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Five Molecules We Would Take to a Remote Island

Thomas U. Mayer[1,*] and Andreas Marx[1]
[1]Konstanz Research School Chemical Biology (KoRS-CB), University of Konstanz, Universitätsstr. 10, 78457 Konstanz, Germany
*Correspondence: Thomas.u.mayer@uni-konstanz.de

Which five molecules would you take to a remote island? If you imagine yourself as a castaway on an island you might pick water, glucose, penicillin, and ethanol in combination with aspirin. However, as a scientist, you may ask yourself which molecules impressed you most by their chemical or biological property, their impact on science, or the ingenuity and/or serendipity behind their discovery. Here, we present our personal short list comprising FK506, colchicine, imatinib, Quimi-Hib, and cidofovir. Obviously, our selection is highly subjective and, therefore, we apologize up front to our colleagues for not mentioning their favorite compounds.

FK506

FK506 is a fascinating molecule because of both its medical impact and its intriguing mode of action. FK506 is a macrolide with immunosuppressant activity produced by the bacterium *Streptomyces tsukubaensis*, giving FK506 the alternative name Tacrolimus (Tsukuba macrolide immunosuppressant). FK506 was identified in 1984 in the course of a systematic search for novel immunosuppressant agents by a Japanese team headed by T. Goto, T. Kino, and H. Hatanaka (Goto et al., 1987; Tanaka et al., 1987). Ten years later, FK506 was already approved for use in liver transplantation, and by now is a widely applied suppressor of allograft rejections. Its medical importance fueled the search for the cellular target of FK506 and succeeded in the identification of FKBP (FK506-binding protein), a peptidyl-prolyl isomerase (PPIase) (Harding et al., 1989; Siekierka et al., 1989, 1990; Standaert et al., 1990). PPIases increase the rate of protein folding by catalyzing the *cis–trans* isomerisation of peptide bonds N-terminal to proline residues. FK506 inhibits FKBP by a substrate mimicking mechanism, whereby the α-keto amide of FK506 serves as a surrogate for the twisted amide of a bound peptide substrate (Rosen et al., 1990). However, the loss of PPIase activity is not the basis of the T cell inhibition by FK506. Instead, the complex of FK506 and FKBP inhibits the phosphatase calcineurin (CaN), also known as PP2B, thereby preventing the dephosphorylation of NF-AT (nuclear factor of activated T cells) that is required for the expression of early T cell activation genes, e.g. IL-2 (Liu et al., 1991). Neither FK506 nor its target FKBP by itself can bind to CaN. Thus, rather than by simply inhibiting the function of its target protein, FK506 induces a gain-of-function by imparting new binding properties to FKBP, which results in the termination of the T cell receptor-initiated immune response at the level of CaN (Figure 1).

For those not yet convinced by FK506's claim to be taken to a remote island, it might be noteworthy that the story of immunosuppressants is even more complicated because the small molecules cyclosporine A (CsA) and rapamycin suppress T cell activation by a related, yet distinct pathway (for review, see Powell and Zheng [2006]). CsA, a cyclic undecapeptide produced by the fungus *Beauveria nivea*, binds to cyclophilin (CpN), a PPIase unrelated in sequence to FKBP. However, both the complexes FK506/FKBP and CsA/CpN converge on calcineurin and thus suppress T cell activation by a common mechanism, i.e., inhibiting the dephosphorylation of NF-AT by CaN. On the contrary, rapamycin, a macrolide chemically related to FK506 and produced by the bacterium *Streptomyces sirolimus*, binds to FKBP-like FK506, but the FKBP/rapamycin complex interacts with mammalian target of rapamycin (mTOR), a serine-threonine protein kinase, instead of CaN and exerts its immunosuppressive activity by inhibiting the response to IL-2 rather than its expression. Thus, FK506 and rapamycin share a common structural element mediating binding to FKBP while having different effector elements, resulting in the inhibition of different signaling pathways (Bierer et al., 1990).

FK506's mode of inhibition is not only intriguing and holds several unexpected twists, but it is also an excellent example of how the combined effort of chemists, biologists, and physicians yielded a highly potent small molecule that is not only invaluable for basic research but also indispensable for modern medicine.

Colchicine

The story of colchicine started in ancient times, when Jason and his famous Argonauts were sent to Colchis, the old kingdom of Georgia, in Asia to retrieve the *Golden Fleece*, which—according to historians—was nothing but a mass of golden crocus. In Europe, extracts of the golden crocus was in great demand to treat gout, which Hippocrates coined podagra (podos = foot; agra = seizure) due to the fact the first inflammations typically attack the foot. Two millennia later, we now know that the active ingredient of the mystic Asian pharmacophore is colchicine, whose value as an anti-inflammatory agent for the treatment of gout is still acknowledged (Figure 2). While the isolation of the alkaloid colchicine from *Colchicum autumnale* (Autumn crocus also known as "Meadow saffron") was first achieved by Pelletier and Caventou in 1820 (Pelletier and Caventou, 1820), its structure was not determined until 1940 (Cohen et al., 1940). Notably, the cytological effect of colchicine was already described in 1889 by Biaggio Pernice, a physician from Palermo (Pernice and Caventou, 1889). He noted "*quasi tutti gli elementi in cariocinesi*," i.e., a dramatic accumulation of mitotic cells in the gastric and intestinal mucosa of

dogs treated with high doses of colchicine. Subsequent studies revealed that colchicine had major effects on the mitotic spindle (Dustin, 1934; Eigsti, 1938; Levine, 1945) and it was in fact colchicine's antimitotic effect that was used to unambiguously determine that 46 is the normal human diploid number of chromosomes rather than the previously believed 48 (Tjio and Levan, 1956). Thus, colchicine has a one-of-a-kind historical record as a drug and a biological probe—even before its target was known. The advent of radioisotopes finally enabled Edwin Taylor and Gary Borisy to identify tubulin as the cellular target of colchicine. Colchicine-^{3}H, prepared by methylation of colchiceine with diazomethane in tritiated water, was found in a noncovalent complex with a macromolecule that showed a correlation with the presence of microtubules (Borisy and Taylor, 1967a, 1967b; Taylor, 1965). Using colchicine-^{3}H and GTP-^{3}H, Taylor and colleagues not only identified a 120kDa dimer (i.e., the α-/β-tubulin-dimer) as the target of colchicine, but also discovered that only one of the two GTP molecules bound per dimer is exchanged (Mohri, 1968; Shelanski and Taylor, 1967; Weisenberg et al., 1968). Today, we know that the cycle of GTP hydrolysis and nucleotide exchange by β-tubulin forms the basis for the key characteristic of microtubules: dynamic instability. This phenomenon describes the stochastic bidirectional switches of microtubules between phases of growth and shrinkage that allows them to quickly explore cellular space, to direct cell movement, and to segregate chromosomes (Desai and Mitchison, 1997).

Does the identification of tubulin finally disclose all mystery of colchicine? Not really. It is still an open question of how colchicine's mode of action explains its major clinical use in gout. Gout is caused by the deposition of uric acid crystals in joints and surrounding tissues. Colchicine might prevent the formation of uric acid crystals by modulating the pH of the tissue, act as an anti-inflammatory agent by suppressing the activity of neutrophils, or inhibit the invasion of immune cells into damage tissue—to name a few potential explanations. Unlike other microtubule-binding drugs, colchicine is not used in cancer chemotherapy, probably due to its high toxicity (Jordan and Wilson, 2004). However, colchicine is widely applied in plant breeding to produce diploid gametes resulting in plants with stronger flower colors. The binding of colchicine to tubulin has several intriguing features. It is a two-step process that includes a fast reaction yielding a low-affinity complex followed by a slow, unimolecular step to form the poorly reversible colchicine-tubulin (TC) complex (Engelborghs, 1998). The TC complex incorporates at the end of growing microtubules where it prevents curved tubulin from adopting a straight conformation. This conformational constraint prevents the stabilization of lateral contacts between tubulin subunits resulting in loss of dynamic instability and the depolymerization of microtubules at low and at high TC concentrations, respectively (Panda, et al., 1995, Ravelli, et al., 2004). Colchicine binds to a single site on β-tubulin (colchicine site), which is shared by a large number of molecules structurally unrelated to colchicine, e.g., podophyllotoxin, combrestatin-A4. The high specificity of colchicine for tubulin fueled the search for an endogenous molecule that regulates microtubule dynamics via the colchicine site. However, identified candidates have not been widely accepted. Thus, the story of colchicine has still to be continued.

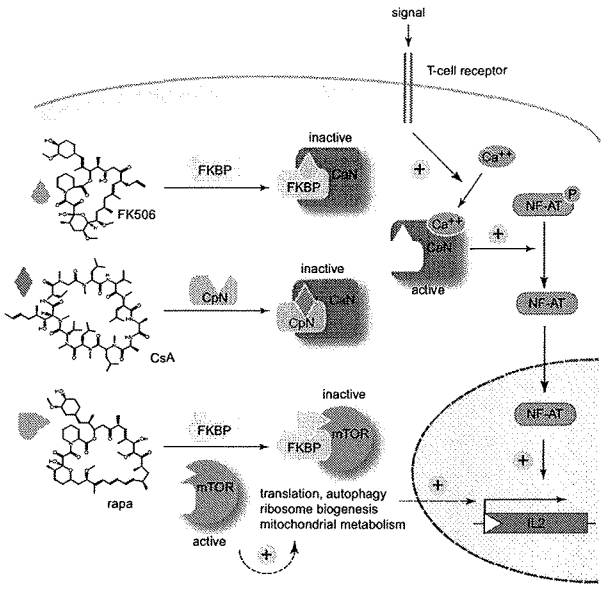

Figure 1. Simplified Diagram of T Cell Receptor Mediated Signal Transduction Pathway Resulting in the Expression of Interleukin 2
Receptor activation triggers a transient rise in intracellular calcium levels, and thus the activation of the phosphatase calcineurin (CaN). Dephosphorylated NF-AT enters the nucleus and induces the expression of IL2. Note that FK506 and rapamycin share common structural elements mediating binding to FKB, yet have distinct effector elements. FK506 binding protein, FKBP; cyclophilin, CpN; mammalian target of rapamycin, mTOR.

4-[(4-Methylpiperazin-1-yl)methyl]- *N*-[4-methyl-3-[(4-pyridin-3-ylpyrimidin-2-yl)amino] phenyl]benzamid

This is better known as gleevec, glivec, imatinib, STI571, or the magic bullet for the treatment of chronic myelogenous leukemia (CML) (Figure 3). CML is a malignancy of a pluripotent hematopoietic stem cell. Ninety-five percent of CML patients are positive for the Philadelphia (Ph) chromosome, which was named after the location of the two research institutes where it was first discovered and described (Nowell, 1962; Rowley, 1973). The Ph-chromosome is the result of t(9;22)(q34;q11), i.e., the reciprocal translocation between the large arms of chromosome 9 and 22. The molecular consequence of this translocation is a replacement of the first exon of the proto-oncogene c-Abl with sequences from the BCR ("break point cluster region") (Heisterkamp et al., 1985; Konopka et al., 1984; Shtivelman et al., 1985). c-Abl encodes a nonreceptor tyrosine kinase that has tightly controlled activity in normal cells. In contrast, the protein product of the Bcr-Abl fusion shows constitutive tyrosine kinase activity. Thus, BCR-Abl allows cells to proliferate in a cytokine-independent manner. Furthermore, BCR-Abl's inhibitory effect on DNA repair induces genomic instability, which might be causative for the feared blast crisis in CML.

The story of imatinib began in the early 1990s at Ciba-Geigy (now Novartis)

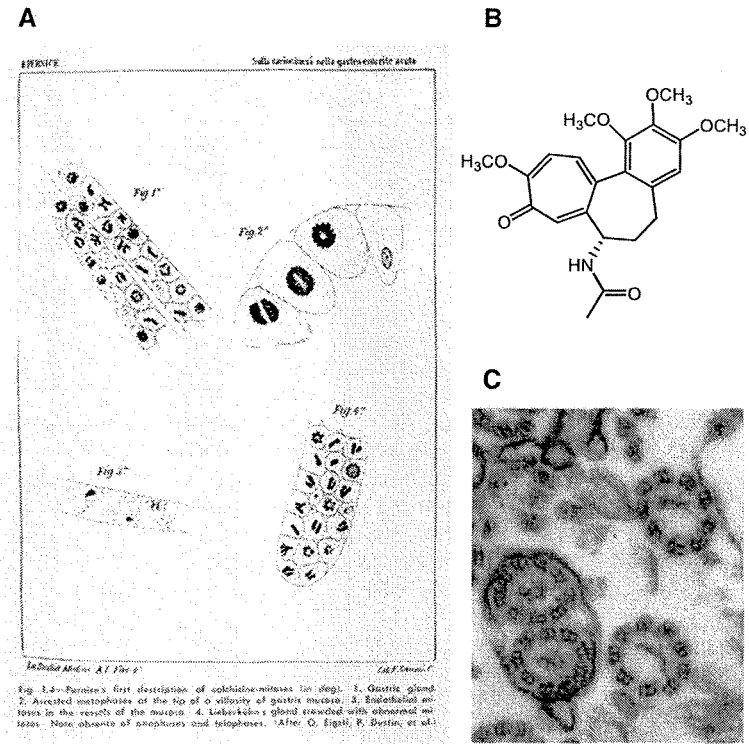

Figure 2. Colchicine and Its Effect on Mitosis
(A) First description of colchicine's effect on cells in mitosis by the Italian physician Biaggio Pernice, 1889 (image was obtained through the Biodiversity Heritage Library).
(B) Chemical structure of colchicine.
(C) Electron micrograph of sperm tail preparation showing the characteristic 9+2 microtubule structure, i.e., two central microtubule doublets surrounded by nine outer doublets (Shelanski and Taylor, 1967).

when a large number of 2-phenylaminopyrimidine derivatives were screened for inhibitory activity against protein tyrosine kinases (PTKs). The originally identified lead compound inhibited Abl-kinase in vitro; however, its primary cellular target was the platelet-derived growth factor receptor (PDGF-R) PTK. Using this compound as a lead structure, rational drug design led to the identification of imatinib, which inhibited the autophosphorylation of Abl and PDGF-R with equal potency (Buchdunger et al., 1996). Importantly, colony formation assays with bone marrow cultures from CML patients demonstrated that imatinib inhibited the growth of BCR-Abl positive cells to an extent of 92%–98% (Druker et al., 1996). Normal colonies were minimally affected, despite the fact that imatinib inhibited in vitro BCR-Abl and c-Abl with an identical IC_{50}. Extensive animal testing, including a CML-mouse model, confirmed imatinib's high efficiency as well as low toxicity and paved the way for the first clinical trials just few years after its discovery. Today, imatinib mesylate is approved as first-line treatment for CML. Several patients, however, develop drug resistance partly due to amplification or mutation of the BCR/ABL gene, suggesting that combined therapies including imatinib should be considered (Waller, 2010).

Imatinib is an ATP-competitive inhibitor, raising the question of how this molecule can be selective for the PTKs Abl, PDGF-R, and c-kit, another PTK. The crystal structure of Abl in complex with an imatinib variant revealed that the inhibitor recognizes the inactive conformation of the activation loop of Abl (Schindler et al., 2000). For most kinases the activation loop controls catalytic activity. Importantly, the "active" conformation of the loop, stabilized by phosphorylation, is highly similar in all known structures of active kinases, while the "inactive" conformation displays great diversity. Thus, despite the highly conserved nucleotide-binding pocket of kinases, the characteristic conformation of the activation loop in its inactive state allows imatinib to be highly selective for its targets while being essentially inactive against other PTKs and serine/threonine kinases.

Why do we consider Imatinib worth taking to a remote island? First, it is an excellent example of the synergistic research between academia and industry. The combined efforts of Brian Druker, Nicholas Lydon, and Charles Sawyers were awarded with the Lasker-DeBakey Award in 2009. Second, its intriguing mode of action identified kinases as druggable, despite the high sequence conservation of their catalytic center. Third and most importantly, it shifted the role of physicians from checking blood counts and delivering cruel news to efficiently treating CML patients.

Quimi-Hib

Immunogens used in many vaccination strategies derive from constituent parts of the pathogen. For instance, the vaccine against *Haemophilus influenzae* type b (Hib), a pathogen that causes bacterial meningitis and pneumonia that was introduced around 1990 and reduced incidences by more than 95%, is based on a capsular polysaccharide isolated from the pathogen. The usage of synthetic antigens, and in particular carbohydrate conjugates in which the immunogen is synthesized chemically with atom scale precision, holds great promise for further developments along these lines (Astronomo and Burton, 2010). This is predominately due to the controlled production of a homogenous compound that minimizes batch-to-batch variability and thereby increases quality control standards. This might be accompanied with lower production costs in comparison with conventional vaccines. According to the World Health Organization (http://www.who.int/mediacentre/factsheets/fs294/en/index.html), high costs are the cause for the slow introduction of the Hib vaccination in developing countries. A major leap forward in this field represents the development of the first synthetic conjugate polysaccharide vaccine that was reported 2004 by a Cuban-Canadian research team (Verez-Bencomo et al., 2004). They first developed a high yielding route for the synthesis of a Hib polysaccharide

fragment, an oligomeric polyribosylribitol phosphate (Figure 3). These oligomers with an average of eight repeating units were reproducibly obtained in high yields (80%). The antigen was conjugated to suitable carriers and immunogenicity studies performed in animals, adults, children, and infants. After several clinical trials, a 99.7% success rate was obtained, leading to commercial production as a vaccine (Quimi-Hib by Heber Biotech). This makes the polyribosylribitol phosphate conjugates to the first successful synthetic carbohydrate antigen. Given the great potential of medicinal chemistry nowadays and, in particular, carbohydrate chemistry, the approach of using synthetic antigens should allow the introduction of artificial carbohydrate analogs for increasing the immunogenicity of the conjugates in future.

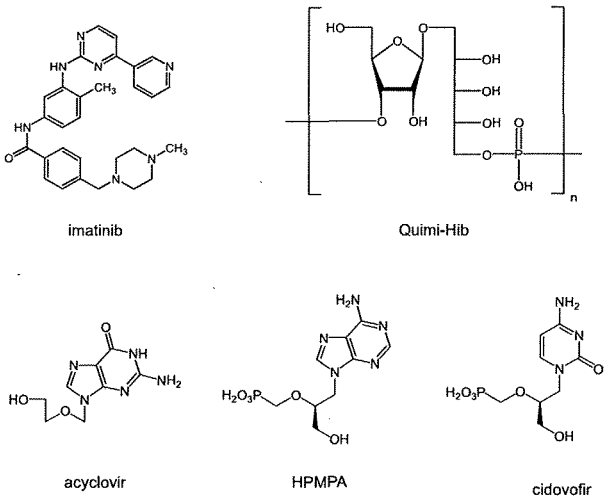

Figure 3. Chemical Structure of Compounds Mentioned in the Main Text

(S)-1-(3-Hydroxy-2-phosphonylmethoxypropyl) cytosine, Better Known as Cidofovir

Compared to the structures that we have discussed before, cidofovir impresses by its structural simplicity (Figure 3). Cidofovir is an acyclic nucleoside phosphonate with very broad antiviral activity. Acyclic nucleosides may be described as nucleoside analogs with opened, simplified sugar surrogates. How was it discovered? The history of acylic nucleoside analogs had its coming of age with the discovery of acyclovir as a selective inhibitor of herpes simplex virus (HSV) replication (De Clercq, 2008). In order to gain antiviral activity, nucleosides need to be phosphorylated to the triphosphates by three kinase-promoted steps. The resulting triphosphate is then used predominantly by the viral enzymes for incorporation of the modified nucleotide in the nascent DNA, and thus selectively impedes viral replication. Interestingly, although acyclovir is an acyclic guanosine analog, it was found by Elion et al. (1977) that the viral thymidine kinase more efficiently promotes the first phosphorylation step compared to the cellular enzymes. This makes acyclovir selective for virus-infected cells. Further phosphorylation is promoted by the cellular kinases, resulting in the triphosphates that impede viral replication. In 1986, De Clercq, Holý, and colleagues reported on the discovery of a new class of broad-spectrum anti-DNA virus agents: nucleoside phosphonates. (*S*)-9-(3-Hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA) (Figure 3) is active against all tested DNA viruses (polyoma, papilloma, adeno, herpes, pox) (De Clercq et al., 1986). Cidofovir is a closely related cytosine derivative of HPMPA that has been further developed clinically and has a remarkable broad-spectrum antiviral activity. It has been shown to be efficacious in the treatment of many DNA virus infections, including that of acyclovir-resistant HSV and varicella-zoster virus. Interestingly, since cidofovir has shown promising effects in animal models, it was predicted to be effective in the prophylaxis and therapy of smallpox in humans, a threat of bioterrorism. Thus, cidofovir is the sole antiviral drug that has been reserved and stockpiled for possible use in the therapy and prophylaxis of smallpox and the complications of smallpox vaccination (De Clercq and Holý, 2005).

Why are these phosphonates so effective? As depicted above, most nucleoside antiviral reagents get phosphorylated by host kinases in order to generate antiviral activity. The resulting triphosphates are selective substrates for the viral DNA polymerases and, after incorporation of the acyclic nucleotide, further DNA synthesis is obviated, a mechanism termed chain termination. In contrast to other antiviral nucleoside analogs, the phosphonates do not need the first phosphorylation step promoted by a kinase, which is often a bottleneck and counteracts the activity of the compounds.

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