Eur J Med Chem (1995) 30, 387–394

Molecular model of an interaction between factor Xa and DX-9065a, a novel factor Xa inhibitor: contribution of the acetimidoylpyrrolidine moiety of the inhibitor to potency and selectivity for serine proteases

S Katakura, T Nagahara, T Hara, S Kunitada, M Iwamoto

Exploratory Research Laboratories 2, Daiichi Pharmaceutical Co Ltd, 1-16-13, Kita-Kasai, Edogawa-ku, Tokyo 134, Japan

(Received 18 October 1994; accepted 21 December 1994)

Summary — Molecular modeling of a complex between factor Xa (FXa) and DX-9065a, a novel FXa inhibitor, has demonstrated a salt-bridge interaction of the amidinonaphthalene moiety of the inhibitor to the S1 site pocket of the enzyme and a crucial carboxyl group of the inhibitor for the FXa/thrombin selectivity. In the present study, we synthesized some DX-9065a derivatives and studied their interaction modes with FXa, trypsin and thrombin to evaluate the role of the acetimidoylpyrrolidine moiety. The docking study of the inhibitor in the FXa model provided a molecular model of the inhibitor–FXa complex showing a hydrophobic interaction of the pyrrolidine ring with an aryl binding site of FXa, and a hydrogen bond between the acetimidoyl group and the backbone carbonyl oxygen of Glu97. Comparison between the interaction mode of DX-9065a with FXa and those with trypsin and thrombin explained the inhibitory potencies of the enzymes.

molecular modeling / factor Xa / DX-9065a / thrombin / trypsin

Introduction

Factor Xa (FXa) is a trypsin-like serine protease involved in the cascade of blood coagulation. Although FXa is a promising target for anticoagulant agents [1], no synthetic inhibitor of FXa has been developed yet. In an earlier study, we reported a novel FXa inhibitor, (2*S*)-2-[4-[[(3*S*)-acetimidoyl-3-pyrrolidinyl]oxy]phenyl]-3-(7-amidino-2-naphthyl)propanoic acid hydrochloride pentahydrate, DX-9065a (fig 1) [2]. This compound has a potent inhibitory activity for FXa and high selectivity for FXa over other serine proteases.

In a previous paper, we investigated the binding mode of DX-9065a by computer molecular modeling [3]. A docking study of DX-9065a into a three-dimensional model of FXa, based on the X-ray crystallographic data for trypsin [4], indicated that the amidinonaphthalene moiety of the inhibitor binds to the S1 site of FXa with a salt-bridge interaction. Furthermore, the Glu192 of thrombin corresponding to the Gln192 of FXa evokes an electrostatic repulsion of the carboxyl group of the inhibitor, which explains a mechanism of the FXa/thrombin selectivity of DX-9065a. However, DX-9065a has two basic terminals in the structure, suggesting that the acetimidoylpyrrolidine and the amidinonaphthalene moieties significantly participate in the potent inhibitory activity against FXa.

The acetimidoylpyrrolidine moiety also may contribute to the selectivity of DX-9065a for FXa from thrombin. The interaction of the moiety with a site of thrombin may fix the direction of the carboxyl group of DX-9065a, which leads to an increase in the electrostatic repulsion between the carboxyl group of the inhibitor and Glu192 of the enzyme. Thereby, DX-9065a cannot bind to thrombin. In addition, a loss of such an electrostatic repulsion between trypsin and DX-9065a suggests that the carboxyl group does not contribute to the selectivity for FXa from trypsin, and the acetimidoylpyrrolidine moiety should be involved in the selectivity.

In the present study, we determined a contribution of the acetimidoylpyrrolidine moiety of DX-9065a in

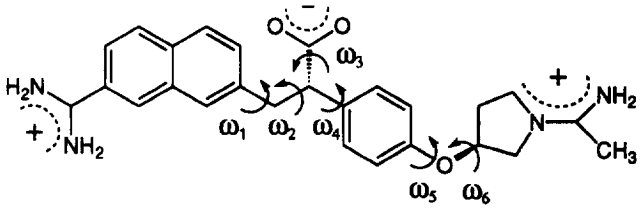


Fig 1. Structure of DX-9065a. Torsion angles rotated during a conformational search are shown with arrows.

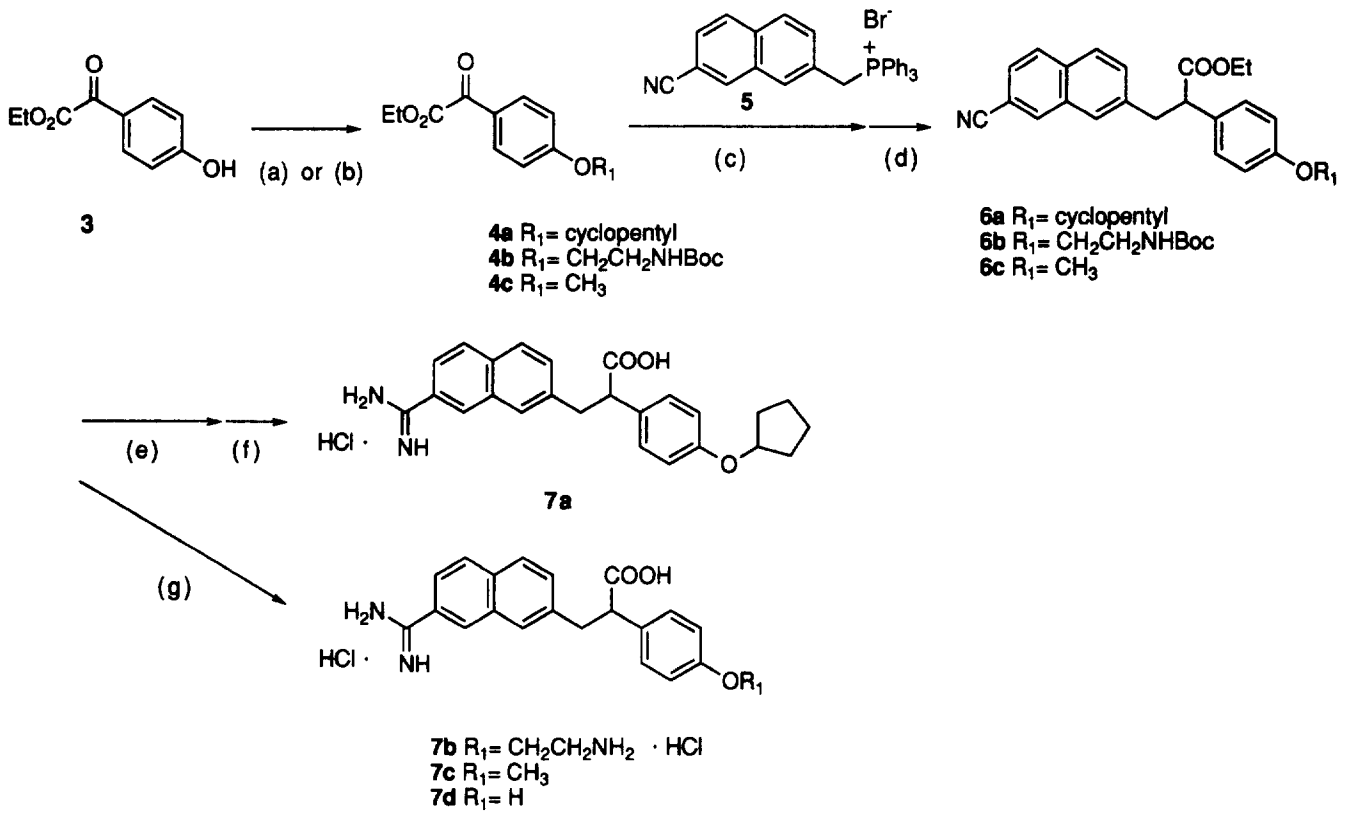
the potency and selectivity for FXa by a molecular modeling technique. First, we synthesized a series of compounds **7a–d** in order to evaluate the interactions of the acetimidoylpyrrolidine moiety with FXa, thrombin and trypsin. Second, diverse low-energy conformers of DX-9065a were generated by the conformational search using a Metropolis Monte-Carlo method. Finally, on the basis of the SAR of the series of inhibitors, docking studies of these conformers into FXa, thrombin and trypsin were performed.

Chemistry

Compounds **6a–c** were prepared by the method described previously (scheme 1) [5]. Treatment of compounds **6b,c** with hydrochloride in ethanol and then with an ammonia/ethanol solution gave the amidine derivatives, which were hydrolyzed with hydrochloric acid to afford compounds **7b,c**. Acid hydrolysis of the amidine intermediate synthesized from **6a** gave the phenol derivative **7d**. Compound **7a** was prepared along the following pathway with a modification: protection of the amidino group with *tert*-butoxy dicarbonate, alkali hydrolysis with sodium hydroxide and deprotection with trifluoroacetic acid.

Molecular modeling of serine proteases

The three-dimensional structure model of the heavy chain of human FXa was constructed on the basis of trypsin, a highly homologous serine protease, as described previously [3]. A recent X-ray crystallographic analysis of FXa revealed the geometric similarity of the α-carbons of FXa to those of thrombin, except for an insertion loop in thrombin, Tyr60A to Thr60I [6]. On the basis of this report, the coordinates from Asp170 to Ile176 of the FXa model, which were topologically different from those of thrombin, were replaced by the corresponding coordinates of thrombin, Leu170 to Ile176. Side chains of the resulting model were optimized manually to remove steric clashes, and then the FXa model was obtained by



Scheme 1. (a) R_1OH, diethyl azodicarboxylate, PPh_3, THF; (b) R_1I, K_2CO_3, DMF; (c) DBU, THF/EtOH; (d) Pd/C, H_2, EtOH; (e) EtOH/HCl; EtOH/NH_3; *tert*-butoxy dicarbonate, Et_3N, DMF; (f) 1 N NaOH; TFA; (g) EtOH/HCl; EtOH/NH_3; 4 N HCl, Δ.

energy minimization of these residues in 500 steps without moving the other amino-acid residues.

The three-dimensional structure model of human α-thrombin was derived from the coordinates for the structure labeled 1DWE [7] in the Brookhaven Protein Data Bank [8]. Water molecules in the coordinates were removed and the hydrogens were added to the backbone and side chains based on the standard average bond angles and lengths.

The atomic coordinates of bovine trypsin registered as 1TPP [4] in the same data bank were employed as a template of the tertiary model of human trypsin (TRYI). After removal of water molecules and addition of hydrogens, the amino acids of bovine trypsin were replaced with those of human trypsin [9] by using the mutation option of the Quanta protein module. All side chains of the model were optimized by 500-step energy minimization. The numbering system of the enzymes was defined on the basis of topological equivalence with chymotrypsin.

Results and discussion

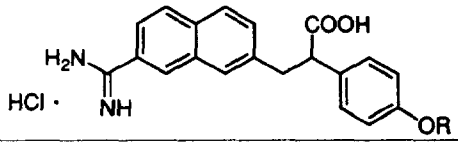
Structure–activity relationships

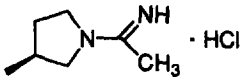
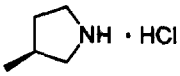
Anti-FXa and anti-thrombin activities of DX-9065a and its derivatives were measured as amidolytic activities for chromogenic substrates S-2222 and S-2338, respectively [2]. In a similar manner, anti-trypsin activity was measured using S-2222 (table I). Pyrrolidine derivative **2** and its acetimidoyl derivative **1** had almost same potency in FXa inhibitory activity [5]. Substitution of the methyl group of **7c** with a cyclopentyl group, forming compound **7a**, exhibited an eightfold increase in inhibitory potency. Replacement of the aminoethyl group of **7b** with the pyrrolidine ring gave compound **2** showing a 35-fold increase in potency. These results indicate importance of a terminal five-membered ring in the FXa inhibitors. The anti-FXa activity of pyrrolidine derivative **2** is 21 times as potent as that of cyclopentane derivative **7a**. Similarly, aminoethyl derivative **7b** is fivefold more potent than **7c**. These results indicate that the terminal amino group of the inhibitors contributes to the increase in potency. Thus, the pyrrolidine ring may be not only a constrained amino group but also a group contributing to a hydrophobic interaction with FXa.

The anti-trypsin activity of compounds **1**, **2**, and **7a–d**, unlike their anti-FXa activity, is almost the same. The hydrophobicity and basicity of the terminal substituents of the inhibitors are unlikely to be associated with the binding affinity for trypsin. Therefore, the FXa/trypsin selectivities of these inhibitors are primarily dependent on the anti-FXa activity.

The inhibitory activity of compounds **1**, **2**, and **7a–d** against thrombin was negligible.

Table I. Inhibitory activities of derivatives of DX-9065a on FXa, thrombin and trypsin.



Compound	*R*	*IC50 (μM)*		
		FXa	*Thrombin*	*Trypsin*
1		0.16	>1000	10
2		0.20	>1000	5.4
7a		4.2	>1000	4.8
7b		7.3	>1000	10
7c	—CH_3	35	>1000	7.5
7d	—H	26	>1000	16

Conformational search of DX-9065a

In order to sample diverse low-energy conformers efficiently, a Metropolis Monte-Carlo search procedure [10, 11] was applied to DX-9065a. The initial structure of DX-9065a was taken from its X-ray crystallographic structure [5]. To generate a conformer, six torsion angles ω_1–ω_6 (see fig 1) were altered randomly within 45° at a pseudo-temperature of 5000K using Boltzmann jump search option of the Quanta program [12]. The generated conformer, whose dihedral root mean square difference from the previous conformer was over 43°, was accepted as a new conformer, which was subsequently optimized by 100-step minimization without changing the ring conformation of the pyrrolidine. This procedure was iterated 5000 times.

Conformers with the torsion angle ω_2 of 180 ± 10° were removed since their acetimidoylpyrrolidine moieties were incapable of binding to FXa. Elimination of conformers with energy levels more than 3 kcal/mol above the minimum gave 144 conformers. The conformational space shows that the acetimidoylpyrrolidine moiety is predominantly located in two

regions, as shown in figure 2. The resulting 144 conformers were docked into the FXa model to study the mode of interaction between the acetimidoylpyrrolidine moiety of DX-9065a and FXa.

Docking studies of DX-9065a

DX-9065a has two basic, amidinonaphthalene and acetimidoylpyrrolidine groups. The previous modeling study of the complex between FXa and DX-9065a demonstrated that the Sl pocket of FXa prefers the planar amidinonaphthalene group to the acetimidoylpyrrolidine group with a spacially expanded structure [5]. Therefore, the amidinonaphthalene part of DX-9065a was adjusted to the S1 site to form a hydrogen-bonded ion pair of the amidino group with the carboxyl group of Asp189 of the enzyme without steric collisions. The docking study showed that 10 of the 144 conformers had a binding mode such that their pyrrolidine rings interacted with a site of FXa corresponding to the 'aryl binding site' [13] of thrombin. The pyrrolidine ring was surrounded with Tyr99, Phe174 and Trp215 without any steric hindrance. This result is consistent with the model proposed by Padmanabhan *et al*, in which the dansyl group of dansyl-GluGlyArg, an FXa-inhibitor, interacts with the hydrophobic site of FXa [6], suggesting the hydrophobic interaction of the pyrrolidine ring with the aryl binding site. The pyrrolidine rings of the other conformers did not have such an interaction with FXa.

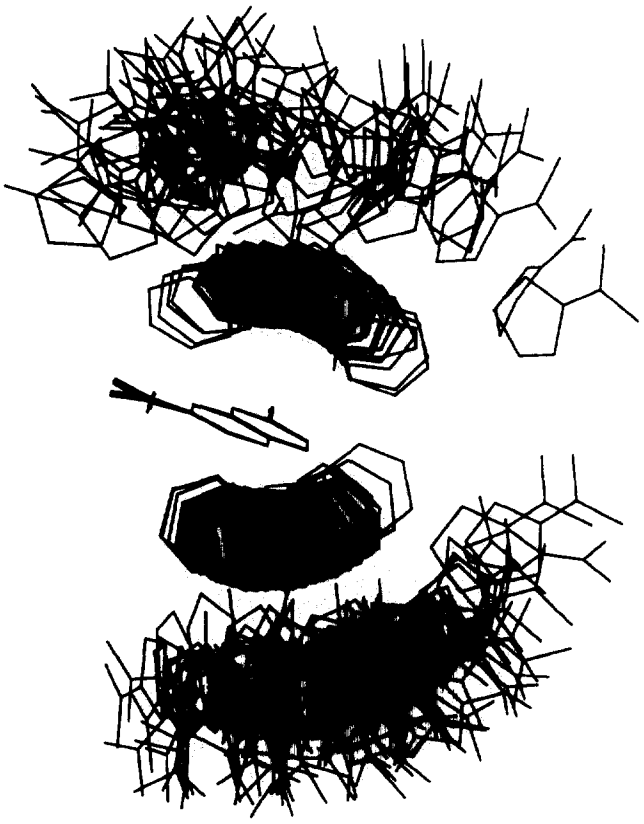


Fig 2. Superimposition of 144 conformers with an energy less than 3 kcal/mol above the minimum. The amidinonaphthalene, benzene and acetimidoylpyrrolidine moieties are displayed.

One of the 10 conformers was selected as a putative active conformer, since the acetimidoyl nitrogen was expected to form a hydrogen bond with the backbone carbonyl oxygen of Glu97. The hydrogen bond could explain the importance of the terminal basic group of the inhibitors **1**, **2** and **7b** in inhibitory potency. Energy-minimization of the complex between this conformer and the FXa model was performed in 500 steps without moving the α-carbons of FXa, to give a plausible model of DX-9065a bound to the enzyme. As shown in figure 3, the carbonyl oxygen of Glu97, which was located near the aryl binding site, appeared to donate a hydrogen bond to the acetimidoyl nitrogen. The distance between the two atoms was observed to be 2.8 Å, which was appropriate for a hydrogen bond. Alternatively, a salt-bridge interaction may be considered as an interaction between the terminal basic center of the inhibitor and the acidic side chain of Glu97. However, the hydrogen bond seemed preferable to the salt-bridge interaction since the orientation of the side chain of Glu97 was unfavorable for the tight interaction with the acetimidoyl group.

In addition, there was no hydrophobic site of FXa interacting with the benzene ring attached to the phenoxypyrrolidine moiety of all the 144 conformers. The ring might be a spacer to fix the pyrrolidine ring on the favorable location.

Trypsin also has a site, which consists of two hydrophobic Leu99 and Trp215 and a hydrophilic Lys175, corresponding to the aryl binding site of FXa. A docking study of DX-9065a in the trypsin model showed that the inhibitor could bind to the enzyme in a similar manner to FXa (fig 4). However, the hydrophobic binding of the acetimidoylpyrrolidine moiety with the site of trypsin may not be very strong as the site is less hydrophobic than that of FXa. This could account for the almost identical antitrypsin activities of **7a** and **7c**. In addition, an electrostatic repulsion between the pyrrolidine nitrogen and the basic side chain of Lys175 could weaken a hydrogen bond between the pyrrolidine nitrogen and the carbonyl oxygen of Glu97, explaining the almost identical antitrypsin activity of the compounds with or without the terminal basic nitrogen. Thus, comparison between the binding modes of DX-9065a with trypsin and FXa

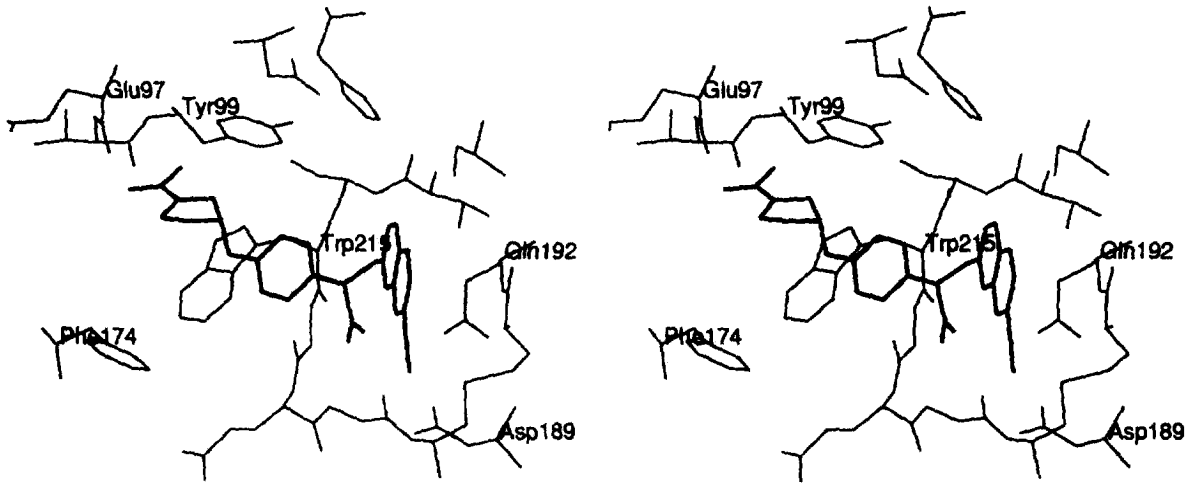


Fig 3. Model structure of the complex between FXa (thin lines) and DX-9065a (thick lines).

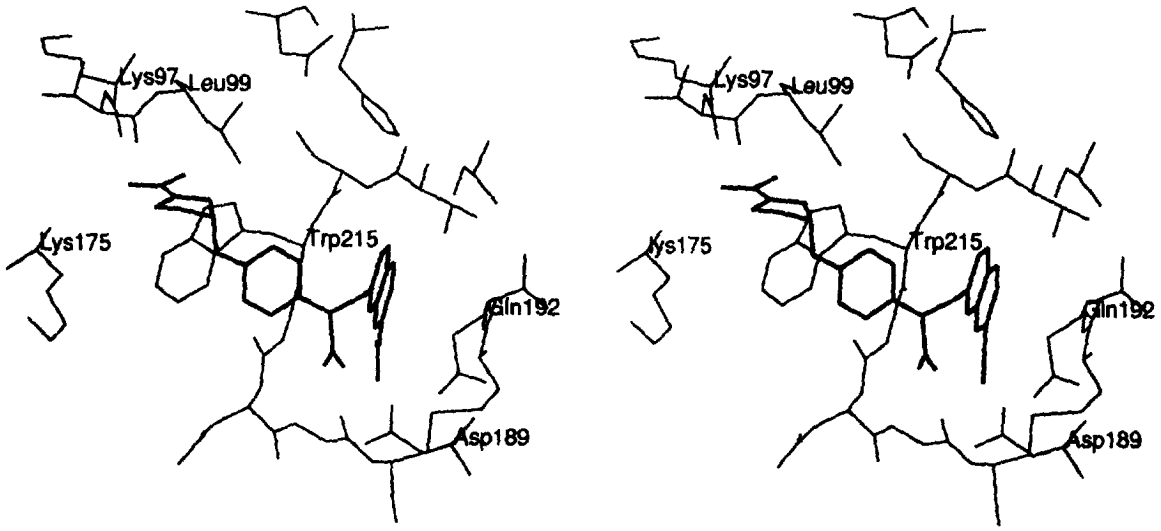


Fig 4. Model structure of the complex between trypsin (thin lines) and DX-9065a (thick lines).

can give an explanation of the FXa/trypsin selectivity, which is ascribed to the acetimidoyl pyrrolidine moiety.

In the previous paper, we proposed the molecular mechanism of the FXa/thrombin selectivity of DX-9065a. An electrostatic repulsion between the carboxyl group of DX-9065a and that of Glu192 of thrombin causes the unfavorable enzyme–inhibitor contact and thereby extinguishes the inhibitory activity against thrombin [3]. Both thrombin and FXa have the aryl binding site which was formed with Leu99, Ile174 and Trp215. A docking study of DX-9065a indicated that the site accommodated the pyrrolidine ring without steric clashes. In addition, thrombin also had Glu97 which seemed capable of forming a hydrogen bond with the amino terminal of DX-9065a. The interaction between the acetimidoylpyrrolidine moiety and the enzyme would fix the orientation of the carboxyl group of DX-9065a, which resulted in the electrostatic repulsion of the carboxyl group to Glu192 of thrombin (fig 5). However, compounds **7c** and **7d** without the acetimidoylpyrro-

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.