Successful Drug Discovery

Volume 2
Edited by
János Fischer and
Wayne E. Childers

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Volume 2
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Contents

Preface XIII

List of Contributors XVII

Part I HDAC Inhibitor Anticancer Drug Discovery 1

1 From DMSO to the Anticancer Compound SAHA, an Unusual Intellectual Pathway for Drug Design 3
   Ronald Breslow
   1.1 Introduction 3
   1.2 The Discovery of SAHA (vorinostat) 4
   1.3 Clinical Trials 7
   1.4 Follow-On Research – Selective HDAC Inhibitors 8
   1.5 Conclusion 9
   References 9

2 Romidepsin and the Zinc-Binding Thiol Family of Natural Product HDAC Inhibitors 13
   A. Ganesan
   2.1 Histone Deacetylases as a Therapeutic Target 13
   2.2 The Discovery and Development of Romidepsin 15
   2.3 The Zinc-Binding Thiol Family of Natural Product HDAC Inhibitors 18
   2.4 Synthetic Analogues of the Zinc-Binding Thiol Natural Products 21
   2.5 Summary 23
   References 24

3 The Discovery and Development of Belinostat 31
   Paul W. Finn, Einars Loza and Elisabeth Carstensen
   3.1 Introduction 31
   3.2 Discovery of Belinostat 32
   3.2.1 Design Strategy 32
   3.2.2 Medicinal Chemistry and SAR 34
## Contents

3.3 Belinostat Biological Profiling 41
3.3.1 Mode of Action and HDAC Isoform Selectivity 41
3.3.2 Antiproliferative and Antitumor Activity 42
3.4 Formulation Development 44
3.5 Clinical Development 45
3.5.1 Clinical Studies Leading to Approval and Other Clinical Investigations 45
3.5.2 Pharmacokinetics 49
3.5.3 Safety and Tolerability 51
3.6 Conclusions 52

References 53

4 Discovery and Development of Farydak (NVP-LBH589, Panobinostat) as an Anticancer Drug 59

**Peter Atadja and Lawrence Perez**

4.1 Target Identification: From p21Waf1 Induction to HDAC Inhibition 59
4.2 Program Flowchart Assays for Drug Discovery 61
4.3 Hit-To-Lead Campaign: Trichostatin A to LAK974 63
4.4 Lead Optimization: LAK974 to LAQ824 64
4.5 Profiling LAQ824 for Cancer Therapy 66
4.6 Preclinical Development of LAQ824 70
4.7 LAQ824 Follow-Up 72
4.8 Discovery of LBH589 73
4.9 Safety Profile for LBH589 74
4.10 Pan-HDAC Inhibition by LBH589 76
4.11 Cancer Cell-Specific Cytotoxicity of LBH589 76
4.11.1 Toxicity and Safety Studies with LBH589 78
4.11.2 Early Clinical Activity of LBH589 in CTCL 78
4.11.3 Large-Scale Cell Line Profiling to Discover Lineage-Specific LBH589-Sensitive Cancer Indications 79
4.11.4 Clinical Profiling of Heme Malignancies for LBH589 Activity 80
4.11.5 Phase II Study of Oral Panobinostat in Hodgkin Lymphoma 81
4.11.6 Phase IB Clinical Studies in Multiple Myeloma 82
4.11.7 Phase III Registration Study in Relapsed or Refractory Myeloma 82
4.11.8 Conclusion and Future Perspective 83

References 85

5 Discovery and Development of HDAC Subtype Selective Inhibitor Chidamide: Potential Immunomodulatory Activity Against Cancers 89

**Xian-Ping Lu, Zhi-Qiang Ning, Zhi-Bin Li, De-Si Pan, Song Shan, Xia Guo, Hai-Xiang Cao, Jin-Di Yu and Qian-Jiao Yang**

5.1 Introduction 89
5.1.1 Epigenetics and Cancer 89
5.1.2 Epigenetic Drugs 90
5.2 Discovery of Chidamide 93
5.2.1 Identification of Chemical Scaffold 93
5.2.2 Design and Screening New Selective Benzamide HDAC Inhibitors 93
5.2.3 Molecular Docking of Chidamide with HDAC2 95
5.3 Molecular Mechanisms of Chidamide 97
5.3.1 Selectivity 97
5.3.2 Induction of Cell Cycle Arrest, Apoptosis and Differentiation of Tumour Cells 98
5.3.3 Reversal of Epithelial to Mesenchymal Transition 99
5.3.4 Stimulation of Innate and Antigen-Specific Antitumour Immunity 99
5.3.5 Multiplicity of Anticancer Mechanisms by Chidamide 100
5.4 Animal Studies 101
5.5 Clinical Development 101
5.5.1 Pharmacokinetics and Pharmacodynamics 101
5.5.2 Unmet Medical Needs for Peripheral T-Cell Lymphoma (PTCL) 102
5.5.3 Efficacy Assessment of Chidamide in PTCL Patients 103
5.5.4 Safety Profile 105
5.6 Future Perspective 106
References 108

Part II Steroidal CYP17 Inhibitor Anticancer Drug Discovery 115

6 Abiraterone Acetate (Zytiga): An Inhibitor of CYP17 as a Therapeutic for Castration-Resistant Prostate Cancer 117
Gabriel M. Belfort, Boyd L. Harrison and Gabriel Martinez Botella
6.1 Introduction 117
6.2 Discovery and Structure–Activity Relationships (SAR) 119
6.3 Preclinical Characterisation of Abiraterone and Abiraterone Acetate 126
6.3.1 Pharmacology 126
6.3.2 Pharmacokinetics 127
6.3.3 Toxicology 128
6.4 Physical Characterisation 129
6.5 Clinical Studies 129
6.6 Conclusion 132
References 133

Part III Anti-Infective Drug Discoveries 137

7 Discovery of Delamanid for the Treatment of Multidrug-Resistant Pulmonary Tuberculosis 139
Hidetsugu Tsubouchi, Hirofumi Sasaki, Hiroshi Ishikawa and Makoto Matsumoto
7.1 Introduction 139
Sofosbuvir: The Discovery of a Curative Therapy for the Treatment of Hepatitis C Virus

Michael J. Sofia

8.1 Introduction 163
8.2 Discussion 165
8.2.1 Target Rationale: HCV NS5B RNA-Dependent RNA Polymerase 165
8.2.2 Rationale and Design of a Liver Targeted Nucleotide Prodrug 168
8.2.3 Prodrug Optimization and Preclinical Evaluation 171
8.2.4 Prodrug Metabolism 175
8.2.5 Clinical Proof of Concept of a Liver Targeted Nucleotide Prodrug 176
8.2.6 The Single Diastereomer: Sofosbuvir 176
8.2.7 Sofosbuvir Preclinical Profile 177
8.2.8 Sofosbuvir Clinical Studies 179
8.2.9 Viral Resistance 182
8.3 Conclusion 183
References 184
Part IV  Central Nervous System (CNS) Drug Discovery  189

9  The Discovery of the Antidepressant Vortioxetine and the Research that Uncovered Its Potential to Treat the Cognitive Dysfunction Associated with Depression  191
   Benny Bang-Andersen, Christina Kurre Olsen and Connie Sanchéz
9.1  Introduction  191
9.2  The Discovery of Vortioxetine  192
9.3  Clinical Development of Vortioxetine for the Treatment of MDD  200
9.4  Uncovering Vortioxetine’s Potential to Treat Cognitive Dysfunction in Patients with MDD  201
9.4.1  Early Preclinical Evidence that Differentiated Vortioxetine from Other Antidepressants  201
9.4.2  Vortioxetine’s Primary Targets and Their Putative Impact on Cognitive Function – Early Preclinical Data  202
9.4.3  Hypothesis-Generating Clinical Study of Vortioxetine’s Effects on Cognitive Symptoms in Elderly Patients with MDD  203
9.4.4  Substantiation of a Mechanistic Rationale for the Procognitive Effects of Vortioxetine in Preclinical Models and Its Differentiation from SSRIs and SNRIs  204
9.4.5  Confirmation of the Cognitive Benefits of Vortioxetine in Two Large Placebo-Controlled Studies in Adults with MDD  205
9.4.6  Additional Translational Evidence of the Effect of Vortioxetine on Brain Activity During Cognitive Performance  208
9.5  Conclusion  208
References  210

Part V  Antiulcer Drug Discovery  215

10  Discovery of Vonoprazan Fumarate (TAK-438) as a Novel, Potent and Long-Lasting Potassium-Competitive Acid Blocker  217
   Haruyuki Nishida
10.1  Introduction  217
10.2  Limitations of PPIs and the Possibility of P-CABs  218
10.3  Exploration of Seed Compounds  220
10.4  Lead Generation from HTS Hit Compound 1  220
10.5  Analysis of SAR and Structure–Toxicity Relationship for Lead Optimization  223
10.6  Selection of Vonoprazan Fumarate (TAK-438) as a Candidate Compound  224
10.7  Preclinical Study of TAK-438  226
10.8  Clinical Study of TAK-438  228
10.9  Discussion  229
10.10  Conclusion  230
References  232
11 Discovery and Development of Nintedanib: A Novel Antiangiogenic and Antifibrotic Agent 237

Gerald J. Roth, Rudolf Binder, Florian Colbatzky, Claudia Dallinger, Rozsa Schlenker-Herceg, Frank Hilberg, Lutz Wollin, John Park, Alexander Pautsch and Rolf Kaiser

11.1 Introduction 237
11.2 Structure–Activity Relationships of Oxindole Kinase Inhibitors and the Discovery of Nintedanib 238
11.3 Structural Research 244
11.4 Preclinical Pharmacodynamic Exploration 246
11.4.1 Kinase Inhibition Profile of Nintedanib 246
11.4.2 Oncology, Disease Pathogenesis and Mechanism of Action 246
11.4.3 Idiopathic Pulmonary Fibrosis, Disease Pathogenesis and Mechanism of Action 249
11.5 Nonclinical Drug Metabolism and Pharmacokinetics 250
11.6 Clinical Pharmacokinetics 251
11.7 Toxicology 252
11.8 Phase III Clinical Data 253
11.8.1 Efficacy and Safety of Nintedanib in IPF 253
11.8.2 Efficacy and Safety of Nintedanib in NSCLC 255
11.9 Other Oncology Studies 256
11.10 Conclusions 257

References 258

Index 267
Preface

The first volume of Successful Drug Discovery has been well received and the International Union of Pure and Applied Chemistry (IUPAC) supported its continuation.

The main goal of this book series is to help experts of drug research and development both in academia and industry with case histories described by their key inventors or recognised experts whose contributions can also serve as teaching examples.

This year marks the tenth anniversary of the approval of vorinostat, the first marketed histone deacetylase inhibitor (HDAC). This event inaugurated a stream of HDAC inhibitor approvals and confirmed the validity of this drug target and of epigenetic modulation as a viable therapeutic mechanism. To celebrate this important milestone the volume presents a number of HDAC inhibitor drug discovery stories.

The editors of the second volume focused on the following six parts:

I. HDAC Inhibitor Anticancer Drug Discovery

Part Editor: A. Ganesan (University of East Anglia, Norwich, UK)

1. **Vorinostat**
   Ronald Breslow (Columbia University, USA) describes the discovery of vorinostat, which is a pioneer HDAC inhibitor whose discovery started from dimethylsulfoxide as a lead molecule.

2. **Romidepsin**
   A. Ganesan (University of East Anglia, UK) gives an overview of the discovery of romidepsin, a depsipeptide natural product. High-throughput screening led to an anticancer drug that proved to be a potent inhibitor of class I HDACs.

3. **Belinostat**
   Paul W. Finn and coworkers (University of Buckingham, UK) report on belinostat, which is a potent pan-inhibitor of class I and II HDACs. It was approved in 2014 for the treatment of peripheral T-cell lymphoma.

4. **Panobinostat**
   Peter Atadja and coworker (Novartis Institute for Biomedical Research, US & China) present the story of how a functional high-
throughput screen looking for inducers of cyclin-dependent kinase 2 (CDK2) inhibitor p21 provided hits that were identified as HDAC inhibitors, ultimately resulting in the discovery of panobinostat.

5. **Chidamide**

Xian-Ping Lu and coworkers (Shenzhen Chipscreen Biosciences, China) describe the discovery and development of chidamide which is a novel benzamide type inhibitor of class I HDACs and class IIb HDAC10.

II. Steroidal CYP17 Inhibitor Anticancer Drug Discovery

Part Editor: Juan-Miguel Jimenez (Vertex Pharmaceuticals, UK)

6. **Abiraterone acetate**

Gabriel Martinez Botella and coworkers (SAGE Therapeutics, USA) have written a chapter on the discovery of abiraterone acetate, which is a key therapeutic in the treatment of metastatic castrate-resistant prostate cancer.

III. Anti-infective Drug Discoveries

Part Editor: John Proudfoot (Boehringer Ingelheim, Ridgefield, USA)

7. **Delamanid**

Hidetsugu Tsubouchi and coworkers (Otsuka, Japan) summarise the discovery of delamanid, which is a new drug for the treatment of multidrug-resistant pulmonary tuberculosis.

8. **Sofosbuvir**

Michael J. Sofia (Arbutus Biopharma, USA) describes the discovery of sofosbuvir, which has become the backbone agent of combination curative therapy for hepatitis C virus infection.

IV. Central Nervous System (CNS) Drug Discovery

Part Editor: Helmut Buschmann (Aachen, Germany)

9. **Vortioxetine**

Benny Bang-Andersen and coworkers (Lundbeck, Denmark and USA) give an overview of the discovery of vortioxetine, a new multimodal antidepressant drug with serotonin modulator and stimulator activity.

V. Antiulcer Drug Discovery

Part Editor: Jörg Senn-Bilfiger (Konstanz, Germany)

10. **Vonoprazan fumarate**

Haruyuki Nishida (Takeda, Japan) describes the discovery of vonoprazan fumarate, which is a novel, potent and long-lasting potassium-competitive acid blocker showing several advantages over proton pump inhibitors.

VI. Cross-Therapeutic Drug Discovery (Respiratory Diseases/Anticancer)

Part Editor: Stefan Laufer (University of Tübingen, Germany)

11. **Nintedanib**

Gerald J. Roth and coworkers (Boehringer Ingelheim, Biberach, Germany) summarise the discovery and development of nintedanib, which represents a pioneer discovery of a cross-therapeutic research for the treatment of solid tumours and idiopathic pulmonary fibrosis.
The editors and part editors thank the advisory board members: Magid Abou-Gharbia (Temple University, USA), Kazumi Kondo (Otsuka, Japan), John A. Lowe (JL3Pharma LLC, USA), Barry V.L. Potter (Oxford University, UK) and Anette Graven Sams (Lundbeck, Denmark). Special thanks are due to the following reviewers who helped both the authors and the editors: Jan Heeres, Manfred Jung, Sándor Mahó, Tom Perun (Division Chemistry and Human Health of IUPAC) and Ron Weir (Interdivisional Committee on Terminology, Nomenclature and Symbols of IUPAC).

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Part I
HDAC Inhibitor Anticancer Drug Discovery
Chapter 1
From DMSO to the Anticancer Compound SAHA, an Unusual Intellectual Pathway for Drug Design

Ronald Breslow

1.1 Introduction

This is an account of aspects of a collaboration between Ronald Breslow (originally Professor of Chemistry at Columbia University, also a member of the Biological Sciences Department, now University Professor at Columbia) and Paul Marks (originally Professor of Human Genetics and Medicine, Dean of the Faculty of Medicine, then Vice President for Health Sciences and Director of the Comprehensive Cancer Center at Columbia University, then President and Chief Executive Officer at Memorial Sloan Kettering Cancer Center, now President Emeritus and Member of the Sloan Kettering Institute) in the invention and development of suberoylanilide hydroxamic acid (SAHA), an effective anticancer agent that has been in human use for years after approval in the United States, Canada and more recently Japan. The Breslow group designed new potential molecules and carried out their syntheses in the Columbia University chemistry department, and submitted them to Paul Marks and Richard Rifkind at the Columbia Cancer Center, and later at the Sloan Kettering Institute for Cancer Research, for biological evaluation. Paul Marks instituted the collaboration, based on some work by Charlotte Friend of Mount Sinai School of Medicine.

This is the way most modern pharmaceuticals are created in pharmaceutical companies or in academic medicinal departments. Biologists may be aware of a promising area for drug development, medicinal chemists then design and create candidate molecules and send them to the biologists, who then evaluate them. With promising results, the chemists continue to create new, perhaps better, candidates while the biologists extend testing to animals and then to humans. Successful medicines are then approved for human use.

Normally the chemists are aware of compounds that have some promise, based on binding studies, and they can design around those structures. In the case of SAHA, the initial lead, dimethylsulfoxide (DMSO) 1, was very far from a potential medicine so the design was based on a series of hypotheses. Even so, the eventual structure of SAHA proved to be ideal as a binder to the biological target, although this is not how it was discovered. Thus the editors of this volume have invited
me to describe the unusual intellectual history that led to its structure. I am a
physical organic chemist who had designed and created new molecules for novel
properties, such as unusual conjugative stability or instability, or effective catalytic
enzyme mimics, but not medicinal properties. However, I have a Master’s degree
in Medical Science from Harvard University in addition to my Ph.D. in Chemistry,
and I had been a consultant with pharmaceutical companies for many years. There
I proposed both new synthetic approaches to their target compounds and also
possible alternative medicinal targets themselves.

A few years ago, Paul Marks and I wrote a short review describing the work
of both our labs in the development of SAHA [1], but the present chapter will
concentrate only on the chemical approach that led to drug development. Thus it
does not describe in detail the brilliant biological work done by Paul Marks and
Richard Rifkind. The references are only those in which Paul Marks and I are both
authors, and it will not cover the many papers and a book produced by the Marks
lab alone and several papers from only our lab that related the SAHA story to our
other work.

1.2
The Discovery of SAHA (vorinostat)

Stem cells have two functions. They multiply to form additional stem cells, and
they differentiate to adult tissue cells with specialised functions. In 1966 Paul
Marks approached me with the information that Charlotte Friend had seen
something remarkable [2, 3]. When a suspension of murine erythroleukemia cells
(MELC) was treated with dimethylsulfoxide (DMSO) (1) at 280 mmolar approx-
imately 60% of the cells underwent cytodifferentiation to normal erythrocytes.
This was the first example in which such a process occurred, and it suggested
a new approach to cancer treatment generally. Of course such a required con-
centration was totally impractical for a medicine, so it was important to find
more potent analogs of DMSO. Marks and I agreed to collaborate and build a
research programme based on this finding. The Breslow lab with my students
and postdocs would conceive and create new compounds that would be tested
by Marks and his associates for cytodifferentiation of erythroleukemia cells, as
DMSO had done, but with more practical doses. Marks would also further evalu-
ate promising leads with biological testing. This led to the discovery of SAHA. In
time Marks and Breslow and Richard Rifkind formed a company, ATON Pharma
Inc. It received the patent rights from Columbia University and Sloan Kettering
and funded the Phase I human trials for SAHA.

Many small molecule linear and cyclic amides were examined. N-Methylacet-
amide (2) was fivefold more effective than DMSO, but still not effective enough
to be a practical drug [4]. Thus the chemists decided to create linked dimers
of acetamide, to take advantage of the well-known chelate effect that leads to
stronger binding, and thus should require lower doses for anticancer effective-
ness. Double binders have entropy advantages over single ligands if both ends
contribute to the binding. This involved the hope that there were more binding sites than a single one for the initial compounds, and thus linking them together could be useful. The first compound, hexamethylene bis-acetamide (HMBA, linked at the nitrogen atoms) (3), was indeed one order of magnitude (tenfold) more potent than simple acetamide, and changing the linking groups from three methylenes up to nine made it clear that a six methylene chain – the first we tried – was the optimum [5–7]. This preference will eventually be seen and understood when we describe SAHA. We also prepared a dimer of acetamide linked at the methyl groups, suberoyl-bis-\(N\)-methylamide (4), and it also showed tenfold stronger binding than simple acetamide [8]. Various dimers including dimers of DMSO were also examined [8, 9]. HMBA had extensive biological study, and indeed some human trials were performed with HMBA [10–13]. There were some useful responses in cancer patients, but the doses required were too high to be well tolerated in human patients. When even trimers and tetramers of acetamide were not more effective [14, 15], we concluded that simple amides were not bound strongly enough.

We were already thinking that the target could be an enzyme, perhaps a metalloenzyme, to explain the strong preference for particular lengths of our compounds. Since DMSO and the amides had polar groups that could be metal ligands, we decided to go to even better metal ion binders. We synthesised a bis-amide like 4 but with hydroxyl groups instead of methyl groups, creating compound 5 that we called suberoyl-bis-hydroxamic acid, SBHA [14]. Hydroxamic acids were known to be strong binders to metal ions. Compound 5 was more effective than was HMBA, compound 3, suggesting that indeed there was a metal ion in the biological target. Again the six-methylene chain length was optimal. However, the chance that a receptor protein would have two metal ions that distance apart seemed unlikely, so we decided to replace the hydroxyl of one hydroxamic group with a hydrophobic phenyl group to see if it could make an even better binder. This would bind to a metal ion with its hydroxamic group while binding to a hydrophobic region of a protein with the phenyl group. This was speculation, but it turned out to be correct.

Figure 1.2 5 suberyl-bis-hydroxamic acid (SBHA), 6 suberylanilide hydroxamic acid (SAHA).
We created SAHA, suberoylanilide hydroxamic acid 6 [14]. It inhibited histone deacetylases was approximately sixfold more potent than was SBHA in the MELC assay and also in various other tests [15–17]. Again we varied the chain length, and the six-methylene linker was optimal. We and others have replaced the phenyl group with many other larger hydrophobic units, which made compounds much more strongly bound, but in animal studies the more strongly bound analogs showed increased toxicity. This represents a fundamental problem not always recognised by medicinal chemists.

A binding constant is a ratio of two rate constants, the second-order rate constant for binding over the first-order rate constant for dissociation. It is often difficult to increase the rate of binding, which is limited by the collision rate. Strong binding instead often reflects slower dissociation, the first-order process, as the attractive interactions must be broken. Thus strong binders are often bound to biological receptors for a longer time. Putting it another way, for effectiveness a drug must normally be 50% or so bound to the receptor, and with strong binders a smaller dose is needed for 50% binding. If the strong binding reflects slower dissociation, the drug will be present on the biological targets for a long time. In the case of SAHA, physicians have found that unpleasant or dangerous side effects are minimised in human patients if the drug is present for only 8 h or so before excretion, so SAHA is administered once a day. With tenfold slower dissociation the drug would be present for 80 h, and side effects could be serious. With any SAHA analog significantly more strongly bound – and we looked at several with subnanomolar dissociation constants – adverse toxic side effects appeared in animal tests that could not be overcome by cutting back the dose.

SAHA proved to be an effective drug against a variety of cancers, as Paul Marks and our other collaborators established. In some cases the cancer cells differentiated into normal cells, as had happened with DMSO in the Charlotte Friend experiments. Examples included human colon (HT-29) and adult leukemia (HL-60) cells. The National Cancer Institute (NCI) then examined SAHA in sixty different human cancer cell types and saw stasis (lack of growth) with all, and about equal occurrences of either cytodifferentiation to normal cells or apoptosis (programmed cell death, not simple toxicity). SAHA also caused cytodifferentiation of MCF-7 breast adenocarcinoma cells into normal functioning breast milk cells. Very many cancers have been examined with SAHA.

The scientific question is, of course, how does SAHA cause these effects? A strong clue came from the work of Yoshida with two other cytodifferentiating agents, trichostatin A and trapoxin B. He showed that they induced cytodifferentiation by inhibiting the enzyme histone deacetylase (HDAC) [18]. The structure of trichostatin A 7 is similar to that of SAHA, although it is a less attractive drug. We saw that SAHA was also an inhibitor of HDAC and that the potency of various SAHA derivatives as HDAC inhibitors ran parallel to their biological anticancer effectiveness. We created a derivative 8 of SAHA with an azido group on the phenyl para position and tritium labeling in the phenyl, and irradiated it with HDAC in solution. The azido group lost nitrogen to form a reactive nitrene that then attached it to HDAC, so it was clear that HDAC was the binding tar-
get [19]. Finally, X-ray crystal structures were obtained in the lab of Pavletich that showed the detailed structure of the complex of SAHA and of trichostatin A with HDAC [20]. SAHA bound into HDAC by inserting into a pore with the phenyl group bound to a surface hydrophobic face of the protein while the hydroxamic acid group bound to a Zn\(^{2+}\) metal ion that was part of the HDAC protein. The six methylenes were the perfect length to reach between these two binding sites. We also synthesised a compound called pyroxamide 9 in which a pyridine ring replaced the phenyl ring of SAHA, and it had similar properties to SAHA [21].

\[
\text{SAHA}\hspace{1cm}\text{pyroxamide}
\]

Figure 1.3

The enzyme histone deacetylase binds an acetylated lysine from the protein histone at the zinc of HDAC, which catalyzes the hydrolysis of the acetyl group – hence histone deacetylase. The structure of SAHA bound to HDAC almost perfectly matches the structure of an acetylated lysine group of histone bound into the pore of the protein, with the six-methylene chain mimicking the side chain of an acetylated lysine. Although SAHA was not invented this way, it is ideal as a mimic of the transition state for zinc-catalyzed hydrolysis of an acetylated lysine group from histone. Other work not detailed here shows that particular lysines, when acetylated, can induce differentiation of stem cells or cancer cells, so blocking the deacetylation as SAHA does upregulate (increase) the acetylation level of the histone [22, 23]. Other studies suggest how apoptosis is also triggered by SAHA.

1.3 Clinical Trials

Phase I trials of SAHA in human cancer patients showed that it was well tolerated and that it had useful clinical results. At this point more extensive trials were needed, and several companies were interested in buying ATON for SAHA and its patents and data. Merck and Co bought ATON in 2004, and performed trials that were successful, so Merck obtained approval for the human use of SAHA against disease, first in the United States in 2006, then in Canada in 2009 and more
recently in 2011 in Japan. SAHA has been used in clinical trials against many cancers, and it is still in active use in chemotherapy treatment of cancer patients.

1.4 Follow-On Research – Selective HDAC Inhibitors

Humans have eleven different zinc-dependent HDACs, with different structures and different selectivities for the cleavage of acetyl groups from various proteins. SAHA has rather broad selectivity among them, but a particular enzyme called HDAC6 is selective for the removal of acetyl groups from the protein tubulin; it is less effective against acetylated histone, for example. Thus the name histone deacetylase (HDAC) is a misnomer, since some of these deacetylases have other protein targets. The first example of an inhibitor of HDAC6 was a compound called tubacin, created by Schreiber in 2003 [24]. Kozikowski has been studying enzymes with such selectivity, and has made some compounds that are selective in blocking hydrolytic removal of the acetyl group from acetylated tubulin. He has suggested that HDAC6 has a shorter distance from the surface of the protein to the hydroxamic acid site. We have two very promising compounds that are quite selective for HDAC6 alone among the HDACs. They were designed to have a shorter distance between the surface and the HDAC group than SAHA has, and both have a branching group that prevents the compound from penetrating further into the protein cavity.

One compound we called HPOB, 10, selectively inhibits HDAC6 catalytic activity in vivo and in vitro [25]. Paul Marks compared it with Schreiber’s tubacin and saw that HPOB was 51.8-fold selective for HDAC6 versus HDAC1 while tubacin was only 4.3-fold selective. As we described, HPOB has very good biological properties, and is very promising in combination therapy to enhance the potency of various anticancer drugs. A second compound we call HPB, 11, we described in a paper just published in Proceedings of the National Academy of Sciences [26]. It is a little less selective than HPOB but it has even better anticancer properties, including lack of side effects, in animal studies. Both compounds need human evaluation before they can be seen as true improvements over SAHA. We are planning such studies.

![Figure 1.4](https://example.com/f14.png)

**Figure 1.4** $N$-hydroxy-$4$-$[(N(2$-hydroxyethyl)$)-2$-phenylacetamido$]-methyl$benzamide$ (HPB)$, $4$-$[(hydroxyamino)carbonyl]$$-N$-$(2$-hydroxyethyl)$-$N$-phenyl$-benzeneacetamide$ (HPOB)$.