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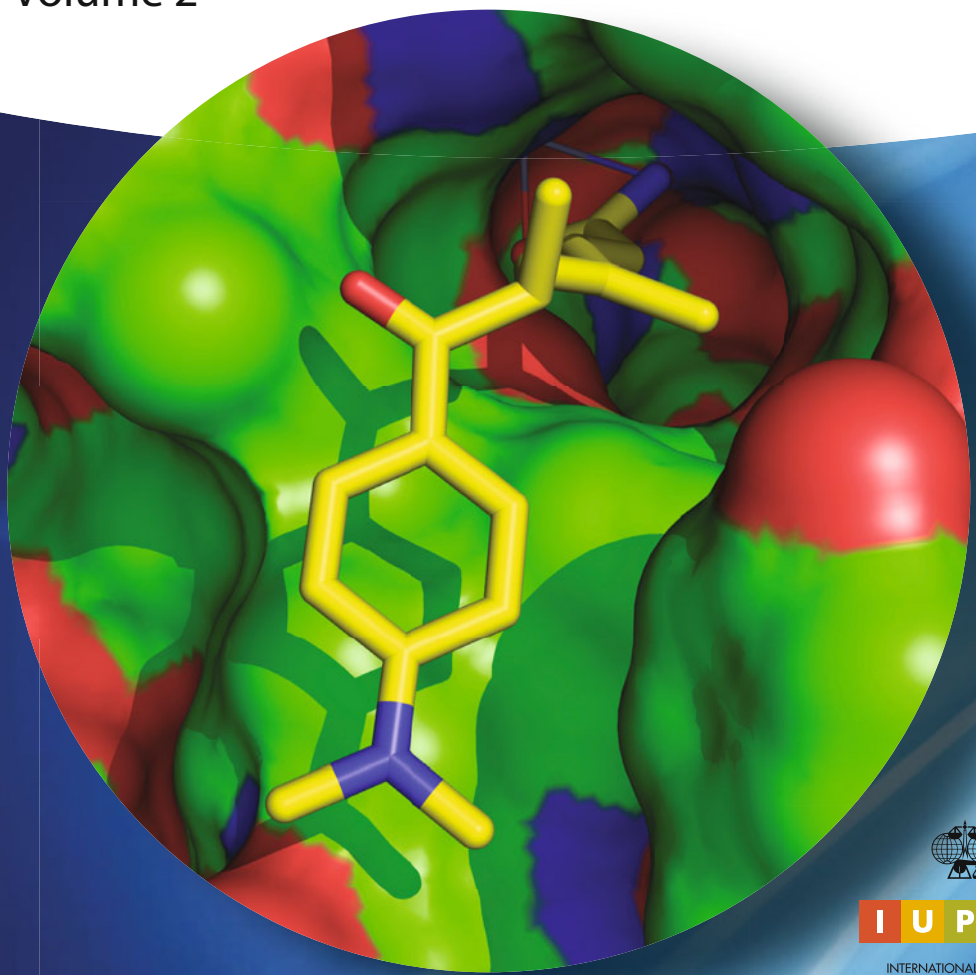
Edited by János Fischer and Wayne E. Childers

Successful Drug Discovery

Volume 2



IUPAC
INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

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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 desribed 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 inibitor 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 (Shenzen 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

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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 approximately 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 concentration 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 evaluate 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*-Methylacetamide (**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 effectiveness. Double binders have entropy advantages over single ligands if both ends

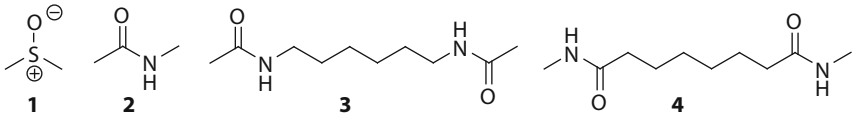

Figure 1.1 **1** *N*-methylacetamide, **2** dimethylsulfoxide (DMSO), **3** hexamethylene bis-acetamide, **4** suberoyl-bis-*N*-methylamide.

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 one 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.

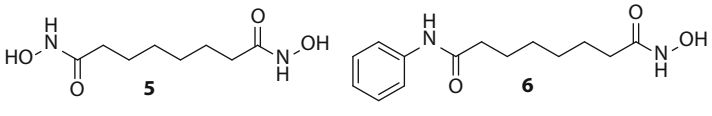

Figure 1.2 **5** suberyol-bis-hydroxamic acid (SBHA), **6** suberyolanilide hydroxamic acid (SAHA).

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 cytodifferention to normal cells or apoptosis (programmed cell death, not simple toxicity). SAHA also caused cytodifferention 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].

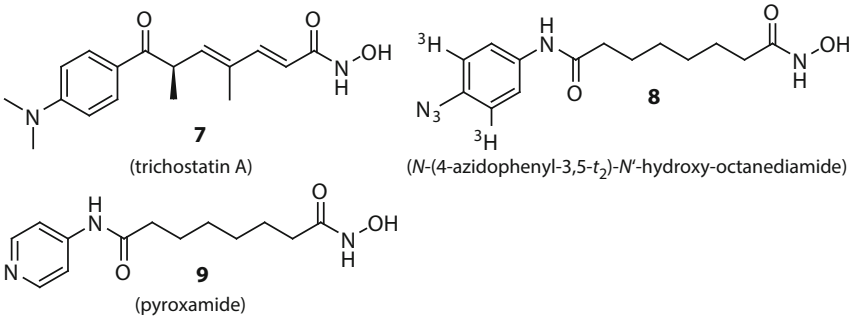

Figure 1.3

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