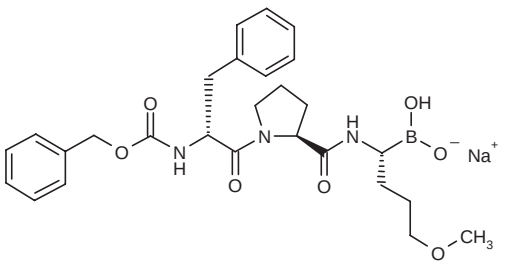
# Flovagatran Sodium

*Thrombin Inhibitor*
*Anticoagulant*

TGN-255

2(*R*)-[*N*-(Benzyloxycarbonyl)-D-phenylalanyl-L-prolylamino]-4-methoxybutylboronic acid sodium salt

InChI=1/C27H35BN3O7.Na/c1-37-17-9-15-24(28(35)36)30-25(32)23-14-8-16-31(23)26(33)22(18-20-10-4-2-5-11-20)29-27(34)38-19-21-12-6-3-7-13-21;/h2-7,10-13,22-24,35H,8-9,14-19H2,1H3,(H,29,34)(H,30,32);/q-1;+1/t22-,23+,24+;/m1./s1



$C_{27}H_{35}BN_3NaO_7$
Mol wt: 547.3836

CAS: 871575-98-3

CAS: 871576-03-3 (free acid)

EN: 353226

**Abstract**

Flovagatran sodium (TGN-255) is a potent, reversible, low-molecular-weight, synthetic direct thrombin inhibitor that has demonstrated promising pharmacokinetic properties and biological activity in preclinical studies. At a mean plasma concentration of 12 μg/ml, flovagatran prolonged the activated clotting time (ACT) and thrombin time (TT) by 480 and 245 s, respectively, in dogs. Phase I trials in healthy volunteers showed that infusion of flovagatran increased thrombin clotting time 4.5-7.5-fold over baseline values. The studies also indicated that the TT ratios and flovagatran plasma concentrations were well correlated. In light of its good safety profile and promising antithrombotic effects, flovagatran is set to enter phase III clinical development.

## Synthesis

Flovagatran sodium can be synthesized as follows:

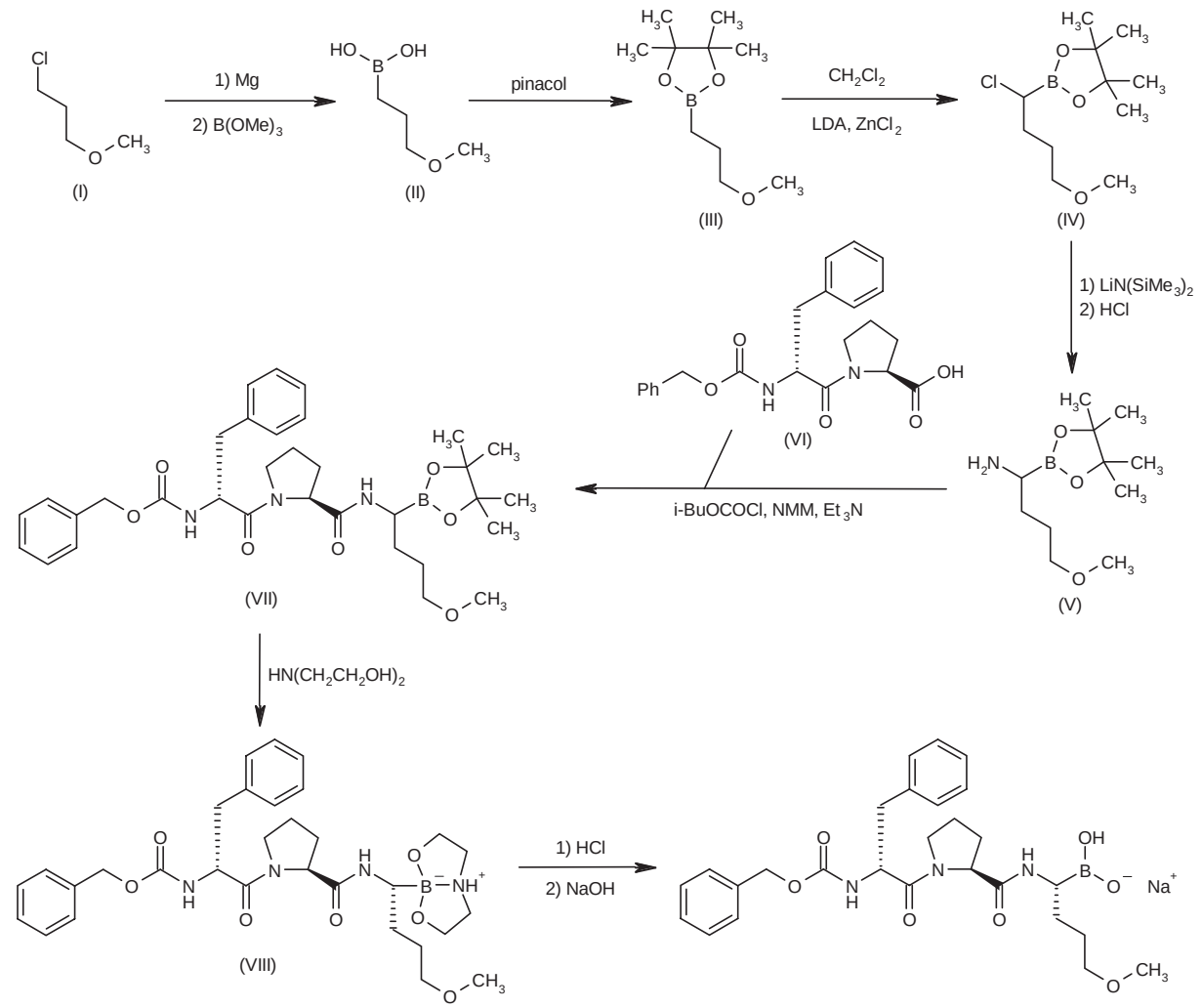
3-Methoxypropyl chloride (I) is converted to the corresponding Grignard reagent by means of Mg in THF, followed by reaction with trimethyl borate and quenching with aqueous sulfuric acid to produce 3-methoxypropylboronic acid (II). Subsequent treatment of boronic acid (II) with a toluene or heptane solution of pinacol gives the pinacol boronate (III). One-carbon insertion into (III) is accomplished by treatment with dichloromethane, LDA and zinc chloride to furnish the 1-chloro-4-methoxybutylboronate (IV). Substitution of the chloride (IV) with lithium hexamethyldisilazide in THF, followed by desilylation with HCl in cold *n*-heptane, leads to the aminoboronate (V), which is then coupled with *N*-benzyloxycarbonyl-D-phenylalanyl-L-proline (VI) via activation as the mixed anhydride with isobutyl chloroformate to afford the tripeptide analogue (VII). The diastereomeric mixture of pinacol boronates (VII) undergoes transesterification and epimerization with diethanolamine in boiling diethyl ether to provide the diethanolamine adduct (VIII). Subsequent acidic hydrolysis of adduct (VIII) gives the boronic acid flovagatran, which is finally isolated as the corresponding sodium salt by treatment with sodium hydroxide in acetonitrile/water (1-3). Scheme 1.

## Background

Thrombin, an extracellular trypsin-like serine protease, plays a dual role in the development of thrombotic events, acting as both a regulator of fibrin clot formation and as a potent inducer of platelet activation and aggregation. Also known as activated factor II (FIIa), thrombin is the final enzyme in the coagulation cascade. The first event initiating blood coagulation after tissue injury is the exposure of blood to tissue factor (TF), a transmembrane protein that binds activated factor VII (FVIIa), which is present in trace amounts in circulating blood. This complex catalyzes the activation of FIX and FX. FIXa then binds to FVIIIa to form a complex that activates FX. The principal role of FXa after being activated by TF-FVIIa is to generate small amounts of thrombin in the proximity of platelets, enhancing their

Y. Wang. 8106 Runnymeade Dr., Frederick, MD 21702, USA. N. Mealy, N. Serradell, J. Bolós, E. Rosa. Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

**Scheme 1: Synthesis of Flovagatran Sodium**




activation. Moreover, FXa promotes coagulation by binding to FVa on membrane surfaces to form the prothrombinase complex. This complex first converts prothrombin (FII) to thrombin (FIIa), which then converts fibrinogen to fibrin. Fibrin then polymerizes, forming the strand network of the developing clot. Through positive feedback and activation of FVa, FVIIIa and FXI, thrombin enhances its own production (4-6) (see Fig. 1).

Because of the crucial role of thrombin in the coagulation cascade and platelet activation, the enzyme is a primary target for the development of anticoagulant and antithrombotic drugs. Both direct and indirect thrombin inhibition strategies are currently available. Indirect thrombin inhibition via suppression of precursor coagulation proteins is associated with undesirable properties such as bleeding and interpatient dosing variability. In contrast, selective direct thrombin inhibitors are not associated with these undesirable effects since they bind directly to thrombin and block its interactions with its substrates. Low-molecular-weight heparins (LMWH) indirectly inhibit thrombin by strongly catalyzing the function of antithrombin (7). Although there is less interpatient variability with LMWH, they are ineffective against clot-bound thrombin and may cause thrombocytopenia (8, 9). In contrast, direct thrombin inhibitors act independently of antithrombin, so they can inhibit thrombin bound to fibrin or fibrin degradation products (9, 10). Direct thrombin-inhibitory drugs can block the action of the enzyme by binding to three domains: the active site or catalytic site and two exosites (11, 12).

Flovagatran sodium (TGN-255) is a novel, potent, selective, reversible, low-molecular-weight direct thrombin inhibitor with a good pharmacodynamic, pharmacokinetic and safety profile. It is initially being developed for preventing coagulation in hemodialysis patients at risk of developing heparin-induced thrombocytopenia (HIT). Flovagatran has completed a phase II trial for preventing coagulation in hemodialysis patients, and is in earlier clin-

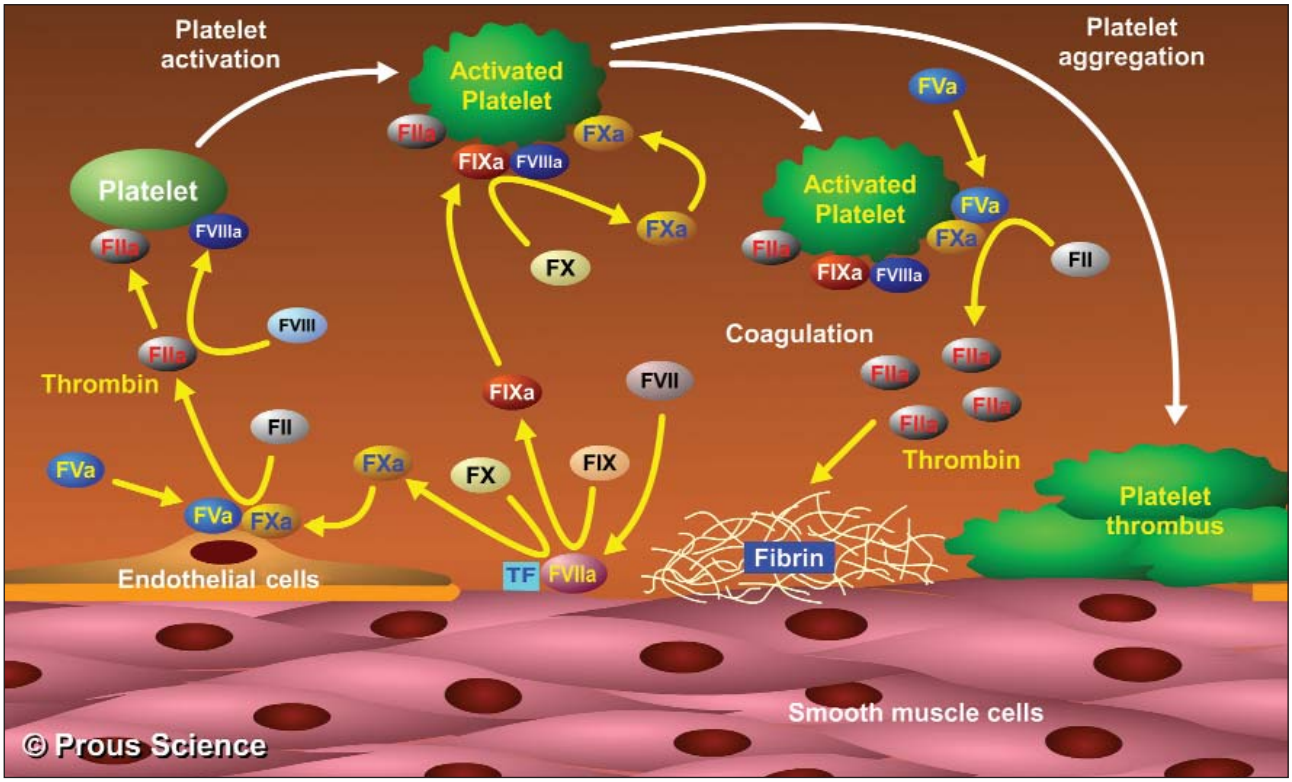



Fig. 1. Dual role of thrombin in coagulation and platelet aggregation. After damage of vessel endothelium, contact occurs between blood and tissue factor (TF) exposed on subendothelial cells. This leads to the activation of small amounts of prothrombin (FII) to thrombin (FIIa), which locally activates platelets. Thrombin activates FVII, which in turn directly activates FX on the surface of locally activated platelets. FXa promotes coagulation by binding to FVa on the platelet membrane surface to form the prothrombinase complex. This complex converts large amounts of prothrombin to thrombin, which converts fibrinogen to fibrin. Activated platelets adhere to and aggregate on the damaged vessel wall. Together with the polymerized fibrin strand, aggregated platelets form a stable thrombus. Subscribers to the on-line version of *Drugs of the Future* and/or Integrity® can access the animation: Coagulation Cascade Events Triggered After Atherosclerotic Plaque Rupture.

ical development (phase I) for preoperative anticoagulation in coronary artery bypass graft (CABG) surgery and for preventing thrombosis during percutaneous coronary intervention (PCI). A phase III program consisting of three randomized, multinational studies has been designed to evaluate the efficacy and safety of flovagatran for the prevention of clotting in hemodialysis patients who can receive heparin, as well as those with HIT (13, 14).

## Preclinical Pharmacology

*In vitro* experiments were conducted to compare the properties of flovagatran and melagatran. Both compounds displayed potent inhibition of thrombin ($K_i$ = 7-22 and 4 nM, respectively, for flovagatran and melagatran) and excellent selectivity relative to plasmin and factor Xa ($K_i$ = 7800-15,000 and 1730-2900 nM, respectively); flovagatran, but not melagatran, also showed 30-fold selectivity relative to trypsin ($K_i$ = 711 nM). Doubling of the thrombin time (TT) in human plasma was observed at concentrations of flovagatran and melagatran of 960 and 24 nM, respectively, whereas the activated partial thromboplastin time (aPTT) was doubled at respective concentrations of 15,000 and 918 nM. The high plasma protein binding of flovagatran (90%) was suggested to account for the higher concentration required to double the TT compared to melagatran (15, 16).

Flovagatran induced a linear, concentration-dependent increase in the ecarin clotting time (ECT) in human plasma samples over a broad range of concentrations, with an increase to 370 s (from 25 s at baseline) at 5.6 μg/ml, similar to other direct thrombin inhibitors, including bivalirudin (to 380-480 s at 10-15 μg/ml), lepirudin (to 270-320 s at 4-5 μg/ml), argatroban (to 317-440 s at 1-2 μg/ml) and melagatran (to 330-450 s at 1-1.6 μg/ml). It is suggested that, in addition to activated clotting time (ACT), measurement of ECT may be useful for monitoring anticoagulation with direct thrombin inhibitors (17).

The anticoagulant effects of flovagatran were studied *in vivo* using a model of cardiopulmonary bypass (CPB) and simulated mitral valve repair in dogs. Flovagatran was associated with excellent anticoagulation during the surgery and little bleeding, and the animals showed good cardiac function after the bypass procedures. Analysis of plasma samples revealed that flovagatran at a dose of 2.5 mg/kg by bolus injection plus 10 mg/kg/h by infusion provided plasma concentrations of 10-20 μg/ml, and at a mean plasma concentration of 12 μg/ml, it prolonged the

ACT and TT by 480 and 245 s, respectively. ECT was also increased by 5-6-fold throughout the CPB procedure. The analysis also showed that plasma concentrations declined rapidly after termination of flovagatran infusion (18, 19).

### Pharmacokinetics and Metabolism

The pharmacokinetics of flovagatran were evaluated in a double-blind phase I study performed in 16 healthy male volunteers who were randomized to receive infusions of placebo or flovagatran 25 or 40 mg/h for 24 h. The drug was rapidly absorbed and plasma concentrations rapidly increased after the start of infusion. The disposition of flovagatran showed a short distribution phase with a half-life of 8 min and a systemic half-life of about 2.3 h (20, 21). Similar results were obtained in another double-blind, ascending-dose study in healthy male subjects who received placebo or flovagatran as a bolus dose of 7 mg followed by 3-h infusion of 25 mg/h, a bolus of 10 mg followed by a 3-h infusion of 40 mg/h or a 3-h infusion of 40 mg/h. Plasma levels increased rapidly (2 min) following boluses, with a short distribution phase (half-life = 6 min) and a systemic half-life of 1.7 h (22). Data from these two trials indicated good systemic exposure and distribution, with a volume of distribution of about 40 l, and steady state was achieved within 4-6 h of starting infusions; 14% of the dose was excreted in the urine as unchanged drug (20, 21, 23).

The pharmacokinetics of flovagatran were also studied in an open-label, heparin-controlled, crossover study in 12 healthy male volunteers who received flovagatran as a bolus dose of 25 mg followed by a 3-h infusion of 40, 80 or 160 mg/h, or heparin as a bolus of 5000 IU followed by 15 IU/kg/h by 3-h infusion. Flovagatran demonstrated dose-proportional pharmacokinetics. A short half-life of 1.7 h was reported (24-26).

Results from an open-label, multicenter phase II study in 28 patients on stable hemodialysis demonstrated that plasma concentrations of flovagatran declined rapidly after cessation of the infusion. Flovagatran concentrations were higher in the extracorporeal circuit than in the patients and 5% was removed by dialyser (27, 28).

### Clinical Studies

The safety and pharmacodynamics of flovagatran were also evaluated in the above-mentioned double-blind phase I studies in healthy volunteers. Infusion of flovagatran produced a dose-related increase in TT, with a mean maximum increase to 4.5-7.5-fold over baseline values. After the cessation of the infusion, the TT decreased to 3-fold the baseline values within 30 min. The effect on aPTT was less marked and of shorter duration (mean maximum increase of 1.55-2-fold baseline levels). The effect on TT was highly predictable. No adverse events were observed in these studies (20-23, 29).

In the heparin-controlled, crossover study, the elevation in TT ratios was considerably higher (6.9-19.7 times) than for aPTT ratios (1.7-2.4 times) across flovagatran dose groups. In contrast, heparin showed a less selective effect on TT ratios. ACT ratios were increased by a mean of 1.9-2.7 times on flovagatran and 1.7 times on heparin. The relationship between TT ratio and flovagatran plasma concentrations was well correlated, and no significant adverse events were reported (25, 26).

Pharmacodynamic and safety results from the phase II trial in patients undergoing stable hemodialysis also demonstrated that continuous infusion of flovagatran was well tolerated at all dose levels tested, with no hemorrhages. As in the other studies, TT increased rapidly (4-6-fold over baseline) and decreased rapidly following cessation of flovagatran infusion; less marked changes in aPTT were observed. Increasing doses of flovagatran were associated with decreased extracorporeal circuit clotting, with no clotting seen at 25 mg/h and above (28).

### Drug Interactions

The effect of acetylsalicylic acid (aspirin) plus clopidogrel on the pharmacokinetics and pharmacodynamics of flovagatran was evaluated in an open-label, randomized, crossover phase I trial. Twelve healthy male volunteers received flovagatran alone, aspirin plus clopidogrel or flovagatran plus aspirin plus clopidogrel. During the infusion, the plasma concentration of flovagatran was maintained above 2 μg/ml and combination with aspirin plus clopidogrel did not affect the pharmacokinetic or pharmacodynamic properties of flovagatran at the doses tested. The data support further evaluation of flovagatran in combination with aspirin and clopidogrel (30).

### Source

Trigen, Ltd. (UK).

### References

1. Combe-Marzelle, S.M., Kennedy, A.J., Krimmer, D., Withington, R., Allen, G.D. (Trigen, Ltd.). *Peptide boronic acid compounds useful in anticoagulation*. US 2005282757.

2. Boucher, O.V.A. (Trigen, Ltd.). *Peptide boronic acid inhibitors*. US 2006084592.

3. Madge, D.J., Dolman, M., Combe-Marzelle, S.M. et al. (Trigen, Ltd.). *Peptide boronic acids useful in making salts thereof*. EP 1466916.

4. Walsh, P.N. *Platelet coagulation-protein interactions*. Semin Thromb Hemost 2004, 30: 461-71.

5. Narayanan, S. *Multifunctional roles of thrombin*. Ann Clin Lab Sci 1999, 29: 275-80.

6. Heemskerk, J.W., Bevers, E.M., Lindhout, T. *Platelet activation and blood coagulation*. Thromb Haemost 2002, 88: 186-93.

7. Pineo, G.F., Hull, R.D. *Unfractionated and low-molecular-weight heparin. Comparisons and current recommendations*. Med Clin North Am 1998, 82: 587-99.

8. Kleinschmidt, K., Charles, R. *Pharmacology of low molecular weight heparins*. Emerg Med Clin North Am 2001, 19: 1025-49.

9. Weitz, J.I., Hudoba, M., Massel, D., Maraganore, J., Hirsh, J. *Clot-bound thrombin is protected from inhibition by heparin-antithrombin III but is susceptible to inactivation by antithrombin III-independent inhibitors*. J Clin Invest 1990, 86: 385-91.

10. Hauptmann, J., Sturzebecher, J. *Synthetic inhibitors of thrombin and factor Xa: From bench to bedside*. Thromb Res 1999, 93: 203-41.

11. Tulinsky, A. *Molecular interactions of thrombin*. Semin Thromb Hemost 1996, 22: 117-24.

12. Das, J., Kimball, S.D. *Thrombin active site inhibitors*. Bioorg Med Chem 1995, 3: 999-1007.

13. *Trigen presents phase II results for its intravenous direct thrombin inhibitor, TGN 255, for the prevention of clotting during haemodialysis*. Trigen Press Release, July 17, 2006.

14. *Trigen opens IND for the initiation of a phase III programme for flovagatran (formerly TGN 255), its novel intravenous direct thrombin inhibitor (DTI)*. Trigen Press Release, February 6, 2007.

15. Chahwala, S., Chander, C. *Identification and in vitro characterization of TGN 255, a novel synthetic intravenous low molecular weight direct thrombin inhibitor*. 20th Congr Int Soc Thromb Haemost (ISTH) (Aug 6-12, Sydney) 2005, Abst P2306.

16. Chahwala, S., Chander, C., Dupe, R. *Anti-coagulant activity of TGN 255, a novel synthetic direct thrombin inhibitor*. 20th Congr Int Soc Thromb Haemost (ISTH) (Aug 6-12, Sydney) 2005, Abst P0516.

17. Chahwala, S., Chander, C., Blackney, M. *Ecarin clotting time profile of TGN 255, a novel synthetic intravenous direct thrombin inhibitor*. 20th Congr Int Soc Thromb Haemost (ISTH) (Aug 6-12, Sydney) 2005, Abst P1715.

18. Chahwala, S., Kennedy, A., Dupe, R., Nelson, D., Fossum, T., Chander, C. *Anti-coagulant profile of TGN 255 during simulated mitral valve repair using cardiopulmonary bypass*. Am J Cardiol [17th Annu Symp Transcatheter Cardiovasc Ther (Oct 17-21, Washington, D.C.) 2005] 2005, 96(7, Suppl. 1): Abst TCT-314.

19. Kennedy, A., Dupe, R., Nelson, D., Chahwala, S., Chander, C., Fossum, T. *The utility of TGN 255 in a model of coronary pulmonary bypass and simulated mitral valve repair in the dog*. 20th Congr Int Soc Thromb Haemost (ISTH) (Aug 6-12, Sydney) 2005, Abst P2305.

20. Combe, S., Allan, G., Kennedy, T. *A phase 1 double-blind 24-hour intravenous infusion study with TGN255, a direct thrombin inhibitor*. Am J Cardiol 2004, 94(6A, Suppl.): Abst TCT-417.

21. Combe, S., Allen, G., Kennedy, T., Patrick, G.M. *A phase 1 double-blind 24 hour intravenous dosing study of a synthetic direct thrombin inhibitor, TGN255*. J Am Soc Nephrol 2004, 15: Abst F-PO523.

22. Combe, S., Allen, G., Kennedy, T., Patrick, G. *A phase 1 double-blind, intravenous ascending dose study of a synthetic direct thrombin inhibitor, TGN255*. J Am Soc Nephrol 2004, 15: Abst F-PO524.

23. Combe, S., Allen, G., Kennedy, A. *Pharmacokinetics of TGN 255, a novel intravenous low molecular weight direct thrombin inhibitor, in healthy volunteers*. 20th Congr Int Soc Thromb Haemost (ISTH) (Aug 6-12, Sydney) 2005, Abst P1714.

24. Combe, S., Chander, C., Allen, G., Kennedy, A. *Pharmacokinetics and pharmacodynamics of TGN 255, a novel intravenous direct thrombin inhibitor in healthy volunteers. Comparison with unfractionated heparin*. 20th Congr Int Soc Thromb Haemost (ISTH) (Aug 6-12, Sydney) 2005, Abst P1116.

25. Combe, S., Allen, G., Kennedy, A. *Comparative pharmacodynamics of TGN 255, a novel direct thrombin inhibitor and unfractionated heparin in healthy volunteers*. Blood [47th Annu Meet Am Soc Hematol (Dec 10-13, Atlanta) 2005] 2005, 106(11): Abst 1864.

26. Combe, S., Chander, C., Kennedy, A. *Comparison of pharmacokinetics and pharmacodynamics of TGN255, a novel intravenous direct thrombin inhibitor, with unfractionated heparin*. Proc Am Thorac Soc [102nd Int Conf Am Thorac Soc (May 19-24, San Diego) 2006] 2006, 3(Abstracts Issue): A612.

27. Combe, S., D'Souza, R., Mysliwiec, M. et al. *Effect of TGN 255, a novel intravenous direct thrombin inhibitor, on the prolongation of ACT in patients undergoing haemodialysis*. 20th Congr Int Soc Thromb Haemost (ISTH) (Aug 6-12, Syndney) 2005, Abst P1614.

28. Combe, S., D'Souza, R., Mysliwiec, M. et al. *A phase IIa study to evaluate the safety, pharmacodynamics and. pharmacokinetics of TGN255 in stable haemodialysis patients*. 43rd Congr Eur Renal Assoc – Eur Dialysis Transpl Assoc (July 15-18, Glasgow) 2006, Abst SP595.

29. Combe, S., Chander, C., Kennedy, A. *Pharmacodynamics of TGN 255, a novel intravenous low molecular weight direct thrombin inhibitor, in healthy subjects*. 20th Congr Int Soc Thromb Haemost (ISTH) (Aug 6-12, Sydney) 2005, Abst P1115.

30. Combe, S., Kennedy, A., Allen, G. *Phase 1 interaction study between TGN255, a direct thrombin inhibitor, and aspirin plus clopidogrel*. Eur Heart J [15th World Congr Cardiol (Sept 2-6, Barcelona) 2006] 2006, 27(Abstract Suppl.): Abst P4572.