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Adventures in Drug Discovery: Potent Agents Based on Ligands for Cell-Surface Receptors

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ABSTRACT

How does one go about discovering new drugs? This question is addressed by descriptions of drug discovery research in three project areas that pertain to antagonist ligands for cell-surface receptors. The molecular targets of interest are protease-activated receptor-1 (PAR-1), vasopressin receptors (V_{1a} and V_2 subtypes), and the fibrinogen receptor (GPIIb/IIIa). I present different approaches to the identification of high-affinity ligands for these receptors, en route to drug candidates. The PAR-1 project resulted in a pharmacological tool compound that facilitated in vivo proof-of-principle studies, whereas the vasopressin and fibrinogen receptor projects resulted in several preclinical development compounds, three of which advanced into human clinical trials.

Introduction

The discovery of new drugs is a monumental struggle with Nature. Perhaps, it has always been that way, but the hurdles to success are more evident and pervasive at the dawn of the 21st century. Indeed, the output of new pharmaceutical products from companies has waned dramatically over the past decade, despite a tremendous influx of capital and human resources into the research and development process.[1,2] This unhappy situation turns out to be somewhat paradoxical. Given the proliferation of distinct molecular targets for intensive study and myriad advanced technologies for surmounting bottlenecks, one might anticipate a smoother pathway. Unfortunately, many new targets are not eminently "druggable", many technologies are only applicable to early phases of drug discovery, and some target-directed compounds may not have sufficient clinical impact on the disease/disorder of interest.

Historically, compounds directed to cell-surface receptors have provided the lion's share of marketed drugs. Such receptors, which are responsible for physiological actions by linking events in the outside world to processes inside living cells through signal transduction, are readily accessible to drug molecules. Basically, the drug does not have to enter cells to exert its influence. A classic case relates to G-protein-coupled receptors (GPCRs), which are activated by various endogenous mediators (e.g., neurotransmitters, chemokines, hormones) and are targets for a broad range of drugs encompassing diverse therapeutic applications.[3−7] Nearly 50% of marketed medicines function via GPCRs, including about 20% of the 50 best-selling ones.[3,4]

In this Account, I present three vignettes about drug discovery research with the common theme of developing potent agents based on ligands for cell-surface receptors. Over the past 15 years, my research group at Johnson & Johnson has pursued cell-surface receptors that use peptides as native effector ligands within the GPCR and integrin superfamilies. The focus herein is antagonists for protease-activated receptor-1 (PAR-1), vasopressin V_{1a}/V_2 receptors, and the fibrinogen receptor GPIIb/IIIa ($\alpha_{IIb}\beta_3$).

In drug discovery, the identification of interesting compounds ("chemotypes") to serve as practicable starting points is a critical factor. A project can be propelled initially by employing high-throughput screening (HTS) or de novo design to find lead structures for optimization. The burden rests with medicinal chemists to generate more potent, drug-worthy compounds by adjusting many desirable, often orthogonal properties. Thus, druggability poses a huge challenge, as one must satisfy a constellation of requirements to attain a drug candidate. In devising antagonists, we adopted different chemical approaches, sometimes relying on peptide mimicry and de novo design, other times relying on existing knowledge about pharmacophores. Optimization cycles were performed to achieve a balance between potency for the target and other salient attributes that define druggability, such as pharmacokinetics, physical properties, pharmacological efficacy, and toxicology. The PAR-1 project yielded a pharmacological tool, and the vasopressin and GPIIb/IIIa projects yielded development compounds that entered human clinical studies.

G-Protein-Coupled Receptors

GPCRs represent a large superfamily of cell-surface receptors that transduce exogenous signals into intracellular responses.[6,8,9] These receptor proteins assume a seven-helix-bundle topology, in which seven α-helices, each containing ∼25 amino acids, span the membrane bilayer, with the N-terminus outside the cell and the C-terminus in the cytoplasm (Figure 1[10]).[5,6,8] Ligands are known for ∼200 of the 700 GPCRs in the human genome.[5,11,12] Generally, a native ligand binds to the extracellular region, which is usually the target area for drug molecules.

Antagonists of PAR-1. The serine protease α-thrombin is a central enzyme in blood coagulation and is involved in various cellular actions, such as platelet aggregation and

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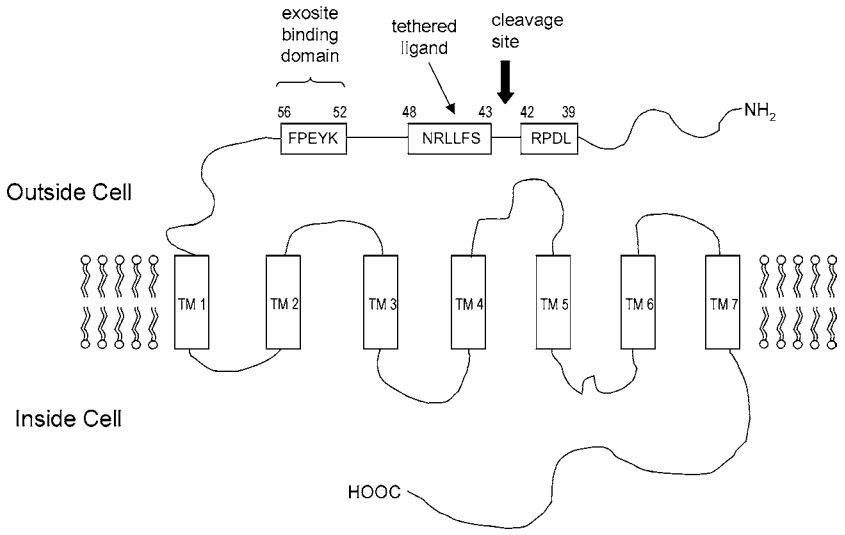


FIGURE 1. Schematic of the GPCR PAR-1 protein (human) in a phospholipid bilayer (side view; transmembrane helices labeled TM1–TM7).[10a] The N-terminus contains a thrombin cleavage site between Arg-42 and Ser-43, tethered-ligand agonist sequence (SFLLRN), and thrombin exosite binding domain. Reprinted from ref 10a with permission. Copyright 2003 Bentham Science Publishers, Ltd.

cell proliferation.[13] An antagonist operating at the cellular level would have potential in treating thrombosis, atherosclerosis, inflammation, and cancer metastasis,[13b,14] without altering thrombin's role in hemostasis. PAR-1, the long-sought platelet thrombin receptor,[15] is an unusual GPCR with an elongated N-terminus that is cleaved by α-thrombin to expose an agonist peptide ligand (Figure 1).[10] In human PAR-1, the receptor-linked ("tethered") activating ligand is the hexapeptide sequence SFLLRN.[10,15] Notably, synthetic SFLLRN-NH_2 (*TRAP-6*) exerts full agonist activity, although its potency on platelets is 1000-fold less than thrombin's.[16,17]

To obtain a PAR-1 antagonist, one must address the "tethered ligand problem". Since the peptide epitope is linked to the receptor, a strong entropy component contributes to the binding free energy, which explains the reduced agonist potency of SFLLRN-NH_2. It would be difficult to compete against this intramolecular binding with a small-molecule ligand. Other challenges arose from the absence of PAR-1 in platelets of different test animals (e.g., rats and dogs) and the presence of additional thrombin receptors, PAR-3[18] and PAR-4,[19] in different species.[10] Since both PAR-1 and PAR-4 exist on human platelets, it is unclear whether a selective PAR-1 antagonist would be a useful antithrombotic drug in humans. Nevertheless, considering the high medical need for antiplatelet drugs to treat thrombotic disorders, we aspired to discover a potent PAR-1 antagonist. Our primary goal was a clinical candidate, but we also hoped to find a pharmacological tool to characterize the physiological actions of PAR-1.

In 1992, we established a research collaboration with COR Therapeutics, Inc. which held rights to pertinent intellectual property.[15] Since HTS of proprietary chemical libraries did not afford a sustainable lead series, we adopted a design approach based on pseudopeptides and peptide mimetics related to the agonist motif SFLLR.[10a,17,20] Our peptide-mimetic design was predicated on structure–

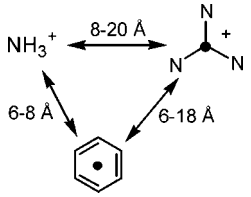


FIGURE 2. Three-point model showing spatial arrangement of key ammonium, phenyl, and guanidinium groups in agonist peptide SFLLRN-NH_2 (doubly protonated form).

function data for PAR-1 agonist peptides, spatial constraints of key groups in SFLLR, and a heterocyclic template to display these functional groups. A pharmacophore model was constructed by analyzing SFLLRN α-helical, β/γ-turn,[12] and β-sheet conformations, with molecular dynamics searches on energy-minimized structures. The distances between amino, phenyl, and guanidino groups were incorporated into a "three-point model" (Figure 2).[10a,21a,b] A suitable spatial arrangement of groups on a molecular scaffold would hopefully furnish worthwhile PAR-1 antagonists.

A breakthrough came from an indole-based series, RWJ-53052 (**1a**) being a prototype (Figure 3).[21b] This compound inhibited human platelet aggregation induced by *both* TRAP-6 (IC_{50} = 0.49 μM) and thrombin (IC_{50} = 2.0 μM) and was selective vs collagen; however, it had modest affinity for PAR-1 in a binding assay.[21a,b] To improve on **1a**, we developed robust solid-phase syntheses for producing numerous derivatives in parallel,[21c,d] then used an iterative approach for optimization: evaluate a set of 12 analogues, synthesize and evaluate a new set of 12, and so on. For example (Figure 3), the amino group of Sieber or Tentagel resin was alkylated to give **2**, which was reacted sequentially with protected L-arginine and protected L-*O*-methyltyrosine to give resin-bound dipeptide **3**. After coupling **4** with **3** to yield resin-bound adduct **5**, the pyrrolidinomethyl group was added, and the resin

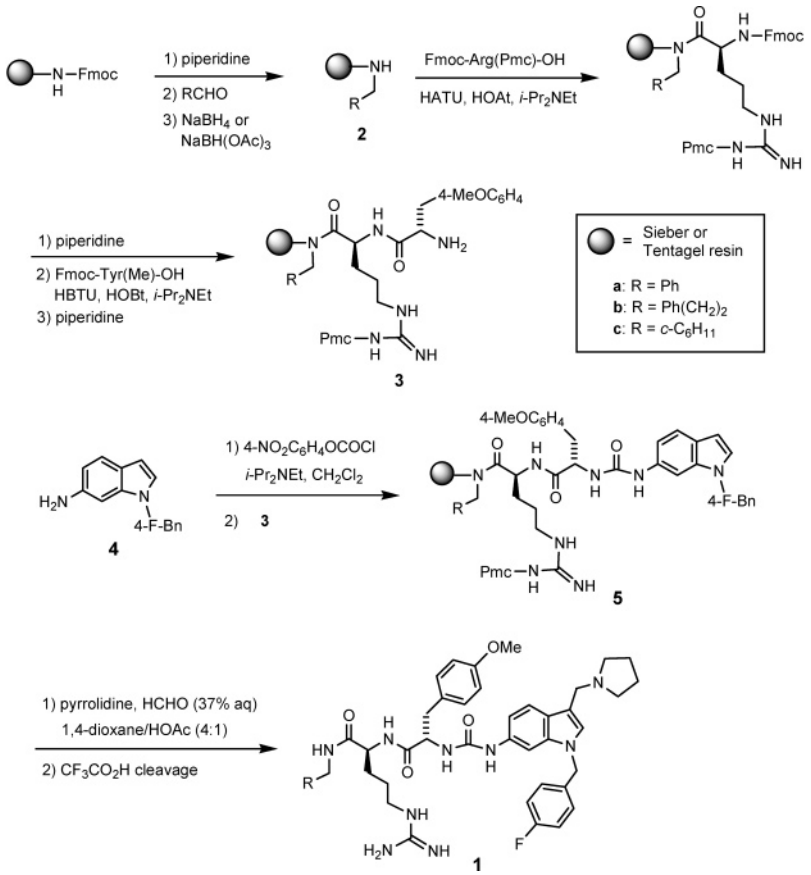
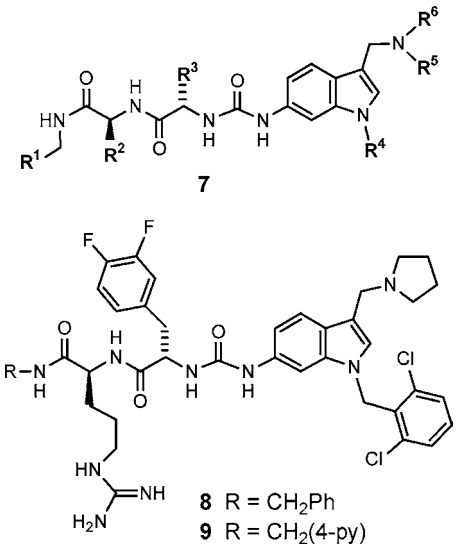


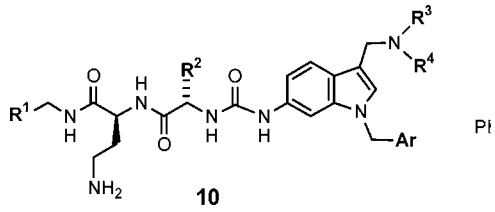
FIGURE 3. Solid-phase synthesis of **1a**−**c** on Sieber or Tentagel resin. Abbreviations: Fmoc, 9-fluorenylmethoxycarbonyl; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; HOBt, 1-hydroxybenzotriazole; HOAt, 1-hydroxy-7-azabenzotriazole; HATU, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

was cleaved to provide **1a**−**c**.[21c] This 10-step sequence supplied a library of diverse analogues **7**, with six variables



(R^1−R^6) permuted, and each compound was tested without purification. In series **7**, we found a preference for R^3 = 3,4-difluorobenzyl and R^4 = 2,6-dichlorobenzyl. Thus, **8** inhibited platelet aggregation induced by thrombin or TRAP-6 with IC_{50} values of 570 or 180 nM, while being selective vs collagen, and **9** showed excellent PAR-1 affinity (IC_{50} = 40 nM).

Substituents were altered using other solid-phase, parallel methods,[21a,b] such as the route in Figure 4, which permuted five variables (R^1−R^4, Ar) in **10**, leading to **11**



(RWJ-56110). Thus, L-2,4-diaminobutyric acid derivative **12** was attached to chlorotrityl resin to give **13**, which was transformed via dipeptide **14** into resin-bound urea **15**. Mannich reaction and resin cleavage yielded **11**. Purified **11** inhibited platelet aggregation induced by thrombin or TRAP-6 (IC_{50} = 340 or 160 nM) and had reasonable PAR-1 affinity (IC_{50} = 440 nM).

We sought to confirm a PAR-1 mechanism with **11**. At *elevated thrombin concentrations*, its potency in blocking thrombin-induced human platelet aggregation was attenuated, whereas its potency in blocking TRAP-6 was not.[21a] This observation relates to the dual thrombin receptor system, PAR-1/PAR-4, in human platelets.[19b,21a] Since **11** inhibited Ca^{2+} signaling at high thrombin concentrations in *rat smooth muscle cells*, which have PAR-1 but lack PAR-4, **11** *can fully compete with the PAR-1 tethered-ligand*. Additionally, in flow cytometry studies

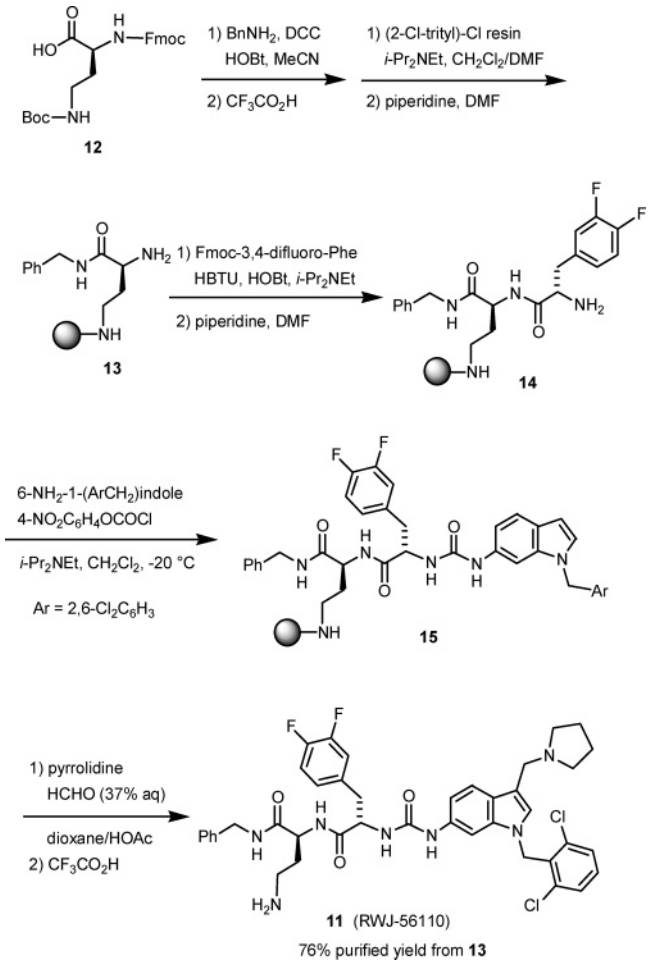
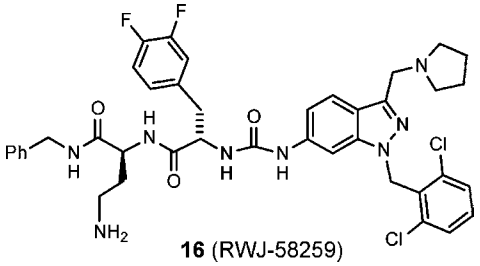


FIGURE 4. Solid-phase synthesis of **11**. Abbreviations: see Figure 3; DCC, 1,3-dicyclohexylcarbodiimide.

with platelet progenitor cells and specialized antibodies,[22] **11** prevented receptor internalization by competing effectively with the tethered ligand.[23] The PAR-1 selectivity of **11** was confirmed in myofibroblasts from mice deficient in PAR-1 that were transfected with human PAR-1, PAR-2, or PAR-4.[21a]

Intravenous administration of **11** to guinea pigs dose-dependently inhibited *ex vivo aggregation* in platelet-rich plasma induced by thrombin or TRAP-6. However, to avoid hypotension at high doses, we switched to corresponding indazole **16** (RWJ-58259), a potent PAR-1 antagonist (thrombin-induced aggregation IC_{50} = 370 nM; binding IC_{50} = 150 nM) with an improved therapeutic index.[21b] In the guinea pig protocol, **16** fully inhibited ex vivo thrombin-induced aggregation at an intravenous dose of 0.3 mg/kg.[24]



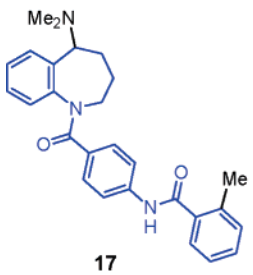
16 (RWJ-58259)

In a rat vascular restenosis model, involving balloon angioplasty with perivascular administration for 14 days,[24] **16** caused a marked reduction in neointimal thickness, consistent with PAR-1's importance in vascular injury. Given the difficulties in studying PAR-1 antagonism with various animal models of thrombosis,[10a,19b] we resorted to an antithrombotic study in cynomolgus monkeys, whose platelets possess PAR-1 and PAR-4 in analogy to humans.[25] Intravenous infusion of **16** attenuated or prevented thrombotic occlusion in 100% of electrolytically injured carotid arteries. This protection against injury-induced thrombus formation constitutes a robust in vivo proof-of-principle for a PAR-1 antagonist and supports antithrombotic utility in humans.

From 1200 dipeptide ureas and related compounds, we identified **11** and **16** as potent, selective PAR-1 antagonists.[21] With these pharmacological tools, we elucidated fundamental mechanistic aspects and obtained in vivo proof-of-principle, which laid a foundation for the pursuit of development compounds. The therapeutic value of a PAR-1 antagonist as an oral antithrombotic drug remains to be established through extensive human clinical studies.

Antagonists of Vasopressin Receptors. Arginine-vasopressin (AVP) is a cyclic nonapeptide hormone with multiple biological actions, including smooth muscle contraction, glycogenolysis, and renal water reabsorption.[26] Its actions are mediated by specific GPCR subtypes: V_{1a} receptors induce smooth muscle contraction, V_{1b} receptors induce corticotropin release, and V_2 receptors induce antidiuresis. Our interest was directed to V_{1a} and V_2 receptors[27] in that AVP maintains plasma volume and osmolarity through renal epithelial V_2 receptors and enhances vascular tone through V_{1a} receptors.[28] Since circulating AVP levels may be high under pathological conditions, resulting in water retention and hyponatremia,[29] drugs for selective water excretion ("aquaresis") could be useful for treating edema, such as in liver disease and congestive heart failure.[30]

To initiate drug discovery research in 1998, we established binding assays based on recombinant human V_{1a} and V_2 receptors[31] and cellular functional assays to determine agonist/antagonist action.[32] Suitable compounds would proceed to in vivo pharmacology. Since the nonpeptide vasopressin antagonists OPC-31260 (**17**, mozavaptan)[33] and VPA-985 (**18**, lixivaptan)[34] contain a key



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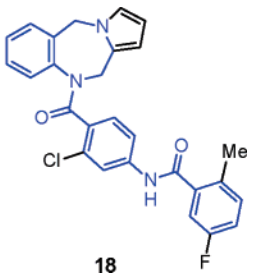

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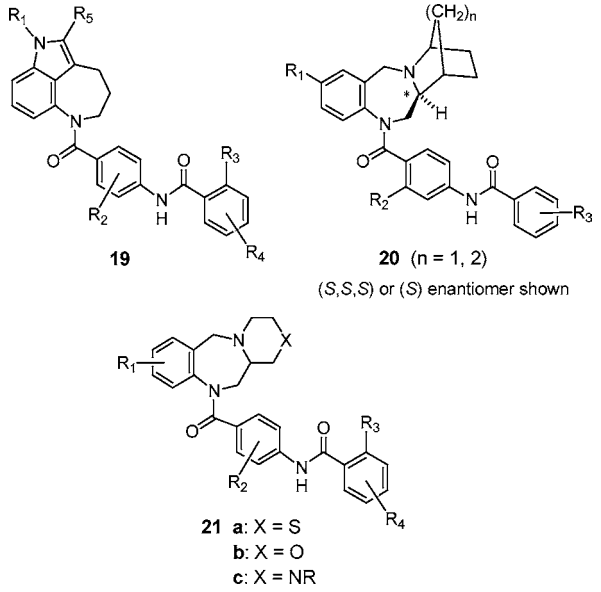
pharmacophore (blue highlighting), we considered novel chemotypes **19**–**21**.[27,35] Such molecules can be problematic as oral drugs because of high molecular weight (>450



Da), excessive hydrophobicity (log $P > 4$), and limited aqueous solubility,[36] but the basic amine center(s) in **20** and **21** could be beneficial.

Compounds in indoloazepine series **19**[35a] did not bind well to the V_{1a} receptor, but some had low nanomolar K_i values in V_2 binding. Potent V_2 ligand **19** ($R_1/R_2/R_4/R_5$ = H, R_3 = Ph) showed good antagonism in the V_2 functional assay (K_i = 70 nM). However, poor oral absorption and low aqueous solubility were concerns.

To access benzodiazepines **20**,[35b] we employed a diastereoselective, acid-catalyzed hetero-Diels–Alder reaction[37] (Figure 5). For example, imine **22** and cyclopentadiene yielded exo-cycloadducts **23** (n = 1), the (*S*,*S*,*S*)-bicycle predominating with (*R*)-(+)-benzylamine. Hydrogenation/hydrogenolysis of the major diastereomers gave α-amino esters **24** (>95% ee), which were converted into targets **20** without loss of stereochemical integrity. In vitro assessment revealed some potent V_2 receptor binders, such as **20a**–**e** (Table 1). Certain compounds with (*S*)-configuration (starred stereocenter) were dual V_{1a}/V_2 antagonists in receptor binding; for example, **20d** had V_{1a}/V_2 K_i values of 1.5/4.8 nM. In functional assays, **20c** was particularly potent, with V_{1a}/V_2 K_i values of 23/13 nM. Despite limited oral bioavailability (fraction of dose absorbed, *F*, was 10%), **20a** and **20b** exhibited good aquaretic activity in rats on oral dosing at 0.3 mg/kg.[35b]

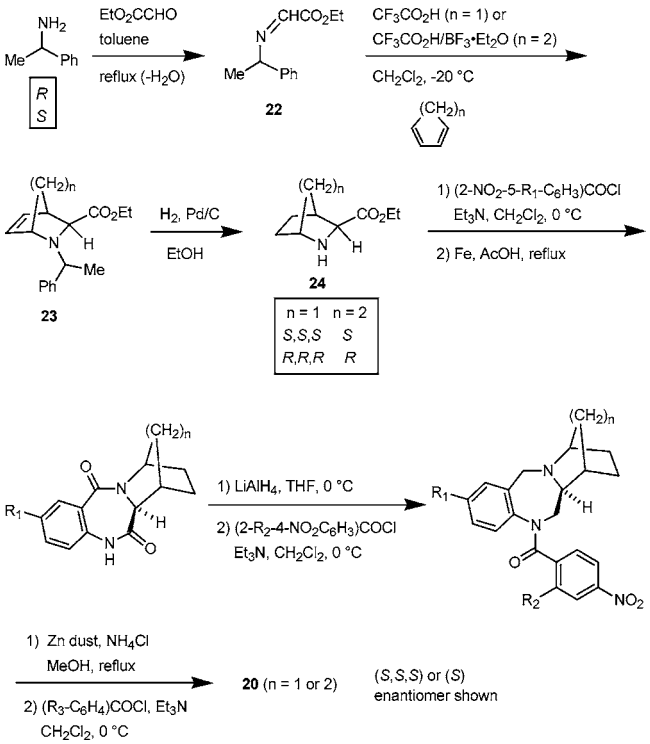


FIGURE 5. Synthesis of **20**.

Table 1. Vasopressin Binding and Functional Data for Benzodiazepines 20

compd	R_1	R_2	R_3	n	config[a]	V_{1a} binding K_i, nM	V_2 binding K_i, nM	V_{1a} funct K_i, nM	V_2 funct K_i, nM
20a	H	Cl	2-Ph	1	*S,S,S*	7.0	1.8	130	9.0
20b	H	Cl	2-Ph	1	*R,R,R*	>500	7.0		30
20c	Cl	H	2-Tol[b]	1	*S,S,S*	7.0	2.3	23	13
20d	Cl	H	2-Cl	1	*S,S,S*	1.5	4.8	130	32
20e	Cl	H	2-Cl	2	*S*	10	6.0	620	520

[a] Absolute configuration: for n = 1, *S,S,S* or *R,R,R*; for n = 2, *S* or *R*. [b] Tol = 4-tolyl.

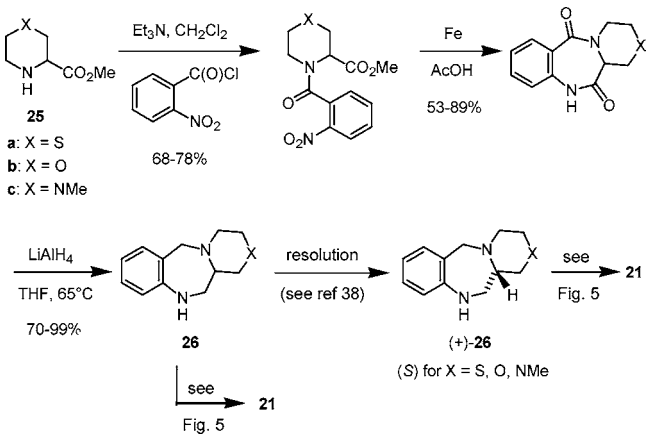
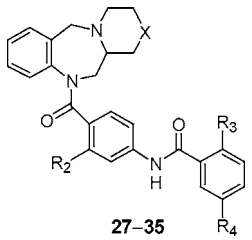


FIGURE 6. Synthesis of **21a**–**c**; resolution of **26a**–**c**.

Better prospects for clinical candidates emerged from series **21a**–**c** (Figure 6).[35c–e] We assembled requisite intermediates **26a**–**c** from **25a**–**c** and appended benzoylbenzamide side chains (Figure 5) to give **21a**–**c**, with scalemic targets coming from resolution of **26a**–**c**.[35c,d,38] Thiazino series **21a** was explored first, mainly via racemic targets (Table 2, **27**–**29**).[35c] (*S*)-(+)-**28** and (*R*)-(−)-**28** had marked V_2 receptor affinity (K_i = 3.2 and 25 nM), and the excellent V_2 functional activity (K_i = 15 nM) with 90-fold selectivity for (*S*)-(+)-**28** was encouraging. Oral administration of (*S*)-(+)-**28** to hydrated conscious rats elicited dose-dependent aquaresis, determined by *increased urine output* and *decreased urine osmolality* (increased dilution).[35c]

For oxazino series **21b**, we mostly prepared (Figure 6) and studied the (*S*)-(+) enantiomers, which were usually better than the (*R*)-(−) counterparts (Table 2, **30**–**32**).[35c] (*S*)-(+)-**32** and (*R*)-(−)-**32** had marked V_2 affinity, but (*S*)-(+)-**32** was 15-fold more potent, with a K_i of 0.9 nM!

Table 2. Vasopressin Binding and Functional Data for Examples of 21a−c



27–35

compd[a]	X	R_2	R_3	R_4	V_{1a} binding K_i, nM	V_2 binding K_i, nM	V_{1a} funct K_i, nM	V_2 funct K_i, nM
27	S	Cl	Ph	F	*b*	37	14000	70
(+)-**28**	S	H	Ph	H	84	3.2	1400	15
(−)-**28**	S	H	Ph	H	290	25	1300	40
(+)-**29**	S	Cl	Ph	H	250	3.7	14000	17
(+)-**30**	O	Cl	Me	F	100	2.8	2000	12
(+)-**31**	O	Cl	Ph	F	∼300	11	6400	12
(+)-**32**	O	Cl	Ph	H	24	0.9	420	3.0
(−)-**32**	O	Cl	Ph	H	640	13	>15000	170
(+)-**33**	NMe	Cl	Ph	H	>2900	12	>28000	47
(−)-**33**	NMe	Cl	Ph	H	*b*	20		17
34	NH	Cl	Ph	H	*b*	21		130
35	N*i*Pr	Cl	Ph	H	*b*	*b*		
18[c]					44	2.3	6000	23

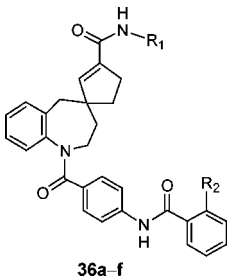
[a] Racemate, unless otherwise noted. [b] Inactive (<30% inhibition at 100 nM). [c] Reference standard lixivaptan (VPA-985).

(*S*)-(+)-**32** also had relatively strong V_{1a} affinity (K_i = 24 nM), so V_2-binding selectivity was just 25-fold. In general, R_3 = Ph imparted good-to-excellent V_2 affinity and V_2 functional antagonism (K_i = 2−20 nM), with good V_2 selectivity.

In rats, (*S*)-(+)-**32** showed excellent oral bioavailability (*F*, 68%; plasma elimination $t_{1/2}$, 3.7 h) and dose-dependent aquaresis on oral administration with remarkable potency: at 1 mg/kg, urine output increased 700% and urine osmolality decreased 60% (vs controls). For comparison, a 1-mg/kg oral dose of lixivaptan (**18**) modified urine output +200% and osmolality −50%.[34] (*S*)-(+)-**32** was also orally efficacious in dogs, cynomolgus monkeys, and cirrhotic rats.[39] On the basis of an array of preclinical data, (*S*)-(+)-**32** (RWJ-351647) was advanced into human clinical studies. On oral dosing in humans, it had excellent pharmacokinetics and was an aquaretic agent of exceptional potency (minimum effective single dose of 5 mg). Thus, (*S*)-(+)-**32** has potential for treating edema in patients.

The physicochemical properties of (*S*)-(+)-**32** were better than those for lixivaptan (**18**), as we hoped.[35c] Nevertheless, in seeking a backup compound for (*S*)-(+)-**32**, we introduced a second basic nitrogen into the tricyclic nucleus.[35d] Pyrazinobenzazepines **21c** were prepared (Figure 6) and evaluated (Table 2, **33**−**35**).[35d,e] (*R*)-(+)-**33** and (*S*)-(−)-**33** showed good V_2 affinity, with little difference, and virtually no V_{1a} affinity. The V_2 binding K_i for (*R*)-(+)-**33** of 12 nM was ∼10-fold weaker than that for oxygen analogue (*S*)-(+)-**32** (0.9 nM).[40] (*R*)-(+)-**33** was also ∼10-fold less potent than (*S*)-(+)-**32** in the V_2 functional assay, but it had remarkable V_2 selectivity (>600-fold). V_2 affinity disappeared when the piperazine nitrogen substituent was larger than Me (**33**) or H (**34**), such as in **35**.[35d] In rats, (*R*)-(+)-**33** exhibited excellent oral bioavailability (*F*, 81%; $t_{1/2}$, 3.8 h) and was an orally effective aquaretic agent.

Table 3. Vasopressin Binding and Functional Data for Spirocyclic Benzazepines 36



36a–f

compd[a]	R_1	R_2	binding IC_{50} (nM) V_{1a}	binding IC_{50} (nM) V_2	funct IC_{50} (nM) V_{1a}	funct IC_{50} (nM) V_2
36a	$(CH_2)_2OH$	Ph	6	11	5	170
36b	$(CH_2)_2OH$	F	4	>1000	99	>1000
36c	$(CH_2)_2NMe_2$	Ph	5	11	4	100
36d[b]	$(CH_2)_2NMe_2$	Ph	2	8	45	36
36e[c]	$(CH_2)_2NMe_2$	Ph	53	71	580	680
36f[b]	$(CH_2)_2NMe_2$	F	4	340	78	650

[a] Racemate, unless noted otherwise. [b] (*R*)-Enantiomer. [c] (*S*)-Enantiomer.

The physicochemical properties of (*R*)-(+)-**33** and (*S*)-(+)-**32** are similar, although their aqueous solubility profiles are different.[35c,d] For (*R*)-(+)-**33**, the solubility is >1.0 mg/mL at pH 2.0 and 0.1 mg/mL at pH 7.4; for (*S*)-(+)-**32**, the solubility is 0.006 mg/mL at pH 2.0 and >1.0 mg/mL at pH 7.4. This distinction might have some bearing on oral drug delivery. After amassing a body of data on (*R*)-(+)-**33** (RWJ-659834), it was advanced into preclinical development as a backup compound for (*S*)-(+)-**32**.

We identified many nonpeptide vasopressin receptor antagonists with potent V_2 action[35a−e] and propelled compounds into development; however, potent V_{1a} receptor antagonism was elusive. Since medical opinion leaders perceived a dual V_{1a}/V_2 antagonist to have wider clinical utility with better potential for treating congestive heart failure, we continued on this project to identify drug-worthy compounds with dual action. By pursuing a spirocyclic benzazepine series (**36a**−**f**, Table 3), we discovered such V_{1a}/V_2 receptor antagonists.[35f−i] Although **36a** and **36c** were remarkably potent in the V_{1a} functional assay, with single-digit nanomolar IC_{50} values, they had moderate V_2 functional potency. The (*R*)-enantiomer of **36c**, **36d**, was notable for its excellent V_{1a} and V_2 affinity, good potency, and balanced ratio in the functional assays. In rats, **36d** had useful oral bioavailability (*F*, 22%; $t_{1/2}$, 6.5 h) and produced dose-dependent aquaresis (10 mg/kg, po: urine output +1100%; urine osmolality −75%). We advanced **36d** (RWJ-339489) into preclinical development as a balanced V_{1a}/V_2 receptor antagonist but ultimately replaced it with backup compound RWJ-676070 (not shown),[35h,i] which entered human clinical studies.

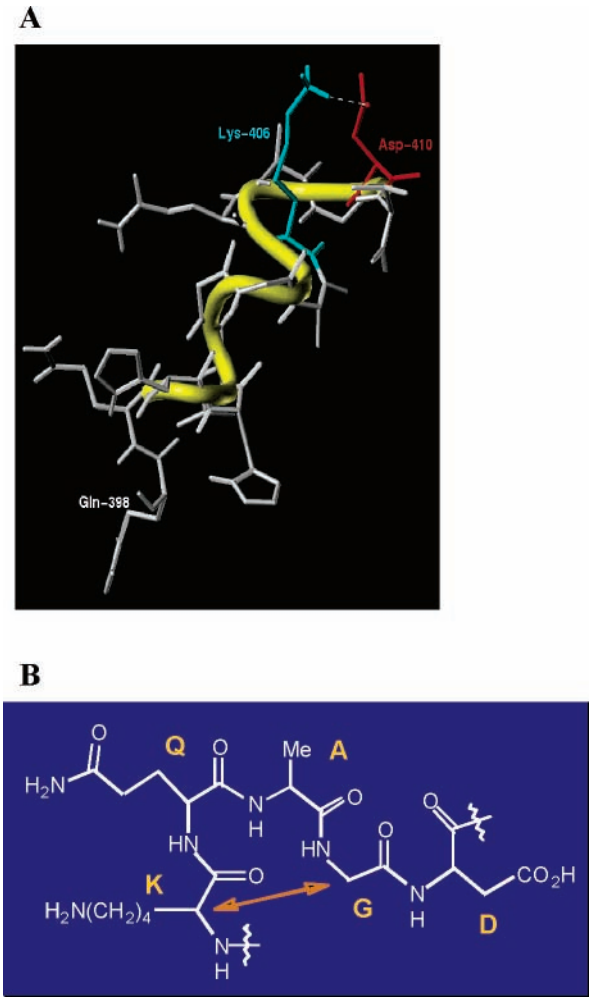


FIGURE 7. (A) Representative solution structure of fibrinogen γ385−411 from a family of conformers derived from NMR distance-geometry calculations and (B) KQADG as oriented in γ385−411 ($C_{\alpha K}-C_{\alpha G}$ distance denoted by arrow).

Integrins

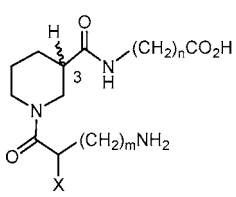
Integrins are a family of heterodimeric transmembrane receptors, which in mammals are comprised of 19 α and 8 β glycoprotein (GP) subunits paired in various α/β combinations.[41] The extracellular portion of an integrin recognizes specific peptide epitopes of a protein ligand, and binding mediates biological processes, such as cellular adhesion and migration.[42] For instance, matrix proteins containing the tripeptide motif RGD bind to the integrins $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$.

Antagonists of GPIIb/IIIa ($\alpha_{IIb}\beta_3$). GPIIb/IIIa, the most abundant integrin on the surface of platelets (~50 000 copies), mediates platelet aggregation in response to diverse activators, such as collagen, adenosine diphosphate (ADP), and α-thrombin.[43] The activated GPIIb/IIIa complex, as the final common step of aggregation, binds the blood glycoprotein fibrinogen to crosslink platelets in a growing thrombus.[44] Thus, compounds that compete with fibrinogen at GPIIb/IIIa can serve as powerful antithrombotic agents.[45] Three intravenous GPIIb/IIIa ("fibrinogen receptor") antagonist drugs are established for preventing thrombosis in an acute-care setting.[46−48] Since adhesion of activated platelets to damaged blood vessels and to each other causes thrombus formation and arterial occlusion (e.g., in myocardial infarction, unstable angina, and stroke), drug researchers envisioned oral agents for chronic antithrombotic therapy.

We pursued oral fibrinogen receptor antagonists by applying a fundamental design approach. Integrin antagonists of this kind have often been based on RGD, an adhesion sequence in the fibrinogen Aα-chain[46c] but also in other adhesive proteins. To foster selectivity, we adopted an alternative approach involving the unique KQAGD sequence in fibrinogen's γ-chain, which also binds to GPIIb/IIIa.[49]

Fibrinogen interacts with sites on GPIIb/IIIa via domains on its α- and γ-chains: RGDF (α95−98), RGDS (α572−575), and HHLGGAKQAGDV (γ400−411).[50] NMR studies on the γ-chain C-terminal peptide γ385−411 identified a turn geometry within KQAGD, γ406−410 (Figure 7).[51] This structure, which has a $C_{\alpha K}-C_{\alpha G}$ distance of 4.5−5.0 Å, suggested the use of certain peptide-mimetic scaffolds bearing moieties related to K and D. We adopted nipecotic acid as a scaffold with K- and D-like units appended to its amine and carboxyl groups, respectively (**37**).[52] Conformational analysis of **37** ($m = 4$, $n = 1$; X = D-NHBoc) revealed a preferred distance of ~6.0 Å between the α-carbons of lysine and glycine. We prepared this compound and its analogues with different m/n spacers, up to ~9 Å, for better coverage.[53,54]

Compounds were evaluated for binding affinity to immobilized GPIIb/IIIa and for inhibition of platelet aggregation.[52] A reasonable result with **37** ($m = 4$, $n = 2$; X = H) led us to **38**, which had good GPIIb/IIIa affinity



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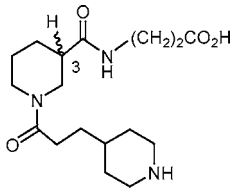
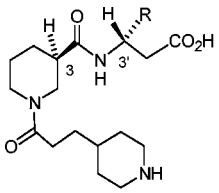


38

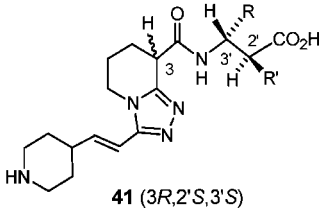
(IC_{50} = 9 nM) and inhibited collagen-induced platelet aggregation (IC_{50} = 140 nM). 3*R*-**38** (Table 4) was tested orally in dogs (ex vivo platelet aggregation) and found to have moderate potency with limited duration.[54] We embarked on a focused optimization campaign that capitalized on the rapid, solid-phase parallel synthesis of analogues, like **39** (Figure 8).[54a] From 250 compounds, we identified several promising nipecotamides, for example, **40b** and **40d** (Table 4). Exploration of improved leads for critical in vivo properties, such as oral absorption, plasma half-life, and duration of pharmacological action, brought 3-pyridyl analogue **40c** to the forefront.[54b,c]

Despite its zwitterionic nature, **40c** (elarofiban, RWJ-53308) had adequate oral pharmacokinetics in dogs (*F*, 16%; $t_{1/2}$, 2 h) and excellent oral pharmacodynamics.[54c] A 3-mg/kg oral dose in conscious dogs strongly inhibited ex vivo collagen- or ADP-induced platelet aggregation for 6 h.[54c] Elarofiban inhibited platelet aggregation in human

Table 4. Nipecotamide (40) and Triazolopyridine (41) Derivatives with Bioactivity



40 (3*R*,3'*S*)



41 (3*R*,2'*S*,3'*S*)

compd	R or R/R′	Fg binding[a] IC_{50}, nM	GFP aggr[b] IC_{50}, nM	oral duration (dose)[c]
3*R*-**38**[d]	H	4.8	270	120 (3)
40a	thien-2-yl	0.1	90	
40b	MDPh[e]	0.5	20	180 (3)
40c	pyridin-3-yl	0.4	60	360 (3)
40d	quinolin-3-yl	0.2	20	150 (1)
40e	PhC≡C	0.2	80	<30 (1)
41a[f]	pyridin-3-yl/H	0.2	61	>360 (1)
41b[f]	H/$NHCO_2Bn$	0.3	41	>360 (1)
41c[f]	quinolin-3-yl/H	0.4	57	270 (1)
xemlo[g]		2.0	300	>180 (3)

[a] Inhibition of fibrinogen (Fg) binding to GPIIb/IIIa. [b] Inhibition of gel-filtered platelet (GFP) aggregation. [c] Duration in dogs (min) at the oral dose given in parentheses (mg/kg) to achieve ≥50% inhibition of collagen-induced (3*R*-**38**, **40a**−**e**) or ADP-induced (**41a**−**c**) platelet aggregation ex vivo. [d] Same as **40** with R = H. [e] 3,4-Methylenedioxyphenyl. [f] Two diastereomers due to *R*/*S* at 3-position, which is stereolabile. [g] Xemlofiban reference standard (Sheppard, L. P. *IDrugs* **1998**, *1*, 257−263).

platelet-rich plasma in response to multiple agonists (collagen, ADP, TRAP-6, arachidonic acid; IC_{50} = 60−160 nM), as expected for a GPIIb/IIIa antagonist.[54c] Given its drug-worthy features, including efficacy in platelet-dependent thrombosis models (dogs, guinea pigs) and excellent safety profile, elarofiban entered human clinical studies for acute and chronic treatment of arterial thrombotic disorders.

To improve on elarofiban, especially for pharmacokinetics and pharmacodynamics, we explored diverse analogues. Favorable results derived from a bicyclic 1,2,4-triazolo[3,4-*a*]pyridine scaffold (Table 4),[55] with **41a** (RWJ-293404) and **41b** (RWJ-58555) having robust metabolic stability, excellent duration of action in dogs (>6 h at 1 mg/kg, p.o.; ex vivo platelet aggregation), and improved oral bioavailability in dogs (*F*, 20−30%, $t_{1/2}$, 4−6 h). Both compounds were advanced into preclinical development as second-generation agents.

Elarofiban progressed successfully through human Phase 2a clinical trials, with good drug exposures and reproducible inhibition of platelet aggregation on oral dosing.[56] Oral elarofiban was well tolerated, with 70% inhibition of aggregation at 3 mg/kg and a prolonged plasma half-life (16−32 h). A 0.03-mg/kg intravenous dose maximally inhibited platelet aggregation, with an ample 3.2-h half-life. This clinical experience supported a regime of sequential intravenous and oral administration to address acute and chronic antiplatelet therapy in patients. At the Phase 2a decision point, we elected not to proceed to full-scale clinical development. Unfortunately, the value of oral GPIIb/IIIa antagonists in chronic antithrombotic therapy was drawn into question by troublesome data from advanced-stage clinical trials from other companies.[57] Consequently, this promising area of therapeutics rapidly vanished from the medical landscape.

Conclusion

In this Account, I tried to convey a flavor of the drug discovery business, from a medicinal chemist's viewpoint, with a trilogy on antagonists for cell-surface receptors. Our research activities constantly operated at the chemistry−biology interface. Since this presentation is very condensed, such that many scientific aspects cannot be fully appreciated, I encourage you to consult the original publications.

Medicinal chemistry is easy to practice, but drug discovery is quite another thing. What percentage of medicinal chemists have invented a marketed drug during their research careers? A very small number, indeed. The percentage increases somewhat for a compound in full development (Phase 2b/3) and is higher for a compound in human clinical trials. The drug hunter requires determination, patience, and persistence to succeed. And like the big-game hunter, good instincts and much luck. There are innumerable perils and pitfalls. It is a very difficult job!

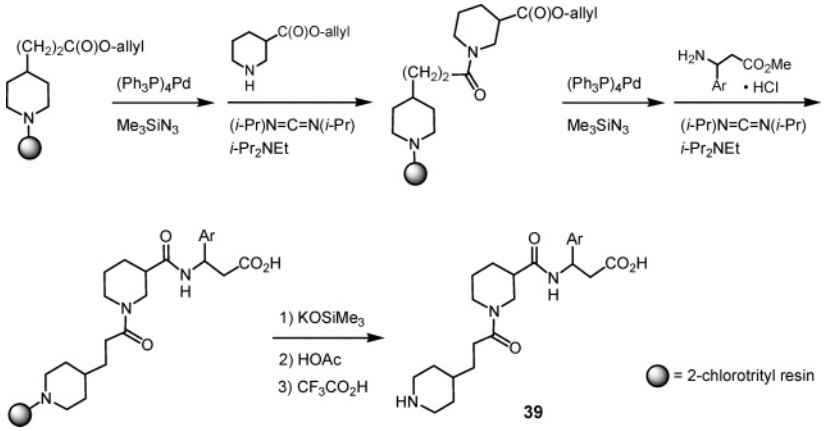


FIGURE 8. Solid-phase synthesis of β-aryl-β-aminopropanoate derivatives **39**.

I thank many excellent scientific colleagues with whom I have collaborated to advance the projects discussed. Their names appear in references 16, 21, 24, 35, 52, 54, and 55.

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