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Hybrid Molecules with a Dual Mode of Action: Dream or Reality?†

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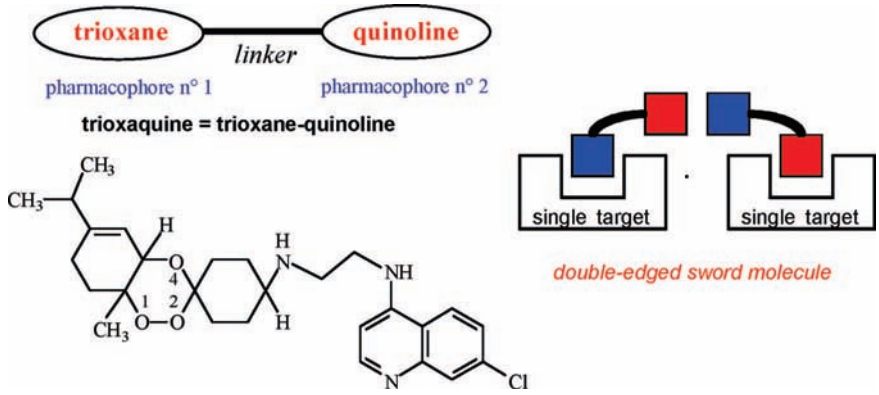
CONSPECTUS



The drug market is still dominated by small molecules, and more than 80% of the clinical development of drug candidates in the top 20 pharmaceutical firms is still based on small molecules. The high cost of developing and manufacturing "biological drugs" will contribute to leaving an open space for drugs based on cheap small molecules.

Four main routes can be explored to design affordable and efficient drugs: (i) a drastic reduction of the production costs of biological drugs, (ii) a real improvement of drug discovery via "computer-assisted combinatorial methods", (iii) going back to an extensive exploration of natural products as drug sources, and (iv) drug discovery by rational drug design and bio-inspired design that hopefully includes serendipity and human inspiration.

At the border between bio-inspired design and rational design, one can imagine preparation of hybrid molecules with a dual mode of action to create efficient new drugs. In this Account, hybrid molecules are defined as chemical entities with two or more structural domains having different biological functions and dual activity, indicating that a hybrid molecule acts as two distinct pharmacophores.

In order to obtain new antimalarial drugs that are affordable and able to avoid the emergence of resistant strains, we developed hybrid molecules with a dual mode of action (a "double-edged sword") able to kill multiresistant strains by oral administration. These hybrid molecules, named trioxaquines, with two pharmacophores able to interact with the heme target are made with a trioxane motif covalently linked to an aminoquinoline entity.

More than 100 trioxaquines have been prepared by Palumed over a period of 4 years, and in collaboration with Sanofi-Aventis, the trioxaquine PA1103-SAR116242 has been selected in January 2007 as candidate for preclinical development.

Introduction

Drug discovery is highly challenging for scientific reasons (difficulties to create new drugs) and also in terms of economical challenges (how to limit the investments and health costs at a reasonable level). The average length of time to develop a drug has increased and is now ranging from 12 to 15 years, compared to an average of 8 years in the 1960s.[1,2] This lengthening of R&D time has dramatically increased the level of capital needed to bring a drug to the market. Estimates are ranging from 0.8 to 1.7 billion of USD depending upon the therapeutic area.[3] The break of these costs is approximately as follows: 10% for discovery,

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15% for preclinical, 15% for manufacturing and process, 55% for clinical trials, and 5% for postmarketing. The development of genomics did not reduce the cost of drug discovery. For example, GlaxoSmithKline spent 7 years on genomic studies (1995–2001) to evaluate more than 300 genes as potential targets for novel antibacterial agents and 70 high-throughput screening (HTS) campaigns were run using large libraries of synthetic chemicals (260 000–530 000 compounds). Few leads were obtained, and the level of success was very low compared to the large efforts invested.[4] A literature survey between 1996 and 2004 shows that more than 125 antibacterial screens on 60 different targets were run by 34 different companies, and none of these experiments has been successful, indicating that the fast track "from gene to drug" does not exist.[5] Despite the reduction of HTS costs, each campaign of library screening costs several millions of USD. If the current results of HTS methods are below the expectations, improvements of these approaches will come from the evolutions of (i) structural proteomics[6] (determination at atomic resolution of protein structures on a genome-wide scale) and (ii) combinatorial chemistry with diversity-oriented syntheses to enrich the chemical space.[7–9] Natural-product chemistry has recently been revisited and will continue to be a powerful source of drug candidates.[10] It is now generally accepted that collections of natural products have a higher probability of delivering hits than typical libraries of molecules generated by combinatorial chemistry.[11] Half of the drugs currently in clinical use are of natural-product origin.[12,13] These natural products could be optimized for desirable drug properties by combinatorial biosynthesis.[14] Computational methods might also be useful as a decision tool for the preparation of molecules to create libraries of chemicals.[15] The use of nuclear magnetic resonance (NMR) spectroscopy to screen potential drug molecules has been in rapid development over the last decade and might be a key tool in the arsenal of biophysical methods for drug discovery and lead optimization.[16] "Chemical genetics" is now a popular metaphor used in drug discovery. Initially coined by Debusk[17] in the title of a review published in 1956, chemical genetics has been in fact successfully promoted by Schreiber since 1998.[18] While classical genetics concerns the study of a gene function by direct removal of a gene coding for a protein (genetic knockout), chemical genetics is defined as a genetic study using chemical tools.[18,19] Chemical genetics is divided into two different methods: "forward chemical genetics" and "reverse chemical genetics" (see Figure 1). These two approaches correspond (i) to the use of a set of different molecules to identify a gene that codes for a protein or (ii) to the screening of a library of molecules with a protein to identify a protein–ligand that will be used in developing zebrafish to observe phenotype modifications, respectively. The fast reproduction rate of zebrafish is a key advantage in chemical genetics compared to the slow reproduction rate of mammals that are mainly used in classical genetics. One should also keep in mind that observations during clinical trials are also "reverse chemical genetics". For example, Sildenafil (Viagra) was initially evaluated on humans for the treatment of heart disease as an inhibitor of cyclic guanosine monophosphate (cGMP) phosphodiesterase, an important enzyme in the control of vasodilatation. Its phenotypic effect on erectile function was discovered during the clinical trials! Target identification should be mainly improved by chemical genetics if nonspecific binding to proteins can be minimized. This problem can be solved by using mammalian cell extracts bearing the target with *Escherichia coli* extracts that are able to saturate nonspecific binding sites.[20] The final goal of chemical genetics will be to produce a complete set of chemical tools to probe each gene product. However, are we sure that small molecules have a real future as drugs?

Small Molecules as Drugs: The Downfall or a Real Future

Besides the drug market of small molecules, new strategies have been developed over the last 2 decades to expand the use of "biological drugs": (i) modulation of gene expression with small DNA or RNA fragments, including interference RNAi as therapeutics,[21–23] (ii) anticancer vaccines (Gardasil has been recently approved to prevent women from developing cervical cancer by targeting four strains of human papilloma virus[24]), and (iii) cellular therapy with human embryonic stem cells, a field that is still a matter of intensive scientific and ethical debates.[25,26]

The future of small molecules has been questioned over the past decade, and their downfall has been predicted several times. We have to keep in mind that the drug market is still dominated by small molecules and that more than 80% of the clinical development of drug candidates for the top 20 pharmaceutical firms is still based on small molecules.[27] The high cost of developing and manufacturing "biological drugs" will contribute toward leaving an open space for drugs based on small molecules, because their development is cheaper. The health costs and the access to new therapies is not only a concern for less-developed countries but also for the U.S.A., Europe, and Japan when taking in consideration the aging of the population that severely increases drug consumption at a rate far above that of the growth of the economy. In addition, the reduction of prescription drug expenditures is one of

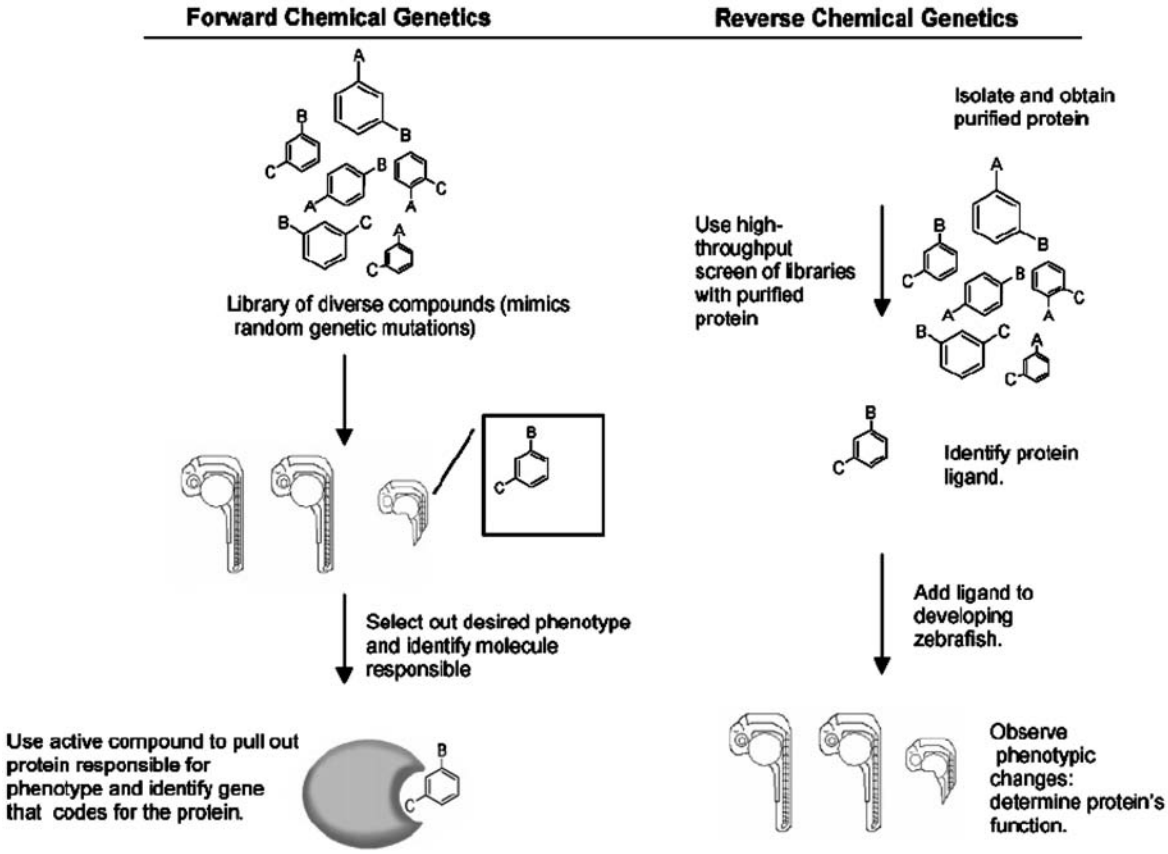

FIGURE 1. Forward and reverse chemical genetics: (i) using a set of different molecules to identify a gene that codes for a protein or (ii) screening a library of molecules with a protein to identify a protein–ligand that will be used in developing zebrafish to observe phenotype modifications. Reproduced with permission from ref 19. Copyright 2006 American Chemical Society.

the main targets to slowdown the increase of health-care costs [the total health costs reached 16% of the gross domestic product (GDP) in 2005 in the U.S.A.].[28] A Swedish survey (2000−2003) evaluated the cost of four biological antirheumatic drugs in southern Sweden (proteins acting as TNFα inhibitors, such as Etanercept, Infliximab, and Adalimumab, or an interleukin-1 receptor antagonist, such as Anakinara). The annual costs of these drugs ranging from 10 800 to 14 400 euros/year, far above 170 euros/year, the average annual drug-cost per person in Sweden.[29] The cost of such sophisticated drugs represents for national health insurances a free medium-size car per citizen per year! These different factors strongly suggest that small molecules have a real future in the drug market, with development and manufacturing costs below that of biological drugs.

Medicinal chemists have made considerable efforts over the last 2 decades to push up the drugability of hits via more elaborated and "rational" designs of drugs. Significant progresses have been obtained in the design of drug candidates active by oral uptake, a noninvasive route of drug administration. To reach this goal, Lipinski's rule is now in mind of all medicinal chemists.[30] To facilitate the screening of lead molecules with a predictable bioavailability by oral route, Lipinski and co-workers developed the concept of "the rule of 5" in 1994−1997. On the basis of a data-mining work with available data on thousands of drugs, these authors selected four (not five) parameters called "the rule of 5" for a simple mnemonic reason: each of the cutoff parameters were close to 5 or a multiple of 5. Easy intestinal absorption and permeability are expected for molecules having less than 5 hydrogen-bond donors, less than 10 hydrogen-bond acceptors, a molecular weight below 500, and a calculated partition coefficient log *P* (*C* log *P*) not greater than 5. The rule of five is now routinely integrated in decision methods for the identification of leads and drug candidates. However, as for any classical rule, some violations to Lipinski's rule have been listed. In particular, the polar surface area parameter is an important factor that governs intestinal passage for small anionic molecules.[31] Drugs targeting proteases or G-protein-coupled receptors are exceeding the limits of the rule of five.[32] The drugable molecules with adapted absorption, distribution, metabolism, and excretion (ADME) parameters constitute a very limited space compared to the vaste universe of the possible structures defined as the "chemical space" (Figure 2).[33]

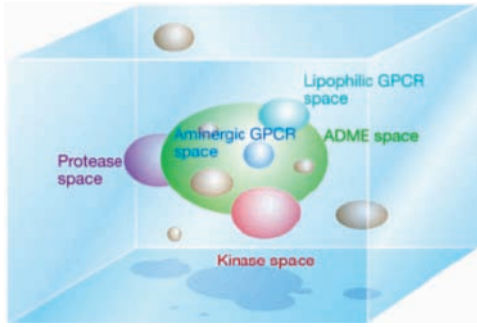

FIGURE 2. Cartoon view of the vast chemical space and the discrete areas that are occupied by some biologically active chemical entities (proteases, kinases, etc.) and, in particular, the region that is defined by molecules with good ADME parameters. Reproduced with permission from ref 33. Copyright 2004 Nature Publishing Group.

The total chemical space with all possible structures, including all possible isomers, is a huge universe far above the up-to-now explored sections of the chemical space with the currently known molecules. Fink and Reymond have recently generated a database containing more than 26 million molecules with up to 11 atoms of carbon, nitrogen, oxygen, and fluorine, which are feasible according to regular synthetic modes.[34] Only 63 850 molecules of this limited chemical space are already available in public databases, i.e., less than 0.24% of the possibilities offered by chemical syntheses; the forest should not be masked by few trees! Large uncharted areas of the chemical space are available for future creative works in the chemistry of small molecules.

Actually, four main different routes can be explored to elaborate affordable and efficient drugs: (i) a drastic reduction of the production costs of biological drugs, (ii) real improvements of drug discovery via "computer-assisted combinatorial methods", (iii) going back to an extensive exploration of natural products as drug sources, and (iv) drug discovery by man-brain, including serendipity, rational-drug design, and bio-inspired design. These last two items are fascinating areas for researchers in universities and pharmaceutical companies.

At the border between bio-inspired and rational design, one can imagine to prepare hybrid molecules with a dual mode of action to create new efficient drugs. Personally, I have been inspired by mechanistic studies on two natural products, bleomycin and artemisinin, anticancer and antimalarial drugs, respectively. This Account will be concentrated on this particular field, hybrid molecules with a dual mode of action, that can be considered as a niche compared to the main streams in drug discovery developed over the past 2 decades in big pharmaceutical companies.

Hybrid Molecules with a Dual Mode of Action

In the present Account, (i) hybrid molecules are defined as chemical entities with two (or more than two) structural domains having different biological functions (chimeric structure is also a possible naming, but the use of hybrid is preferred) and (ii) dual activity indicates that a hybrid molecule acts as two distinct pharmacophores. Both entities of the hybrid molecule are not necessarily acting on the same biological target. These hybrid molecules should be confused with prodrugs. When a drug candidate has a weak bioavailability, the prodrug strategy is highly useful to correct the pharmacokinetic and pharmacodynamic profiles of a valuable lead.[35] The hybrid molecule strategy is different than the fragment-based lead discovery.[36] The fragment-based approach in drug design is the improvement of the biological activity of a molecule fragment by the addition of chemical functions able to bind to adjacent regions of the active site when the protein target is known. The fragment self-assembly approach, consisting of allowing two or three fragments with adapted functionalities to react within the target site to generate *in situ* covalent links, will certainly have a successful development in the future. This approach is very similar to that one developed by Lehn and co-workers to create dynamic combinatorial libraries of chemicals.[37]

Before discussing our work on the design of antimalarial agents based on hybrid molecules with a dual mode of action, we would like to mention that we have been greatly influenced by our previous studies on the mechanism of action of bleomycin, an efficient anticancer agent.[38−40] This glycopeptide, originally isolated as an antibiotic from *Streptomyces verticillus*, is a paradigm for hybrid molecule-based drugs (bleomycin is a very popular research topic: more than 13 287 entries were found in the PubMed databank on March 12th, 2007). This drug has three distinct structural domains: one for DNA binding, a second for metal binding, and a third containing carbohydrates (see Figure 3 for the structure). As such, bleomycin is an excellent example of the design by a microorganism of a hybrid molecule containing three structural entities with three different biological roles. After cell penetration facilitated by the carbohydrate domain, the bithiazole entity and its positively charged terminal chain binds to GC-rich sequences of DNA and an amine-rich domain that strongly chelate redox-active metal ions, such as iron. The BLM–Fe^{III} complex is easily reduced inside cells to BLM–Fe^{II}, which reacts with molecular oxygen, a second electron, and a proton to generate "activated bleomycin". The last intermediate

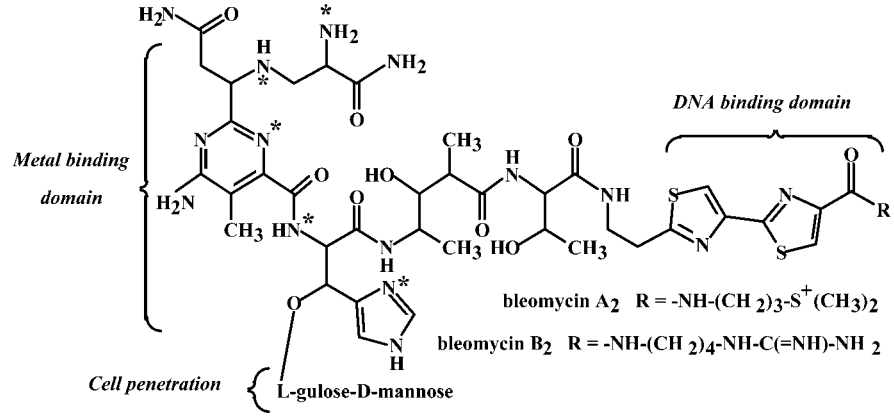

FIGURE 3. Representation of bleomycin with its three different structural domains (asterisks indicate the nitrogen atoms involved in metal binding).

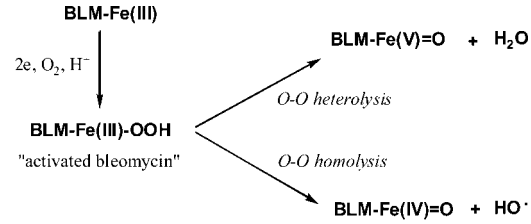

FIGURE 4. Generation of "activated bleomycin" and different possible reactive species in DNA cleavage.

that detected prior DNA cleavage is a low-spin BLM–Fe^{III}–OOH complex, which can abstract a H atom at the C4′ position of deoxyribose units after the homolytic cleavage of the O–O bond or via a high-valent iron–oxo species produced by the heterolysis of the same O–O bond (see Figure 4).[41,42] We contributed to document the formation and its cleaving activity of a high-valent bilayer lipid membrane (BLM)–iron–oxo complex by using $KHSO_5$ as single oxygen atom donor.[43,44] This work on the mechanism of action of bleomycin was at the origin of our interest to design hybrid molecules as potential anticancer drugs. Hybrid metalloporphyrin–ellipticine molecules with a DNA intercalator and a metalloporphyrin also having DNA affinity were able to cleave DNA and to be cytotoxic on leukemia cells at micromolar concentrations as bleomycin.[45,46] The development of these molecules has been stopped because of difficulties to access a suitable murine model for their *in vivo* evaluation.

We became interested in the design of hybrid antimalarial agents after an initial study on the mechanism of action of artemisinin (see Figure 5 for the structure), a drug active on chloroquine-resistant strains of *Plasmodium falciparum*.[47,48] During their erythrocytic life stage, the malaria parasites are using hemoglobin as a source of amino acids, leaving free heme as a waste residue. The liberated heme is polymerized to hemozoin to avoid any oxidative stress because of the redox-active free heme. The interaction of artemisinin with heme generates the homolysis of the O–O bond of the trioxane unit and produces, after β-scission, a C4 radical able to alkylate the meso positions of heme.[49,50] This alkylation of heme by artemisinin has been evidenced in infected mice and not in healthy ones (see Figure 5 for the structures of heme–artemisinin adducts).[51] This alkylating capacity of trioxane-containing antimalarial drugs is not limited to only artemisinin but is also a signature for active artemisinin derivatives or artemisinin mimics.[52,53] The understanding of the strong alkylation capacity of trioxanes when properly designed has inspired our design of antimalarial hybrid molecules. In particular, the access of the trioxane entity to heme should not be blocked by bulky substituents, and the β-scission should give rise to a C-centered radical and not to the release of a H atom (Figure 6).

The comeback of malaria for the last 3 decades is mainly due to the spreading of parasite strains resistant to classical and cheap drugs (chloroquine, pyrimethamine, sulfadoxine, etc.). Monotherapies with new drugs are not recommended by the World Health Organization (WHO) to avoid the fast emergence of new drug-resistant strains. Consequently, combined therapies based on Artesunate (a fast-eliminating drug) and a slow-eliminating drug have been developed over the past decade.[54] However, the cost of these drug combinations is still a problem, and sources of artemisinin are erratic. The challenge is to develop new antimalarial drugs, affordable and able to avoid the emergence of resistant strains.[55] Having these requirements in mind and also considering that free heme within the food vacuole of the parasite is still an attractive target,[56] we decided to create new hybrid molecules with a dual mode of action (a "double-edged sword") able to kill multiresistant strains by oral administration, without having the difficulties to adjust the biodisponibility of two structurally different molecules. These hybrid molecules with two

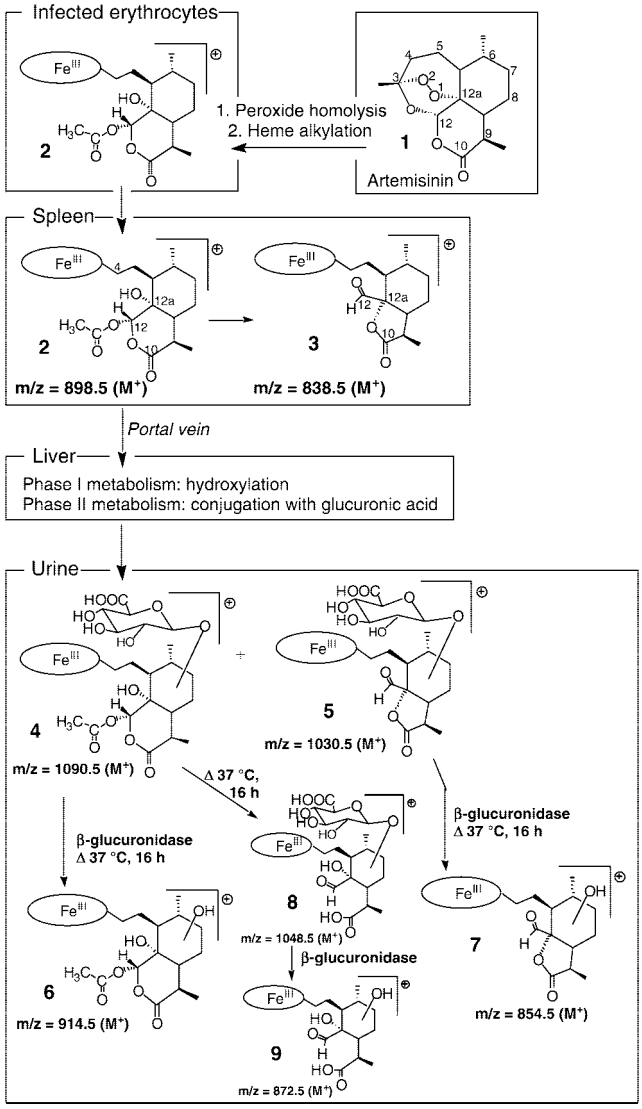

FIGURE 5. Representation of the antimalarial artemisinin with its 1,2,4-trioxane-active motif (the deoxo derivative with one oxygen atom missing in the endoperoxide bridge is totally inactive) and the identified heme–artemisinin adducts in infected mice. Reproduced with permission from ref 51. Copyright 2005 Proceedings of the National Academy of Sciences (PNAS).

pharmacophores able to interact with the target are made with a trioxane motif covalently linked to an aminoquinoline entity and named trioxaquines (see Figure 7 for structures).[57–60] These molecules are highly active *in vitro* on chloroquine-resistant strains of *P. falciparum* (with IC_{50} values ranging from 5 to 50 nM), and they are able to alkylate heme as well as artemisinin derivatives.[61,62] The two separate precursors of trioxaquines have a limited antimalarial activity compared to the whole entity, thus illustrating the synergistic effect of the covalent binding of both precursors.[60] These trioxaquines are active on the young erythrocytic stages of *P. falciparum* as artemisinin derivatives, whereas chloroquine is active on the late stages.[60] Therefore, trioxaquines have all properties of trioxane-containing molecules, but in addition, these hybrid molecules are also able to inhibit the polymerization of β-hematin as chloroquine (artemisinin derivatives are not able to inhibit hemozoin polymerization). These data indicate that both moieties of trioxaquines are able to interact with heme as a common target. The *in vivo* activities of trioxaquines by oral administration are higher than artemisinin itself and very close to that of artesunate with curative doses (CD_{50} values) ranging from 15 to 20 mg/kg per day (dose applied once daily for 4 days on mice infected with *P. vinckei petteri*).[60] On the same model, the CD_{50} values are 19 and above 25 mg/kg per day for artesunate and artemisinin, respectively. The activities of trioxaquines are also very similar to that of the new ozonide-containing antimalarial agent OZ277 developed by Vennerstrom et al.[63] trioxaquine DU1302 is also active by oral route on mice infected by the highly virulent strain *P. yoelii nigeriensis*.[60] Similar to artemisinin, DU1302 is active on gametocytes, the mosquito-transmissible forms of the parasites, which is not

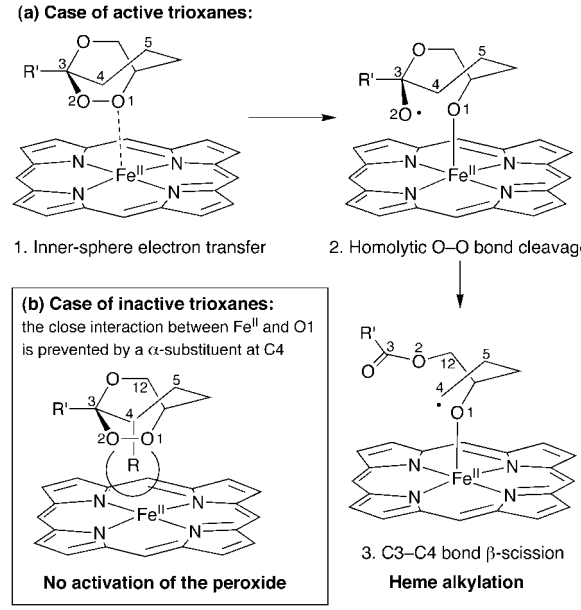

FIGURE 6. Interaction of trioxane-containing antimalarial drugs with heme as a target.

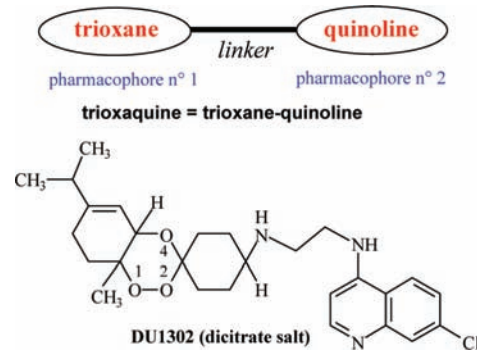

FIGURE 7. Schematic representation of antimalarial trioxaquines and structure of DU1302.

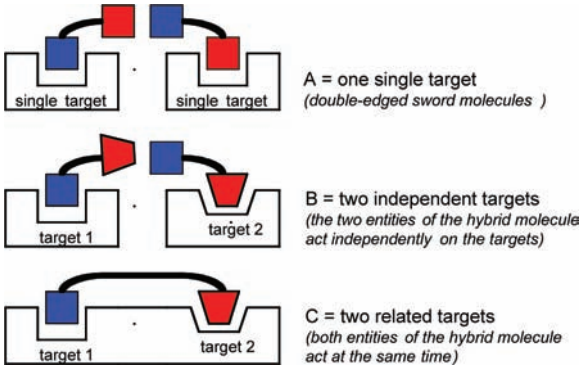

FIGURE 8. Schematic representation of three different possible modes of interaction of hybrid molecules: (A) one single target (for double-edged molecules), (B) two independent targets (each entity of the hybrid molecule acts with its target), and (C) two related targets (both entities of the hybrid molecule act at the same time on two connected targets).

the case for chloroquine. Killing gametocytes is essential to limit the spread of malaria. DU1302, despite a good activity profile *in vitro* and *in vivo*, has not been developed as a drug candidate because of its four different stereoisomers.[59,60] More than 100 trioxaquines have been prepared by Palumed over a period of 4 years, and in collaboration with Sanofi-Aventis, the trioxaquine PA1103-SAR116242 has been selected in January 2007 as a candidate for preclinical development. It should be noted that the large-scale preparation of endoperoxide-containing precursors is a real challenge in medicinal chemistry. Forty years ago, few people believed that a sesquiterpene, such as artemisinin, contained a trioxane motif as an active moiety. Again, the door was opened by Nature, and medicinal chemists have now a source of inspiration to create new peroxide-based drugs.

A frequently asked question: why make a hybrid molecule instead of giving the separate pharmacophores in one tablet? For short answers, one can note (i) the troubles when making a combination of drugs[64] (different solubilities are susceptible to modify the bloodstream uptake, the necessity of fine tuning the formulation to ensure that the blood level of the two drugs should be the same when given in the same tablet, etc.), (ii) that the pharmacokinetic of a hybrid molecule is more predictable, and finally, (iii) that it is possible to use the uptake capacities of one motif to boost the biodisponibility of the second entity. The concept of developing agents that modulate multiple targets simultaneously with the aim of enhancing efficacy has also been recently discussed by Morphy and Rankovic.[65]

Hybrid molecules with a dual mode of action can be classified in three different categories (see Figure 8). The category A concerns a single target, and both entities of the hybrid molecule are able to interact with the target. This is the case of trioxaquines that are "double-edged sword" molecules. Trioxaquines are able to stack with heme via their aminoquinoline entity and can also alkylate heme after a reductive activation: these hybrid molecules have a dual mode of action on the same target. In the second category B, the two entities of the hybrid molecule act independently on two different and nonrelated targets. This case has been recently illustrated by new hybrid antimalarial agents with a covalent attachment of an inhibitor of the PfCRT (*P. falciparum* chloroquine resistance transporter) to a chloroquine motif.[66] Hybrid molecules inspired by Rivastigmine and Fluoxetine are inhibitors of both acetylcholinesterase and serotonin transporters and have been designed for the treatment of Alzheimer's disease.[67] Another example of category B concerns the preparation of efficient antioxidants by linking a chroman moiety of vitamine E and a catechol known for its antioxidant activity.[68] When the linker is labile or designed for being easily cleaved *in vivo*, then a subcategory B can be identified as dual prodrugs. This particular case is illustrated by the example of hybrid antimalarial molecules containing a glutathione reductase inhibitor and a 4-anilinoquinoline.[69] Hybrid anticancer molecules based on the conjugation of taxol (Paclitaxel) to camptothecin, epipodophylotoxin, or colchicines via an amide-containing linker also fall into this subcategory.[70] To illustrate category C, one can mention the preparation of hybrid antiprion molecules obtained by linking acridines to imino-dibenzyl entities.[71] One of these hybrid molecules is one of the most active antiprion compounds yet described, with an EC_{50} value of 20 nM determined with a cell model of prion disease. DNA ligands fall into category C with the high diversity of sequences defined by the base-pair system. Many hybrid molecules have been designed to target specific DNA sequences: some of them are bleomycin mimics (see ref 72 and references therein), but most of them are hybrid DNA-binding molecules made by associating intercalators or minor- or major-groove binders to create efficient tools for the control of gene expression.[73] The hydrid molecule strategy is not recommended when the two targets for each entity are too different. Dual β-lactam–fluoroquinolone derivatives have been limited to phase-I trials and have never been able to pass this clinical stage (a membrane protein and a DNA-binding enzyme in that particular case).[74]

The limited number of examples mentioned above should not be considered as an exhaustive list of hybrid molecules with a dual mode of action but only as a short summary to illustrate the concept and its potential applications.

In conclusion, the design of hybrid molecules with a dual mode of action is a niche in the large field of drug discovery.

It is one possible approach to create drugs via a rational drug design, and it might generate drug candidates at a reasonable price. It is away from repetitive chemistry, and as in "haute-couture", the design should be good from the beginning to quickly get drug candidates with a high score in drug criteria and with the maximum chance of success in clinical trials.

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BIOGRAPHICAL INFORMATION

Bernard Meunier was born in 1947 and educated at the Universities of Montpellier (R. J. P. Corriu) and Paris-Orsay (H. Felkin). After a postdoc at Oxford University, he joined the "Laboratoire de Chimie de Coordination du CNRS" in Toulouse in 1979. He has been Director of Research at the CNRS, Associate Professor at the Ecole Polytechnique (1993−2006), and President of the CNRS (2004−2006). He is currently CEO of Palumed, a small company that he founded in 2000. He is the author of 339 publications and 29 patents. He is a member of the French Academy of Sciences (1999) and the Polish Academy of Sciences (2005).

FOOTNOTES

† This manuscript is dedicated to the memory of Pierre Potier, father of two anticancer drugs.

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