

**ANNUAL
REPORTS IN
MEDICINAL
CHEMISTRY
Volume 32**

*Sponsored by the Division of Medicinal Chemistry
of the American Chemical Society*

Editor-in-Chief: **JAMES A. BRISTOL**

PARKE-DAVIS PHARMACEUTICAL RESEARCH
DIVISION OF WARNER-LAMBERT COMPANY
ANN ARBOR, MICHIGAN



ACADEMIC PRESS

**ANNUAL
REPORTS IN
MEDICINAL
CHEMISTRY
Volume 32**

This Page Intentionally Left Blank

ANNUAL REPORTS IN MEDICINAL CHEMISTRY Volume 32

*Sponsored by the Division of Medicinal Chemistry
of the American Chemical Society*

EDITOR-IN-CHIEF:

JAMES A. BRISTOL

PARKE-DAVIS PHARMACEUTICAL RESEARCH
DIVISION OF WARNER-LAMBERT COMPANY
ANN ARBOR, MICHIGAN

SECTION EDITORS

*DAVID W. ROBERTSON • ANNETTE M. DOHERTY • JACOB J. PLATTNER
WILLIAM K. HAGMANN • WINNIE W. WONG • GEORGE L. TRAINOR*

EDITORIAL ASSISTANT

LISA BAUSCH



ACADEMIC PRESS

San Diego London Boston New York Sydney Tokyo Toronto

This book is printed on acid-free paper. ∞

Copyright © 1997 by ACADEMIC PRESS

All Rights Reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the Publisher.

The appearance of the code at the bottom of the first page of a chapter in this book indicates the Publisher's consent that copies of the chapter may be made for personal or internal use of specific clients. This consent is given on the condition, however, that the copier pay the stated per copy fee through the Copyright Clearance Center, Inc. (222 Rosewood Drive, Danvers, Massachusetts 01923), for copying beyond that permitted by Sections 107 or 108 of the U.S. Copyright Law. This consent does not extend to other kinds of copying, such as copying for general distribution, for advertising or promotional purposes, for creating new collective works, or for resale. Copy fees for pre-1997 chapters are as shown on the title pages. If no fee code appears on the title page, the copy fee is the same as for current chapters.
0065-7743/97 \$25.00

Academic Press

a division of Harcourt Brace & Company

525 B Street, Suite 1900, San Diego, California 92101-4495, USA

<http://www.apnet.com>

Academic Press Limited

24-28 Oval Road, London NW1 7DX, UK

<http://www.hbuk.co.uk/ap/>

International Standard Book Number: 0-12-040532-6

PRINTED IN THE UNITED STATES OF AMERICA

97 98 99 00 01 02 MM 9 8 7 6 5 4 3 2 1

CONTENTS

CONTRIBUTORS ix

PREFACE xi

I. CENTRAL NERVOUS SYSTEM DISEASES

Section Editor: David W. Robertson, DuPont Merck Pharmaceutical Company,
Wilmington, Delaware

1. Recent Advances in Migraine Therapy 1
Theresa Branchek, Synaptic Pharmaceutical Corporation, Paramus, New Jersey; James E. Audia, Eli Lilly and Company, Indianapolis, Indiana
2. Alzheimer's Disease: Recent Advances on the Amyloid Hypothesis 11
Varghese John, Lee H. Latimer, Jay S. Tung, and Michael S. Dappen, Athena Neurosciences, Inc., South San Francisco, California
3. Obesity: Leptin - Neuropeptide Y Interactions in the Control of Body Weight 21
Donald R. Gehlert and Mark Heiman, Eli Lilly and Company, Indianapolis, Indiana
4. Melatonin Receptor Ligands and Their Potential Clinical Applications 31
Cathy D. Mahle, Katherine S. Takaki, and A. John Watson, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, Connecticut
5. Corticotropin-Releasing Hormone (CRH) Receptors and the Discovery of Selective Non-Peptide CRH₁ Antagonists 41
Paul J. Gilligan, Paul R. Hartig, David W. Robertson, and Robert Zaczek, The DuPont Merck Pharmaceutical Company, Wilmington, Delaware
6. Recent Advances in Neurokinin Receptor Antagonists 51
Sander G. Mills, Merck Research Laboratories, Rahway, New Jersey

II. CARDIOVASCULAR AND PULMONARY DISEASES

Section Editor: Annette M. Doherty, Parke-Davis Pharmaceutical Research,
Ann Arbor, Michigan

7. Endothelin Inhibitors 61
Xue-Min Cheng, Kyunghye Ahn, and Stephen J. Haleen, Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan
8. Antithrombotics/Serine Proteases 71
William C. Ripka and George P. Vlasuk, Corvas International, San Diego, California

9. Leukotriene Modulators as Therapeutic Agents in Asthma and Other Inflammatory Diseases 91
Randy L. Bell, James B. Summers and Richard R. Harris, Abbott Laboratories, Abbott Park, Illinois
10. Emerging Opportunities in the Treatment of Atherosclerosis 101
Cheryl M. Hayward and Mark J. Bamberger, Pfizer Inc., Central Research Division, Groton, Connecticut

III. CANCER AND INFECTIOUS DISEASES

Section Editor: Jacob J. Plattner, Abbott Laboratories, Abbott Park, Illinois

11. New Approaches and Agents to Overcome Bacterial Resistance 111
John M. Domagala and Joseph P. Sanchez, Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan
12. Bacterial Genomics and the Search for Novel Antibiotics 121
Leonard Katz, Daniel T. Chu, and Karl Reich, Abbott Laboratories, Abbott Park, Illinois
13. Resistance to Antiretroviral Drug Therapy 131
Akhteruzzaman Molla and William E. Kohlbrenner, Abbott Laboratories, Abbott Park, Illinois
14. Non-HIV Antiviral Agents 141
Steven H. Krawczyk and Norbert Bischofberger, Gilead Sciences, Foster City, California
15. Recent Advances in Antifungal Agents 151
Paul A. Lartey, Abbott Laboratories, Abbott Park, Illinois; Charles M. Moehle, RiboGene, Inc., Hayward, California
16. Angiogenesis Inhibitors 161
Dennis Powell, Jerauld Skotnicki, and Janis Upeslacis, Wyeth-Ayerst Research, Pearl River, New York
17. Chemical Inhibitors of Cyclin-Dependent Kinases 171
Kevin G. Coleman, Joseph P. Lyssikatos, and Bingwei V. Yang, Pfizer Inc., Central Research Division, Groton, Connecticut

IV. IMMUNOLOGY, ENDOCRINOLOGY AND METABOLIC DISEASES

Section Editor: William K. Hagmann, Merck Research Laboratories, Rahway, New Jersey

18. T Lymphocyte Potassium Channel Blockers 181
John C. Kath and Douglas C. Hanson, Pfizer Inc., Central Research Division, Groton, Connecticut; K. George Chandy, University of California Irvine, Irvine, California

19. Male Contraception 191
Donald W. Combs, The R.W. Johnson Pharmaceutical Research Institute, Raritan, New Jersey
20. New Dermatological Agents for the Treatment of Psoriasis 201
Sunil Nagpal and Roshantha A.S. Chandraratna, Allergen Inc., Irvine, California
21. Selective Cyclooxygenase-2 Inhibitors 211
Petpiboon Prasit and Denis Riendeau, Merck Frosst Centre for Therapeutic Research, Point Claire-Dorval, Quebec, Canada
22. Growth Hormone Secretagogues 221
Ravi P. Nargund and Lex H.T. Van der Ploeg, Merck Research Laboratories, Rahway, New Jersey

V. TOPICS IN BIOLOGY

Section Editor: Winnie W. Wong, BASF Bioresearch Corporation, Worcester, Massachusetts

23. Novel Gene Switches for the Regulation of Gene Expression 231
Victoria E. Allgood, Gene Medicine, Inc., The Woodlands, Texas; Eric M. Eastman, Gene Logic, Inc. Columbia, Maryland
24. Agents that Block TNF- α Synthesis or Activity 241
Roy A. Black, Timothy A. Bird, and Kendall M. Mohler, Immunex Corporation, Seattle, Washington
25. Nuclear Orphan Receptors: Scientific Progress and Therapeutic Opportunities 251
David W. Robertson, DuPont Merck Pharmaceutical Co., Wilmington, Delaware; David J. Manglesdorf and Patricia J. Willy, University of Texas Southwestern Medical School, Dallas Texas; and Richard A. Heyman, Ligand Pharmaceuticals, Inc., San Diego, California

VI. TOPICS IN DRUG DESIGN AND DISCOVERY

Section Editor: George L. Trainor, DuPont Merck Pharmaceutical Company, Wilmington, Delaware

26. Discovery and Identification of Lead Compounds from Combinatorial Mixtures 261
Bruce A. Beutel, Abbott Laboratories, Abbott Park, Illinois
27. Electrospray Mass Spectrometric Characterization of Adducts Between Therapeutic Agents and Proteins 269
Catherine Fenselau, University of Maryland Baltimore County, Baltimore, Maryland
28. Nonpeptide Agonists for Peptide Receptors: Lessons from Ligands 277
Elizabeth E. Sugg, Glaxo Wellcome Research and Development, Research Triangle Park, North Carolina

29. Natural Products Research and Pharmaceuticals in the 1990's 285
Stephen K. Wrigley and M. Inês Chicarelli-Robinson, Xenova Ltd., Slough, Berkshire, United Kingdom
30. Inhibition of Cytochrome P-450 and Implications in Drug Development 295
Jiunn H. Lin, Merck Research Laboratories, West Point, Pennsylvania; Anthony Y.H. Lu, Merck Research Laboratories, Rahway, New Jersey

VII. TRENDS AND PERSPECTIVES

Section Editor: James A. Bristol, Parke-Davis Pharmaceutical Research,
Ann Arbor, Michigan

31. To Market, To Market - 1996 305
Paul Galatsis, Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan
- COMPOUND NAME, CODE NUMBER AND SUBJECT INDEX, VOLUME 32 327
CUMULATIVE CHAPTER TITLES KEYWORD INDEX, VOLUMES 1-32 335
CUMULATIVE NCE INTRODUCTION INDEX, 1983-1996 349
CUMULATIVE NCE INTRODUCTION INDEX, 1983-1996, BY INDICATION 363

CONTRIBUTORS

Ahn, Kyunghye	61	Krawczyk, Steven H.	141
Allgood, Victoria E.	231	Lartey, Paul A.	151
Audia, James E.	1	Latimer, Lee H.	11
Bamberger, Mark J.	101	Lin, Jiunn H.	295
Bell, Randy L.	91	Lu, Anthony Y.H.	295
Beutel, Bruce A.	261	Lyssikatos, Joseph P.	171
Bird, Timothy A.	241	Mahle, Cathy D.	31
Bischofberger, Norbert	141	Manglesdorf, David J.	251
Black, Roy A.	241	Mills, Sander G.	51
Brancheck, Theresa	1	Moehle, Charles M.	151
Chandraratna, Roshantha A.S.	201	Mohler, Kendall M.	241
Chandy, K. George	181	Molla, Akhteruzzaman	131
Cheng, Xue-Min	61	Nagpal, Sunil	201
Chicarelli-Robinson, M. Inês	285	Nærgund, Ravi P.	221
Chu, Daniel T.	121	Powell, Dennis	161
Coleman, Kevin G.	171	Prasit, Petpiboon	211
Combs, Donald W.	191	Reich, Karl	121
Dappen, Micheal S.	11	Riendeau, Denis	211
Domagala, John M.	111	Ripka, William C.	71
Eastman, Eric M.	231	Robertson, David W.	251
Fenselau, Catherine	269	Robertson, David W.	41
Galatsis, Paul	305	Sanchez, Joseph P.	111
Gehlert, Donald R.	21	Skotnicki, Jerauld	161
Gilligan, Paul J.	41	Sugg, Elizabeth E.	277
Haleen, Stephen J.	61	Summers, James B.	91
Hanson, Douglas C.	181	Takaki, Katherine S.	31
Harris, Richard R.	91	Tung, Jay S.	11
Hartig, Paul R.	41	Upeslakis, Janis	161
Hayward, Cheryl M.	101	Van der Ploeg, Lex H.T.	221
Heiman, Mark	21	Vlasuk, George P.	71
Heyman, Richard A.	251	Watson, A. John	31
John, Varghese	11	Willy, Patricia J.	251
Kath, John C.	181	Wrigley, Stephen K.	285
Katz, Leonard	121	Yang, Bingwei V.	171
Kohlbrener, William E.	131	Zaczek, Robert	41

This Page Intentionally Left Blank

PREFACE

Annual Reports in Medicinal Chemistry continues to strive to provide timely and critical reviews of important topics in medicinal chemistry together with an emphasis on emerging topics in the biological sciences which are expected to provide the basis for entirely new future therapies.

Volume 32 retains the familiar format of previous volumes, this year with 31 chapters. Sections I - IV are disease oriented and generally report on specific medicinal agents with updates from Volume 31 on antithrombotics, endothelin, neurokinin antagonists, cell cycle regulation, and obesity. As in past volumes, annual updates have been limited to only the most active areas of research in favor of specifically focussed and mechanistically oriented chapters, where the objective is to provide the reader with the most important new results in a particular field. To this end, chapters on topics not reported in at least five years include: migraine therapy, Alzheimer's disease, melatonin, bacterial resistance, bacterial genomics, antiretroviral resistance antifungals, angiogenesis, T lymphocyte potassium channel blockers, male contraception, psoriasis, selective PGHS2 inhibitors, and growth hormone secretagogues.

Sections V and VI continue to emphasize important topics in medicinal chemistry, biology, and drug design as well as the critical interfaces among these disciplines. Included in Section V, Topics in Biology, are chapters on regulation of gene expression, blockade of TNF- α , and nuclear orphan receptors. Each of these areas is likely to lead to novel medicinal agents in the future. Chapters in Section VI, Topics in Drug Design and Discovery, reflect the current focus on mechanism-directed drug discovery and newer technologies. These include chapters on combinatorial mixtures as discovery tools, mass spectrometry of non-covalent adducts, nonpeptide agonists of peptide receptors, natural products, and cytochrome P-450.

Volume 32 concludes with *To Market, To Market* - a chapter on NCE introductions worldwide in 1996. In addition to the chapter reviews, a comprehensive set of indices has been included to enable the reader to easily locate topics in volumes 1-32 of this series.

Over the past year, it has been my pleasure to work with 6 highly professional section editors and 70 authors, whose critical contributions comprise this volume.

*James A. Bristol
Ann Arbor, Michigan
May 1997*

This Page Intentionally Left Blank

SECTION I. CENTRAL NERVOUS SYSTEM DISEASES

Editor: David W. Robertson
DuPont Merck Pharmaceutical Company
Wilmington, DE 19880-0500

Chapter 1. Recent Advances in Migraine Therapy

Theresa Branchek^a and James E. Audia^b
Synaptic Pharmaceutical Corporation^a, Paramus, NJ 07652
and
Eli Lilly and Company^b, Indianapolis IN 46285

Introduction - The search for safe and effective treatments for migraine pain has accelerated in the pharmaceutical industry since the introduction of sumatriptan (1) in the marketplace. This chapter will highlight the proposed mechanisms of action of this acute therapy, the various approaches to improve both efficacy and safety, and future areas of study.

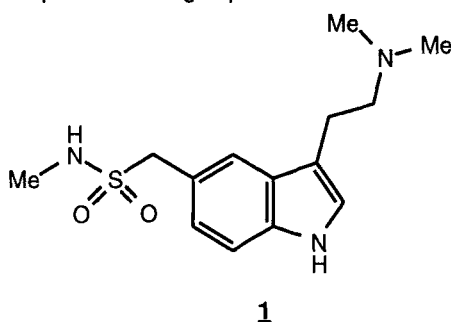
Mechanisms and Models of Migraine - The etiology of migraine is not certain although there are a set of hypotheses concerning various stages of the process of migraine. Migraine is thought to be initiated by a "trigger" of unknown origin. Environmental and physiological factors impinge upon this trigger to set in action the events leading to the migraine episode. It may be that migraineurs have an underlying "neural instability" which facilitates setting off the trigger (1). Once initiated, there are several components. The earliest phase may be a premonitory symptom the evening before a migraine headache. The next may be "aura" consisting of visual field disturbances which may be related to changes in cerebral blood flow; these blood flow changes may alter cortical perfusion. Ultimately, the headache appears. Headache pain has been attributed to several factors including dilation of the cerebral blood vessels (2), cerebral edema (3), neurogenic inflammation (4), and central transmission of pain in the brain itself (5).

The early animal models of migraine were based on the study of vascular changes in response to test compounds. The contraction of isolated blood vessels, such as the dog saphenous vein, was used to discover the activity of sumatriptan (6). Other vascular responses such as blood shunting through the arterial-venous anastomoses of the intact pig using radiolabeled microspheres have also been employed (7). More recently a model based on a "neurogenic" hypothesis has been widely explored (8,9). In this model, the trigeminal ganglion is stimulated electrically, which leads to release of neurotransmitters from the peripheral nerve terminals surrounding blood vessels in the dura. This leads to an increased permeability of the vessels which can be monitored by use of a tracer.

Serotonin Receptor Subtypes - Serotonin (5-HT) receptors have been divided into seven classes based upon pharmacological, structural and signaling properties (10). They are: five 5-HT₁ receptor subtypes, three 5-HT₂ receptor subtypes, one 5-HT₃ receptor, one 5-HT₄ receptor, two 5-HT₅ receptor subtypes, one 5-HT₆ receptor and

one 5-HT₇ receptor. Splice variants or additional subtypes are likely for the 5-HT_{2C} (11) and 5-HT_{2A} (12) and have already been demonstrated for the 5-HT₃ (13), 5-HT₄ (14), and 5-HT₇ receptors (15). The 5-HT₃ receptor is a member of the ligand-gated ion channel superfamily (16). All other cloned 5-HT receptors are members of the G-protein coupled receptor superfamily. The pharmacological properties of these receptors have been extensively reviewed elsewhere (10,17). Only the 5-HT₁ family has been implicated in the acute treatment of migraine. The five 5-HT₁ receptors are termed 5-HT_{1A}, 5-HT_{1B} (formerly called 5-HT_{1Dβ}), 5-HT_{1D} (formerly called 5-HT_{1Dα}), 5-HT_{1E} and 5-HT_{1F} (10,17) and they each contain comparable amino acid chain lengths (421, 377, 390, 365 and 366 respectively for the human sequences). These proteins all share a "homology cluster" displaying mutual conservation of greater than half of the amino acid residues in their transmembrane spanning regions which are likely to contain the ligand binding pocket (18,19). In contrast, the extracellular amino termini and loop regions and carboxyl termini lack significant homology that would enable prediction of their common intracellular coupling to second messengers. All 5-HT₁ receptors couple to G-proteins which leads to inhibition of adenylate cyclase activity (10). The 5-HT_{1A} (20), 5-HT_{1B} (21), 5-HT_{1D} (21), and 5-HT_{1F} (22) subtypes also modulate intracellular Ca⁺⁺ through PTX-sensitive G-proteins. In addition to these pathways, each of the 5-HT₁ receptors, except 5-HT_{1E} (23), has been shown to increase the turnover of inositol phosphates (21,22,24,25). Physiologically, these receptors may act through G-protein-gated channels such as Girk1 (26). The 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} all are thought to inhibit the release of neurotransmitters.

Role of 5-HT_{1B/1D/1-like} Receptors - Sumatriptan (**1**) was developed using classical pharmacological methodology employing the contraction of the dog saphenous vein preparation (27). As modern receptor binding and classical pharmacological methods began to be reconciled, it appeared there was a relationship between the "5-HT_{1-like}" receptor of the dog saphenous vein and the 5-HT_{1D} receptor described in binding



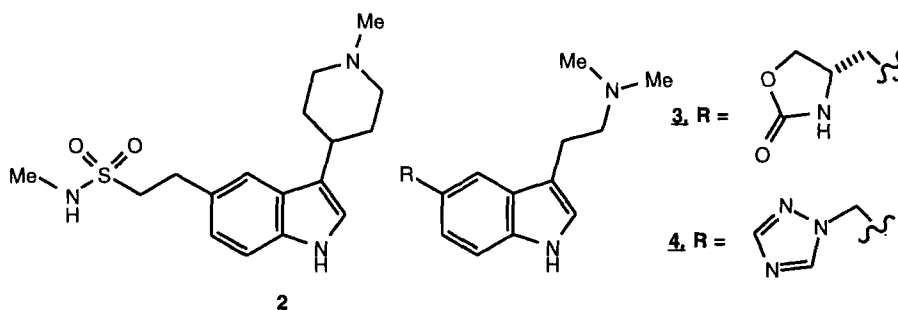
studies (28). Sumatriptan became a selective tool to study 5-HT_{1D} receptors. The advent of molecular cloning further complicated interpretation of these data because two separate genes encoding 5-HT_{1D} receptors were discovered (29), each with high affinity for sumatriptan. Further, in the rat and mouse, one of these receptors, 5-HT_{1Dβ}, had a very different pharmacological profile and was known as a separate subtype, 5-HT_{1B}. Molecular cloning demonstrated that the 5-HT_{1Dβ} and 5-HT_{1B} gene were species homologs (30,31). Their deduced amino

acid sequences were 93% identical over the entire sequence and 96% identical in the transmembrane domains. In fact, a single amino acid difference pinpointed to TM VII (T355N) appears to be responsible for the disparity in the pharmacological profiles of these human and rat 5-HT receptor subtypes (32). The complications in the receptor nomenclature have led to a recent revision (32) and the 5-HT_{1Dβ} receptor is now known as 5-HT_{1B}. Both 5-HT_{1B} and 5-HT_{1D} receptors are distributed in the brain, where they appear to have primarily presynaptic localization (34). In several preparations, these receptors inhibit the release of serotonin from either terminal or somatodendritic processes (35). They also act as heteroreceptors, inhibiting the release of glutamate, acetylcholine and other neurotransmitters (36,37).

How do these receptors and the activity of sumatriptan and other 5-HT₁ agonists described below relate to the models and mechanisms of migraine headache? It is clear that all 5-HT₁ agonists that have been reported to be useful in the treatment of migraine, including naratriptan (**2**), zolmitriptan (311-C90, **3**), and rizatriptan (MK 462, **4**) have substantial affinity for both the human 5-HT_{1D} and 5-HT_{1B} receptor subtypes. It is possible that both activities are therapeutic in alleviation of the migraine episode. Several lines of evidence suggest that the 5-HT_{1B} receptor may be the subtype most closely related to the dog saphenous vein 5-HT₁-like receptor (34). Therefore, these agonists may act on 5-HT_{1B} receptor on the smooth muscle of human cerebral blood vessels to cause vasoconstriction (similar to their activity in the dog saphenous vein). In addition, the 5-HT₁-like agonists may act on 5-HT_{1D} receptors on the terminals of the trigeminovascular system to inhibit neurotransmitter release and to thus stop the neurogenic inflammation (34,38). The clinical utility of compounds acting at only one of these two sites has not yet been reported although it has been suggested that a 5-HT_{1D}-selective compound may be therapeutic and may reduce some of the cardiovascular liabilities of sumatriptan (38, *vide infra*).

Table 1. Affinities of Antimigraine Compounds at Cloned 5-HT_{1D} and 5-HT_{1B} Receptors.

Compound	pK _i , 5-HT _{1D}	pK _i , 5-HT _{1B}	Reference
Sumatriptan (1)	8.5	8.1	39
Naratriptan (2)	8.3	8.7	39
Zolmitriptan (3)	9.2	8.3	40
Rizatriptan (4)	7.7	7.3	39
CP 122,288 (5)	8.1	7.5	39
Avitriptan (8)	8.3	7.7	41
Alniditan (9)	9.0	8.7	42

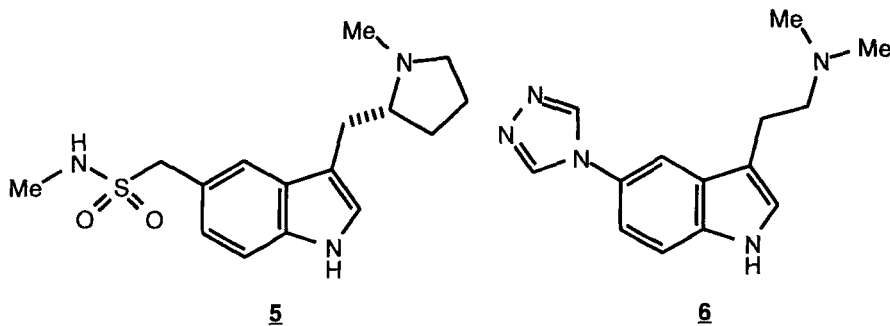


Designing an Improved Sumatriptan - While sumatriptan is effective as a migraine abortive agent, about 15% of patients fail to respond to treatment and up to 40% may suffer from recurrence of headache within 24 hours (43,44). Similar rates of headache recurrence have been observed with other antimigraine treatments. Further, the finding of decreased latency of onset of headache relief after subcutaneous *versus*

oral sumatriptan suggests a possible relationship between plasma drug concentrations and headache relief. A number of approaches for designing improvements beyond sumatriptan in migraine abortive treatment have resulted. These approaches include improvements in pharmacokinetics (45), central penetration, and binding profile for 5HT_{1D} (46) and other receptors such as the 5HT_{1F} (47,48).

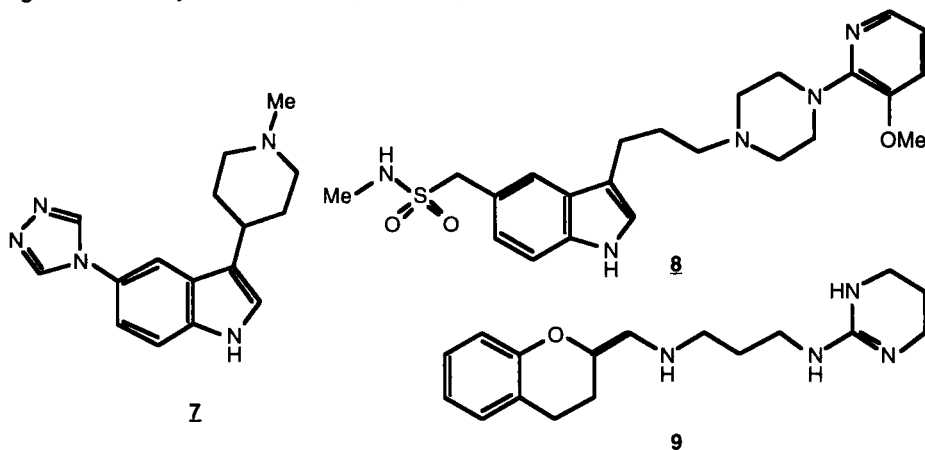
Rizatriptan (**4**) has been demonstrated in preclinical studies to show both increased oral bioavailability and more rapid absorption compared to oral sumatriptan (49). These findings, supported by preliminary clinical pharmacokinetic studies, suggest that this agent might offer greater consistency of effect due to the increased bioavailability. Improvements in onset of efficacy might also occur as a consequence of the more rapid absorption (49).

The observation that sumatriptan fails to prevent headache development upon subcutaneous administration in the aura phase but shows effect after headache, possibly due to diminished integrity of the blood brain barrier during migraine episode, suggests that central penetration might be an important attribute of an effective antimigraine therapeutic (50). Zolmitriptan (**3**) is less hydrophilic than sumatriptan and shows both peripheral and central activities, with activity as a vasoconstrictor (peripherally mediated), as an inhibitor of trigeminally mediated neuropeptide release, and on the central components of the trigemino-vascular system within the nucleus caudalis (51). Preliminary studies suggest that oral zolmitriptan may be useful in preventing migraine when taken during aura (52), supporting the importance of central penetration.

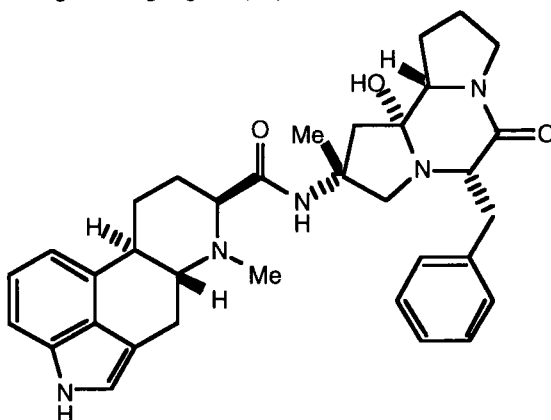


A conformationally restricted analog of sumatriptan, **5** (CP-122,288) selectively inhibits neurogenic inflammation in intracranial tissues at doses which lack vasoconstrictor effects in animal studies (53). With affinity comparable to sumatriptan for 5HT_{1D} and 5HT_{1B} receptors, **5** is 40,000 times more potent in blocking rat dural neurogenic plasma protein extravasation, while similarly potent to sumatriptan as a vasoconstrictor. The clinical experience with this agent should help to elucidate the relevance of each of these activities in migraine intervention. High oral availability is also found with the 5HT_{1D} selective agonist **6** (L-741,604), which also shows reduced plasma clearance compared to either sumatriptan or rizatriptan in preclinical studies (54). The corresponding indolylpiperidine **7** (L-741,519) additionally has an increased *in vivo* half-life relative to the tryptamine analogs. This attribute might prove valuable in reducing the incidence of headache recurrence. Developed as a 5HT_{1B/1D} agonist, avitriptan (BMS-180048, **8**) selectively decreases corotid blood flow due to a reduction in arteriovenous anastomotic blood flow. This agent also potently constricts isolated human coronary artery with effects comparable to those of sumatriptan (55). Chemically unique among the serotonergic antimigraine agents, alniditan (**9**) is a non-

indole with high affinity for 5HT1B, 5HT1D, and 5HT1A receptors, and lacking significant affinity for the 5HT1F (*vide infra*) receptor (56).



Novel Presynaptic Receptor Targets: 5-HT1F - In addition to the 5-HT1B and 5-HT1D receptors, a novel or "orphan" 5-HT receptor called 5-HT1F was cloned and characterized (57). This receptor showed many similarities to 5-HT1B and 5-HT1D receptors with respect to receptor binding properties, coupling to the inhibition of adenylate cyclase activity, and general distribution in the brain, although its abundance as reflected by the mRNA level for this receptors was relatively low. The pharmacological profile of this receptor was not easily related to any of the known pharmacological subtypes measured in native tissues. For example, the 5-HT1F receptor was characterized by the following rank order of agonist potencies: 5-HT > sumatriptan >> 5-CT, forming a unique pharmacological profile. The surprisingly high affinity and potency of sumatriptan for the 5-HT1F receptor subtype lead to speculation that sumatriptan might exert its anti-migraine action through this "orphan" subtype (57). Supporting this notion, the mRNA for this receptor was detected in the nucleus caudalis as well as in other central pain-processing nuclei (58) and was also demonstrated in the trigeminal ganglion (59).



10

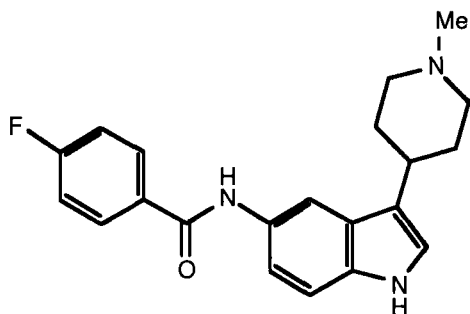
Further support for the 5-HT1F presynaptic mechanism came from a comparison of the activity of "5-HT1D/1B" agonists such as naratriptan, rizatriptan, zolmitriptan, and dihydroergotamine (DHE, **10**) in human migraine and in migraine models with their affinities at the cloned human 5-HT1D, 5-HT1B, and 5-HT1F receptors (60,61). As

shown in Table 2, each compound had substantial activity at the 5-HT_{1F} receptor in addition to affinity at the 5-HT_{1D} and 5-HT_{1B} sites.

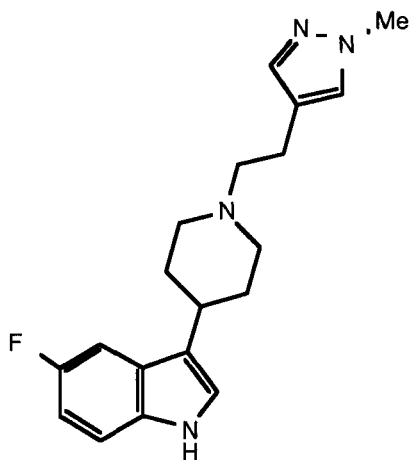
Table 2. Affinities of Compounds at Cloned Human 5-HT_{1F} Receptor (60,61).

Compound	pK, 5HT1F
LY334370 (11)	8.8
LY302148 (12)	8.6
Naratriptan (2)	8.4
Zolmitriptan (3)	7.6
Sumatriptan (1)	7.6
DHE (10)	6.6
Rizatriptan (4)	6.6

Furthermore, comparison of these affinities to the potencies of the same compounds to inhibit plasma extravasation in the guinea pig neurogenic model indicated the best correlation was with the 5-HT_{1F} receptor (60,61). Finally, selective 5-HT_{1F} agonists were designed and evaluated in the cloned receptors as well as in the plasma extravasation model. The activity *in vivo* most closely matched predictions based on their 5-HT_{1F} activity (47,48). Moreover, these 5-HT_{1F} selective agonists were not potent stimulators of contraction of the rabbit saphenous vein, another of the preparations used to hunt for "5-HT_{1D/1B} agonists" (60,61). The 5-HT_{1F} agonist LY 334370 (**11**) is presently under clinical investigation and determine whether a serotonergic presynaptic receptor agonist devoid of vasoconstrictor properties has antimigraine efficacy (60).



11



12

Beyond Serotonin - Several additional pharmacological approaches to the treatment of migraine are in various stages of testing including substance-P antagonists, CGRP antagonists, somatostatin agonists, GABA A antagonists, and others (62). Although the clinical data accumulating on the tachykinin antagonists has not been promising

thusfar, additional clinical data are required to evaluate the potential of these other receptor-based therapies.

However, new insights into possible molecular neurobiological substrates which may underlie migraine pathology have been provided from the study of human genetics of a rare form of migraine, known as familial hemiplegic migraine (FHM). This condition is an autosomal dominant subtype of migraine with aura which is characterized by the occurrence of hemiplegia, or hemiparesis, during the period of aura (63,64). Due to the strong genetic basis for this disease, investigators have sought to find the possibly mutated FHM genes which may be involved in its pathophysiology. The frequency of migraine in patients suffering from another autosomal dominant neurological condition, CADASIL (Cerebral Autosomal Dominant Arteropathy with Subcritical Infarcts and Leukoencephalopathy), is extremely high (65), and this observation led to the hypothesis that the CADASIL gene might be responsible for FHM. Since the CADASIL gene had been mapped to chromosome 19, it was possible that FHM also mapped to this chromosome. Using genetic linkage analysis (66), it was demonstrated that the FHM gene resided within a chromosomal interval containing the CADASIL locus on chromosome 19. This set the stage for cloning efforts which have already yielded two interesting discoveries, a Notch protein and a calcium channel (*vide infra*).

In a recent report, a candidate gene from the critical region of the CADASIL locus was identified (67). This gene was shown to be a member of the Notch gene family, highly homologous to the murine Notch-3. It maps to human chromosome 19p 13.2-13.2. Ten different missense mutations occur in the sequence from over a dozen separate CADASIL families. The mutations are expected to result in an altered folding of the Notch protein because they are additions or mutations of cysteine residues. The functional consequences for the mutant Notch proteins remain to be elucidated. Interestingly, Notch signaling in *Caenorhabditis elegans* is affected by an interacting protein related to the mammalian presenilin genes, PS-1 and PS-2 which may be the keys to early onset familial Alzheimer's dementia (68). Coupled with the role of Notch proteins in *Drosophila* development, these findings suggest that the mutations in the Notch-3 gene in CADASIL patients may be somehow responsible for the "adult-onset" aspect of this neurological condition thus as some kind of "clock". It is also interesting to note that nearly half of the CADASIL patients develop migraine as a first syndrome at about age 20, relatively late compared with the onset of symptoms in common migraine. The more severe aspects of the CADASIL syndrome often take another 20 years to develop (69).

Simultaneous with the discovery of the human Notch-3 gene in CADASIL another gene was isolated from the same chromosomal region, 19p 13. This gene encoded an alpha1 subunit of a type P/Q calcium channel (CACNL1A4, 70) which was mutated in FHM patients. This gene also maps to human chromosome 19p 13.1-p13.2. Four distinct missense mutations in five unrelated FHM families were found. One mutation may affect the voltage sensor (R129Q), a second may affect the ion selectivity filter (T666M), and two others are in S6 segments (V714A; I1811L). The role of these residues is as yet unknown although their absolute conservation in all calcium channel alpha1 subunits suggests a key role. Interesting, mutations at other loci in the same gene are observed in patients displaying episodic ataxia type 2 (EA-2) which is another episodic, autosomal dominant disorder with migraine as part of the syndrome. The mutations in these patients lead to truncated proteins which are unlikely to form functional calcium channels (70).