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PERSPECTIVE

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Assessing Atropisomer Axial Chirality in Drug Discovery and Development

Steven R. LaPlante,*[,†] Lee D. Fader,[†] Keith R. Fandrick,[‡] Daniel R. Fandrick,[‡] Oliver Hucke,[†] Ray Kemper,[‡] Stephen P. F. Miller,[§] and Paul J. Edwards*[,†]

[†]Department of Chemistry, Boehringer Ingelheim (Canada) Ltd., 2100 Cunard Street, Laval, Quebec, H7S 2G5, Canada

[‡]Chemical Development, Non-Clinical Drug Safety, Boehringer Ingelheim Pharmaceutical Inc., 900 Ridgebury Road, Ridgefield, Connecticut 06877, United States

[§]Office of New Drug Quality Assessment, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, 10903 New Hampshire Avenue, Building 22, Room 1446, Silver Spring, Maryland 20993, United States

INTRODUCTION

An underlying goal of drug discovery is to develop safe and stable substances that specifically target essential elements that cause disease. Molecular chirality adds an additional level of specificity and complexity in achieving this objective, as mirror image molecules are distinct substances and must be treated as such. Classical chiral-center enantiomers (Figure 1A) have been shown to differ significantly in biological activity, pharmacodynamics, pharmacokinetics, and toxicity.[1] The cases of thalidomide[2] and perhexiline,[3] whose enantiomers differ dramatically with respect to toxicity and metabolic properties, emphasize the importance of addressing stereochemistry in drug development.

In this Perspective, we address the pharmaceutical implications of a largely overlooked alternative source of drug chirality, atropisomerism,[4] which has the distinct feature of creating molecular chirality as a result of hindered rotation about a bond axis (Figure 1B). Figure 1C shows space-filling models where it is evident that rotation about the vertical axis is hindered because of steric clashes between the bulky R1 and R2 groups with R3 and R4.

Unlike compounds with classical chiral centers, which are often stable and which racemize via a bond breaking and making process, atropisomers racemize via an intramolecular dynamic process that only involves bond rotation. As bond rotation is time-dependent, racemization half-lives for atropisomers can vary dramatically between minutes to years, depending on the degree of steric hindrance, electronic influences, temperature, solvent, etc. Because of this time-dependent feature, drug discovery campaigns can become more complex, or may even be abandoned, when atropisomeric properties are observed. Atropisomerism frequently results as researchers strive to design more compact and conformationally constrained inhibitors. Even for courageous design and synthetic campaigns that attempt to develop atropisomeric compounds, important differences in properties have been reported for enantiomeric pairs, such as in vitro inhibition, crystallization, in vivo racemization rates, and absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties. There are also examples of compounds that were unknowingly developed as a racemic mixture of atropisomers and required chiral detection experiments to finally reveal their existence. Overall, many view atropisomer chirality as a lurking menace with the potential to increase the cost of pharmaceutical research and development and to derail drug

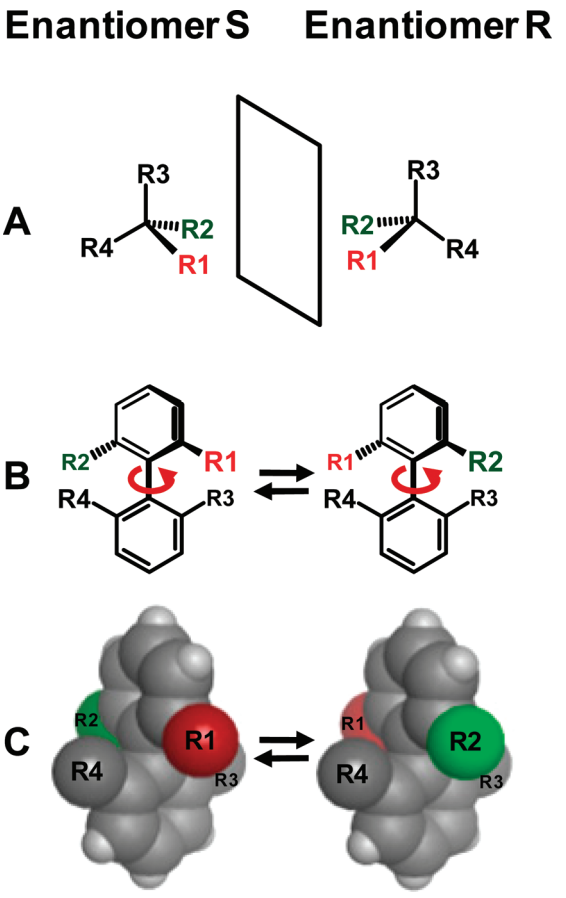

Figure 1. (A) Mirror-image enantiomers *S* and *R* arise from a classical chiral center (atom). (B) Other enantiomers S_a and R_a can arise from hindered rotation that creates a chiral axis. (C) Atropisomeric enantiomers S_a and R_a are shown as space-filling models. Reproduced with permission from *ChemMedChem* (LaPlante, S. R.; Edwards, P. J.; Fader, L. D.; Jakalian, A.; Hucke, O. Revealing atropisomer axial chirality in drug discovery. **2011**, *6*, 505−513, DOI: 10.1002/cmdc.201000485; http://onlinelibrary.wiley.com/doi/10.1002/cmdc.201000485/abstract).[11] Copyright 2011 John Wiley and Sons.

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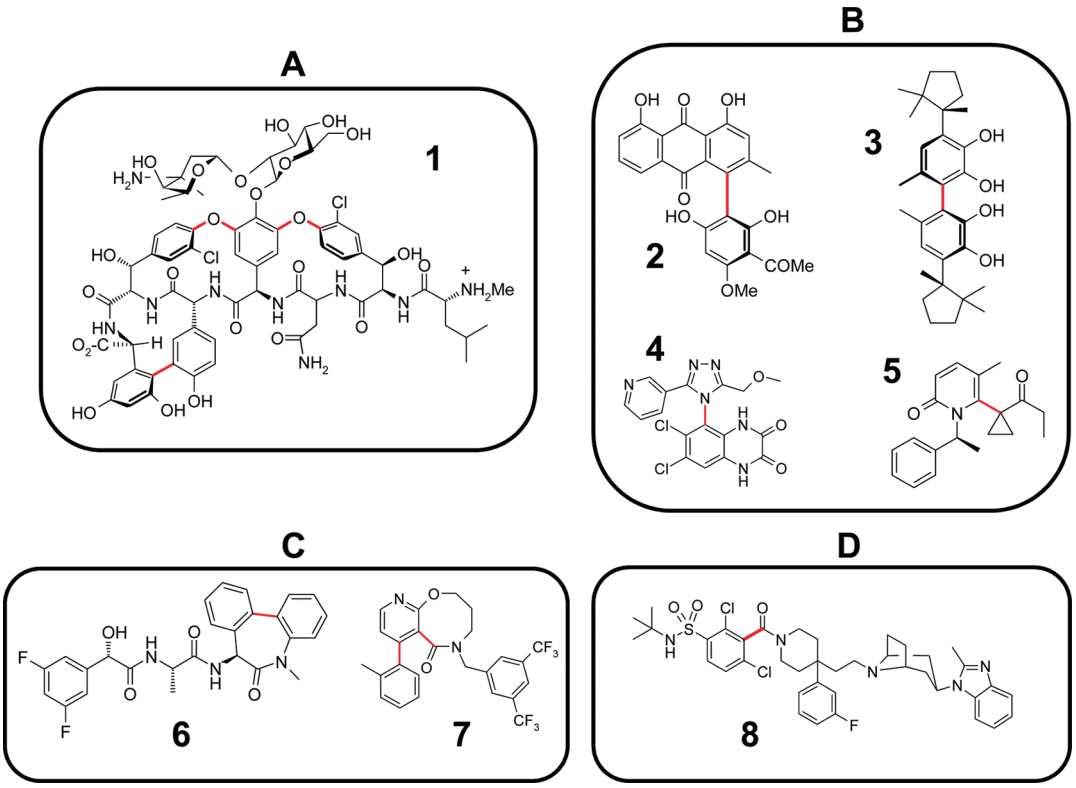


Figure 2. Shown are examples of drugs/candidates that have atropisomeric chirality due to hindered rotation about one or more axes (colored red): (A) large macrocycles; (B) linked 6−6, 6−5, and 6−3 ring configurations; (C) substituted medium-sized seven- and eight-membered rings; (D) hindered amides. Further information and references for the above compounds can be found in the text and Supporting Information of ref 11.

development programs because the racemization potential of atropisomers can undermine the underlying goal of discovering and producing stable drug substances. Furthermore, there are no direct guidelines from regulatory agencies on how to deal with this type of time-dependent chirality. It is therefore surprising that the topic of atropisomerism in drug discovery has not been extensively covered in the literature, despite its prevalence and importance to the pharmaceutical industry.

Herein, we give an overview of the impact that atropisomeric compounds have on drug discovery and development and propose strategies for their management. First, we highlight the variety of chemotypes known to give rise to axial chirality, which also serves as an aid for recognition purposes.[5−10] Figure 2 provides examples of atropisomeric drugs resulting from hindered rotation about an axis. These include large macrocycles[6,7] (Figure 2A), 6−6, 6−5, and 6−3 ring configurations[6a,7,8] (Figure 2B), substituted medium-sized seven- and eight-membered rings (Figure 2C),[9,10a] and hindered amides (Figure 2D). Methods for detecting and computationally revealing atropisomers are also explored, along with interesting examples from drug database searches that have flagged unreported atropisomeric drugs. Practical discovery and development strategies are proposed that can be used as a guide for foreseeing the production of stable chemical substances. These involve an atropisomer classification scheme, based on energy barriers and racemization rates, that should help coordinate efforts that span from early medicinal chemistry to development options. Also, noteworthy properties of atropisomeric enantiomers of drugs are highlighted with respect to large scale substance production and absorption, distribution, metabolism, excretion, and toxicity (environment) (ADMET(E)) pharmacology and hence their ability to be developed as effective pharmaceutical agents.

Overall, this review should help to expose the impact that atropisomers have on drug discovery and development efforts and help to illuminate practical options for managing them. Furthermore, an overview of expectations from the perspective of regulatory agencies is provided for clarification purposes.

■ STERIC HINDRANCE TO ROTATION, ENERGY BARRIERS, AND AXIAL ROTATION RATES

The recognition and management of atropisomers should begin with an understanding of the relationship that exists between axial rotation rates and steric hindrance. Atropisomers are conformers that, because of steric and/or electronic constraints, interconvert slowly enough (by definition, with a half-life of >1000 s) that they can be observed and, in favorable cases, even isolated.[4] As a result, this hindered rotation gives rise to conformational isomers, leading to diastereoisomers or enantiomers, all with the distinctive feature that they can in principle be equilibrated thermally and result in epimerization or racemization, respectively. Thus, atropisomeric stereoisomers should best be dealt with in the same way as stereoisomers with classical chiral centers, but special consideration must be given to the potential for racemization, i.e., isomerization rates and, where necessary, differential conformer populations.

The relationship between axial rotation rates and steric hindrance can be readily conceptualized using Figure 1B and Figure 1C. Rotation about the axis shown in Figure 1B clearly must involve clashing of the large R1 and R2 substituents with R4 and R3.

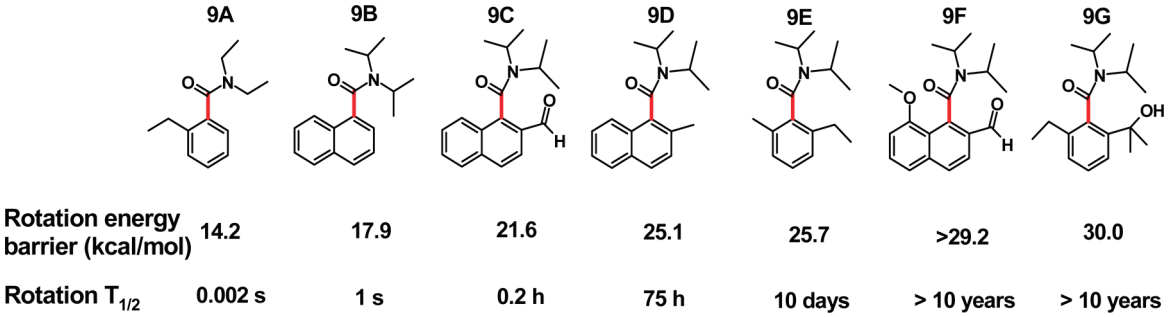


Figure 3. Shown are related compounds having increased steric hindrance about an axis of rotation (red bond) and its correlation with experimentally derived rotation energy barriers and rotation rates in solution.[10]

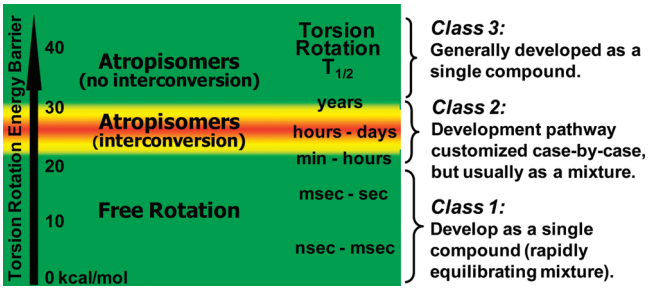


Figure 4. Shown is a qualitative, cross-discipline guide to help correlate axial (torsion) rotation energy barriers, $t_{1/2}$, and compound classes for predicting development strategies. Reproduced with permission from *ChemMedChem* (LaPlante, S. R.; Edwards, P. J.; Fader, L. D.; Jakalian, A.; Hucke, O. Revealing atropisomer axial chirality in drug discovery. **2011**, *6*, 505−513, DOI: 10.1002/cmdc.201000485; http://onlinelibrary.wiley.com/doi/10.1002/cmdc.201000485/abstract).[11] Copyright 2011 John Wiley and Sons.

Figure 3 also illustrates how a series of highly related compounds with incremental degrees of steric hindrance around a rotational axis can result in a wide range of rotational energy barriers (14.2−30.0 kcal/mol) and correspondingly interconversion half-lives ($T_{1/2}$ or $t_{1/2}$) (0.002 s to >10 years).[10]

■ ATROPISOMER CLASSIFICATION AS A CROSS-DISCIPLINE GUIDE

Atropisomeric drugs have the potential of racemization as a result of rotation along a bond axis, which could compromise the ultimate goal of developing stable and consistent chemical substances. This time-dependent feature can have significant impact and must be managed along various stages of discovery and development. Bearing this in mind, we proposed that compounds can be classified into three groups, based on rotational energy barriers, and suggested accompanying development strategies (Figure 4).[11]

Considering the solution properties, the simplest and most common class contains those molecules that do not have classical atropisomer properties (class 1). These compounds possess relatively fast axial rotation rates (on the order of seconds or faster), display no axial chirality, and can be developed as single entities along a traditional development path. Compounds with tortion angle rotational energy barriers $\Delta E_{rot} \gtrsim 20$ kcal/mol have the potential to form atropisomers and are subdivided into classes 2 and 3.

Compounds in class 2 can be complicated to develop given that stereochemical integrity can be compromised over the time course of drug production, administration to patients, and half-lives in vivo. Challenges could also arise in the interpretation of biochemical assays, instability during production and storage, and data analyses during clinical development. In general, compounds with $t_{1/2}$ of interconversion that is much faster than the half-life of in vivo elimination will have consistent exposures controlled by the interconversion rates. In contrast, compounds with longer interconversion half-lives could give rise to significantly different isomeric ratios and, as a result, different in vivo properties (vide infra, e.g., differential elimination rates for the atropisomer pair).[8]

Class 3 compounds are those that experience rates of interconversion on the order of years. These chiral compounds are stable (kinetically inert), meaning that little to no axial rotation or racemization is expected. Development can proceed in a manner similar to conventional stereoisomers that result from chiral centers.

For the sake of simplicity, the classifications were based on the solution properties of compounds. Of course, the rates of conversion and the energy barriers should also be considered for the solid state (crystalline). It is likely that intermolecular noncovalent interactions (hydrogen-bonding, steric effects, etc.) can take place in the solid state which could potentially raise barriers to interconversion relative to that of the solution state. Nonetheless, it would be prudent to also monitor atropisomerism for compounds in the solid state. For example, racemization could occur for crystallization protocols that employ high temperatures. Also, it is probable that racemization could occur in pharmaceutical formulations, and the proportion of atropisomers could potentially be influenced by chiral excipients (vide infra).

■ DEVELOPMENT OF ATROPISOMERS AS A SUBSET OF CHIRAL DRUGS

Drug discovery is a lengthy and expensive process. It is critical that prudent efforts focus on assuring that the final drug product will deliver consistent safety and efficacy. Therefore, atropisomer stereochemistry should be addressed early in drug discovery and development, as it is well-known that molecular chirality profoundly affects many properties of compounds.[1] Additionally, when atropisomers are addressed early in discovery, the risk of a costly failure in late development or clinical trials is minimized.

Given that relatively little has been published regarding the approval of atropisomeric drugs, it is important to note and learn

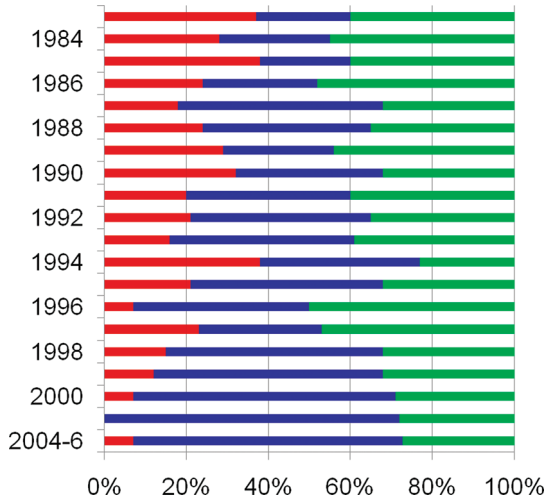


Figure 5. Reports from regulatory agencies that indicate the percentages of chiral and achiral new chemical entities (NCE) that were approved over the years.[3,12] Percentage shown are racemates (red bars, including diastereoisomers), single enantiomers (blue bars, single and multiple chiral center enantiomers), or nonchiral compounds (green bars).

from drug discovery and regulatory agency approval trends for compounds whose chirality were derived from classical atom-centers. The percentage of approved drugs is shown in Figure 5 based on whether they were developed as a racemate (red bars, including diastereoisomers), single enantiomers (blue bars, single and multiple chiral center enantiomers), or nonchiral compounds (green bars). Overall, Figure 5 reveals that since 1990 single enantiomers (blue bars in Figure 5) have clearly dominated over racemates (red bars).[3,12]

Clearly, there has been a distinct trend to develop drugs as stereochemically pure materials, but the development of racemates has also been proven to be a reasonable and feasible strategy,[3,12−14] suggesting that the latter approach could be applied to atropisomers, if justified (vide infra).

Similar trends have been reported for drugs approved between 1988 and 2008 in Japan, and the types of scientific information provided for the racemic drugs have been categorized.[14c] When a drug candidate racemizes rapidly under manufacturing or physiological conditions (as in the case of ibuprofen[13]), development as a racemate is generally appropriate.[12,14] Other considerations from the regulatory perspective are discussed below.

■ OVERVIEW OF REGULATORY ASPECTS OF ATROPISOMER DEVELOPMENT

The U.S. Food and Drug Administration (FDA) Policy Statement for the Development of New Stereoisomeric Drugs[11] does not specifically mention atropisomers. However, as a type of stereoisomer, atropisomers fit within the spirit of that guidance document. Other sources of regulatory guidance for development of stereoisomeric drugs include Health Canada's "Guidance for Industry: Stereochemical Issues in Chiral Drug Development" and the EMA's "Investigation of Chiral Active Substances".[14]

Because the enantiomers of a chiral drug frequently have different biological activities, the FDA policy statement outlines information about the individual enantiomers that is useful for determining whether to develop a single enantiomer or a racemic mixture. Given the technical advances in chiral separations and stereochemically controlled synthesis since the policy statement was published in 1992,[12] many drug candidates can now be feasibly developed as single enantiomers.

The following sections apply the scientific principles in the FDA policy statement to the case of drug candidates that exhibit atropisomeric behavior. Quotes from the policy statement are shown in italics.

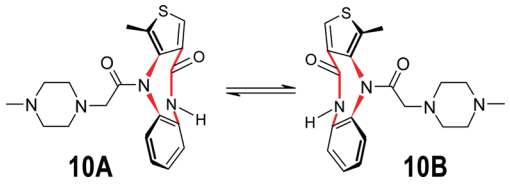
Class 3 Atropisomerism: Very Slow Equilibration ($t_{1/2}$ in Years). Atropisomers that equilibrate very slowly ($\Delta E_{rot} \gtrsim 30$ kcal/mol) are very similar to compounds with classical stereocenter-based chirality. That is, they exist as single enantiomers if there is one axis of chirality and as diastereoisomers if there is more than one axis/center of chirality in the molecule. As with other drugs containing stable stereocenters, it may be feasible to develop many stable atropisomeric drugs as single stereoisomers. As shown by the historical trends in Figure 5, this is the usual mode of development for drugs with stable stereocenters. For a drug candidate developed as a single stereoisomer, the minor enantiomer or diastereoisomer is controlled as an impurity.[12,14]

When it is anticipated that preparation of a single stereoisomer may not be practical at commercial scale, it is important to outline the situation with a suitable scientific justification early in development. As described in the FDA policy statement, data to assess whether development as a single stereoisomer is likely to improve safety and/or efficacy is generally an important part of the scientific justification. For example, "*unless it proves particularly difficult, the main pharmacologic activities of the isomers should be compared in in vitro systems, in animals and/or in humans.*" An assessment of the toxicology of the stereoisomeric mixture in animals can also support the decision to continue to develop the drug as a stereoisomeric mixture or to assess the safety and toxicology of the separate stereoisomers: "*A relatively benign toxicologic profile using the racemate would ordinarily support further development without separate toxicologic evaluation of the individual enantiomers. If, however, there are toxic findings other than those that are natural extensions of the pharmacologic effects of the drug, and especially if they are unusual or occur near the effective dose in animals or near the planned human exposure, toxicologic evaluation of the individual isomers in the study where the toxicity was detected should be undertaken*" and "*if toxicity of significant concern can be eliminated by development of single isomer with the desired pharmacologic effect, it would in general be desirable to do so.*"

As a type of stereoisomer, the recommendations in the FDA policy statement to understand the pharmacokinetic behavior of the isomers are scientifically relevant to stable atropisomers: "*When the drug product is a racemate and the pharmacokinetic profiles of the isomers are different, manufacturers should monitor the enantiomers individually to determine such properties as dose linearity and the effects of altered metabolic or excretory function and drug−drug interactions. To monitor in vivo interconversion and disposition, the pharmacokinetic profile of each isomer should be characterized in animals and later compared to the clinical pharmacokinetic profile obtained in phase 1.*" Finally, the technical challenges that make it difficult to prepare commercial quantities of the individual stable atropisomer are important for a complete scientific understanding of the developmental plans.

When developed as a stereoisomeric mixture, controls on the ratio of the isomers will generally be a part of the overall control strategy for the drug. In summary, for atropisomeric compounds that undergo very slow equilibration, development as a single atropisomeric isomer is generally appropriate from the scientific perspective. However in some circumstances development as an

Scheme 1. Slow Interconversion between the Atropisomeric Enantiomers of Telenzepine 10A and 10B



isomeric mixture can be supported by a scientific justification based on safety, efficacy, and manufacturability.

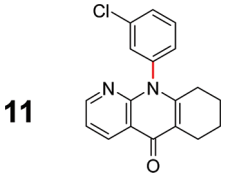
Class 2 Atropisomerism: Moderate Rate of Equilibration ($t_{1/2}$ from Hours to Days). Atropisomers with barriers to torsion−rotation of $\Delta E_{rot} \approx 20-30$ kcal/mol will equilibrate with half-lives of hours to days in solution at room temperature. As outlined in this Perspective, this situation can pose significant challenges for manufacture and quality control and can have complicated effects on absorption and elimination. In some cases it may be possible to modify the structure to obtain a more suitable analogue for development (e.g., design related compounds that have slower or faster axial rotation rates). When this is not practical, information on the activity of the separate atropisomers (where feasible) in an appropriate in vitro or in vivo model may help support the proposed developmental pathway. As with other situations where a mixture is proposed for development, it is valuable to have techniques to monitor the individual stereoisomers in pharmacokinetic samples available early in development.[12] If measurement of the atropisomeric ratio in pharmacokinetic samples is complicated by equilibration during the analysis itself, it may only be possible to obtain qualitative information. Information on the rate of equilibration and the equilibrium ratio (for atropisomeric diastereoisomers) is generally valuable for guiding the scientific and regulatory discussions in preparation for first-in-human studies.

Class 1 Atropisomerism: Rapid Equilibration ($t_{1/2}$ Less Than Minutes). Atropisomers that equilibrate with half-lives on the order of minutes or faster, if selected for development, will of necessity be dosed as an equilibrating mixture. From a scientific perspective, information on the rate of equilibration (and the equilibrium ratio for atropisomeric diastereoisomers) is useful for understanding the spectral and chromatographic properties of the drug substance.

■ ATROPISOMERIC DRUGS: MEDICINAL CHEMISTRY AND MANUFACTURING PERSPECTIVE

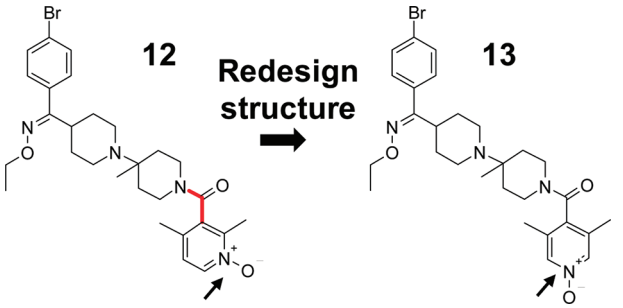
The decision to develop a drug candidate as a purified enantiomer, or a racemate, should be made as early as possible during the medicinal chemistry lead optimization stage. If possible, options for dealing with the atropisomeric phenomenon should be developed and implemented at this early drug design stage, bearing in mind the ultimate goal of producing a stable and consistent drug substance. If the barrier to atropisomerization is high (class 3 compounds, Figure 4), one should aim to develop an atropisomeric drug as a single, pure, stereochemically stable isomer. Telenzepine **10**, a selective muscarinic antagonist that has found use in the treatment of peptic ulcers[15] (Scheme 1), is an example of this class, as its atropisomer interconversion (ring-flipping) is slow, with a $t_{1/2}$ of racemization of 1000 years at 20 °C. It was discovered that there was a 500-fold difference in

Scheme 2. Compound 11[a]



[a] Compound **11** has a fast racemization $t_{1/2}$ of 1.6 min at 37 °C. The hindered axis of rotation is colored as a red bond.

Scheme 3. Substituent Symmetrization as a Means for Minimizing Atropisomers of the CCR5 Antagonist 12 (Left), with the Symmetrical Analogue Shown (Right)[a]



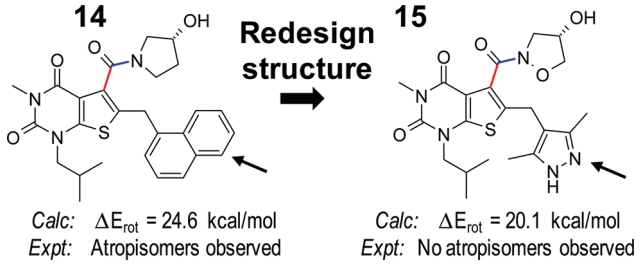
[a] The atropisomeric axis of rotation is colored as a red bond.

activity between the atropisomers of telenzepine at muscarinic receptors in rat cerebral cortex.

If the barrier to atropisomerization of a compound is low (class 2, Figure 4), then one should consider developing the drug as a consistent and reproducible interconverting mixture.[16] This is the case for Sch 40120 (**11**) (Scheme 2), which is an inhibitor of 5-lipoxygenase. This compound has found use in treating acute inflammatory diseases such as psoriasis.[17] The enantiomers of **11** can be observed by chiral HPLC, but they readily interconvert with a half-life of only 1.6 min at physiological temperatures (37 °C). It was therefore argued successfully by Schering Plough that the unavoidably short racemization rate justified the development of this compound as a racemic mixture.[17]

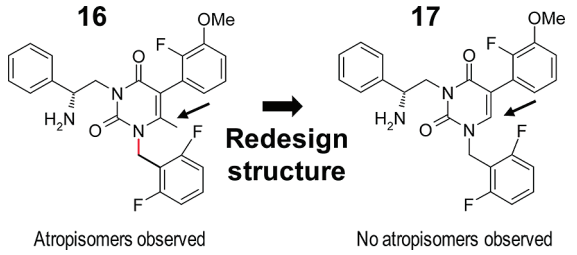
As indicated in Figure 4, other compounds can have atropisomeric interconversion properties that make it challenging to develop these substances as drugs, which themselves should ideally be consistent and stable, both for production purposes and in the body. It may be possible to exercise one of several practical options at the lead optimization stage. For example, the simplest way would be to avoid atropisomerization through the synthesis of related analogues to the compound of interest that have symmetry about the hindered bond. Scheme 3 shows Sch 351125 (**12**), which is a CCR5 antagonist that inhibits HIV entry into host cells.[18] Compound **12** exists as a mixture of four stereoisomers. Atropisomerism, as a result of hindered rotation along the two axes shown in Scheme 3, leads to the presence of two diastereomers each existing as a pair of enantiomers.[18] These entities displayed half-lives for interconversion of ~5 h at 37 °C. To obviate potential problems arising from the presence of the interconverting chiral axis, the symmetrical amide analogues were prepared (see structure **13** in Scheme 3). Symmetry around the Ar−CO bond eliminates diastereoisomerism, and although enantiomeric conformers do still exist (arising from restricted

Scheme 4. Example of Reducing Steric Hindrance to Axis Rotation Helped To Avoid Atropisomeric Chirality[20,a]



[a] Calculated energy barriers to rotation and experimental observations are shown for the hindered axis of rotation colored as a red bond.

Scheme 5. Example of How Reducing Steric Hindrance to Axis Rotation Helped To Avoid Atropisomeric Chirality[a]



[a] The hindered axis of rotation is colored as a red bond.

N−CO rotation), they interconvert rapidly. These symmetrized compounds exhibited comparable efficacy in binding and viral entry assays. However, their overall profiles were inferior to the parent molecule **12** and so work was focused on identifying superior development candidates, such as the symmetrical amide strategy (a dimethylpyrimidine) which became SPRI's vicriviroc (SCH-D).[18]

A related approach stems from the desire to modulate the rate of axial bond rotation through engineering of faster bond rotation such that atropisomers no longer exist. In this way, the compound changes from a class 2 compound to a class 1 compound. Scheme 4 illustrates an example of this approach, where compound **14** on the left is an effective monocarboxylate transporter (MCT1) blocker, and because of slow rotation about the N−CO (colored blue) and Ar−CO bonds (colored red), there exist four separable diastereoisomeric atropisomers.[19] All four compounds possessed different potencies. The half-life rates of interconversion for these compounds ranged from 1 to 12 h at 37 °C, which made it impossible to control the atropisomer ratio under physiological conditions. Conversely, the close analogue **15** on the right of Scheme 4 no longer exhibited atropisomerism, as replacement of the pyrrolidine by an isoxazolidine, as well as the naphthalene by a pyrazole ring, resulted in conformers that interconvert rapidly.

A further example of this strategy is provided in Scheme 5, where removal of the methyl from the central ring resulted in changing from a class 2 compound to class 1 compound.[21] These uracil compounds are potent antagonists of the human gonadotropin-releasing hormone receptor.

Another strategy for dealing with atropisomeric compounds is through the introduction of greater steric encumbrance about the hindered bond, to produce separable atropisomers whose interconversion is negligible (for example, half-lives of the order of millennia). An example of this strategy, in changing from a class 2 compound to a class 3 compound, is shown in Scheme 6. Indicated on the left of the scheme is compound **18** that exists as atropisomers arising as a result of restricted rotation about two bonds that themselves exhibit interdependent, geared rotation.[22] These atropisomers possess half-lives measurable in days at 37 °C and thus allowed the separation, isolation, and structural characterization of these compounds, as well as an assessment of their activities in vivo.

It was thought that development of this NK1 antagonist (**18**, Scheme 6) as a treatment for depression[22] would be complicated by the presence of hindered rotation, so a more conformationally rigid mimic based on the bioactive atropisomer of **18** was designed. Through linking of the amide and the naphthyl moiety in a methylated eight-membered ring, a more rigid class 3 analogue was designed and synthesized (see right-hand side of Scheme 6), which locked the amide into the desired bioactive trans configuration of **19**. Further development led to compound **20** which possessed excellent potency in vitro and in vivo.

Another possible solution to the challenges of class 2 compounds would be to ensure that the drug substance (enantiomeric or diasteoroisomeric mixture) is produced at the thermodynamic stable ratio of isomers. For example, one may want to include a heating step in drug product production to force this stable ratio.

EXPERIMENTAL DETECTION OF ATROPISOMERS

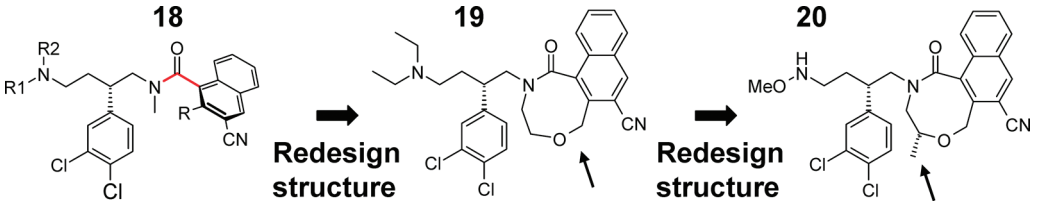
Critical to the management of atropisomeric compounds is the initial recognition of primary structural motifs that give rise to hindered axial rotation. The variety of chemotypes illustrated in this Perspective provides salient examples. There are also less intuitive chemotypes, or structural combinations, that have led to conformation-based chirality. Most often the existence of atropisomerism is initially noted by unusual NMR spectra[24] or HPLC of diastereoisomers taken during the lead optimization stage[25] and/or characterized by single-crystal X-ray crystallography.[26] However, the existence of atropisomers can remain undetected if the compound exists as a pair of enantiomers. For example, in Figure 6A the ^{1}H NMR spectrum of the drug laquinimod (**21**)[28] displays only a single methyl resonance. To reveal atropisomer chirality, we designed and synthesized the related analogue **22** shown in Figure 6B, where a chiral center was added. This resulted in the observation of two resonances for a single methyl, which confirmed the existence of diastereoisomers (i.e., the compound possessed two chiral elements).

Protein binding has also been used to detect the existence of atropisomerism, as the protein pocket introduces a specific chiral template. In our hands, protein binding studies have also been useful for revealing atropisomers and for evaluating relative racemization half-lives for categorization purposes.

Additional analytical tools exist for evaluating atropisomer chirality and racemization rates. Chiral HPLC at room and low temperature are useful techniques. Also, a toolbox of NMR methods are available, including ROESY, EXSY, variable temperature spectra, and chiral-shift reagents. For example, the chiral center and chiral axis of **22** renders **22** as a diastereoisomer and results in the observation of a doubling of resonances in these NMR methods.

We have also noted the existence of atropisomers based on in vitro enzyme inhibition assays. Depending on the racemization

Scheme 6. Example of How Locking the Axes of Rotation Helped To Avoid Axial Atropisomeric Chirality[23,a]



[a] The hindered axis of rotation is colored as a red bond.

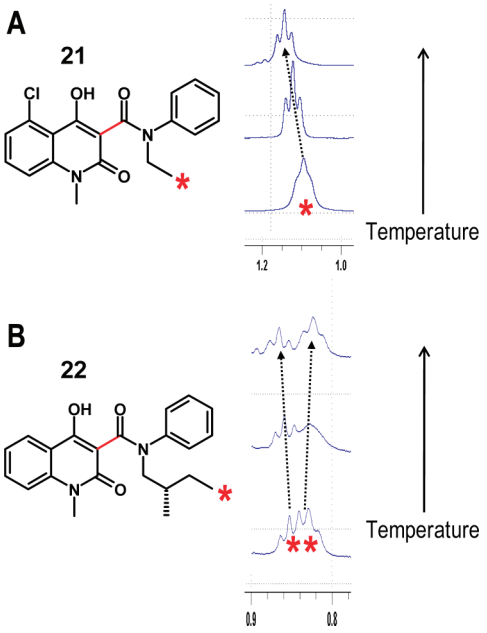


Figure 6. ^{1}H NMR spectra of enantiomeric laquinimod **21** and a diastereoisomer mimetic **22** at increasing temperature. The hindered axis of rotation is colored as a red bond. (A) The atropisomer enantiomer of laquinimod (enantiomer) and the corresponding NMR resonance of the methyl denoted by a red star. (B) A related compound **22** designed to mimic the rotational properties of **21** with the addition of a chiral appendage and the corresponding NMR resonance of the methyl denoted by a red star.

rates and the timing of the assays, we noted that some compounds do not achieve 100% inhibition in dose-response assays. This would result if less than stoichiometric amounts of the active compound were truly dosed, such as would be the case if one atropisomer was significantly less potent (unpublished data in the corresponding authors' laboratories).

In summary, atropisomer chirality can be detected by many methods, and strategies that help to define the existent atropisomeric ratios are necessary. For example, atropisomers are typically assumed to exist in equimolar ratios, such as 1:1. However, atropisomers can adopt unequal ratios that can then have profound effects at various levels of drug discovery. For instance, in cases where an early stage drug discovery program tests mixtures that exist in solution as (for example) a 5:1 mixture, the interpretation of biological assay data may be complicated. A more detailed example of the impact of unequal ratios is discussed here in the subsection entitled, "Atropisomer Drug Substance Production, Crystallization, and Formulation".

COMPUTATIONAL PREDICTION OF ATROPISOMERS

Foreseeing the existence of atropisomerism in compounds of interest is critical at all stages of drug discovery and development. Recently, we showed that a computational approach is suitable for this purpose.[11] In this approach, a relaxed torsion scan (e.g., Figure 7A) simulates rotation along a sterically hindered bond, and the energy is recorded at each increment of rotation. The method used is quantum mechanics, allowing for the calculation of energy values that consider steric and electronic properties, independent of the availability of suitable force field parameters that are required for accurate molecular mechanics calculations. The calculations are straightforward to perform using standard computational chemistry software. The method was initially validated using the seven aromatic amides in Figure 3, because of the availability of a wide range of experimentally determined rotational energy barriers. A good correlation ($r^2 = 0.8$) between the experimental and the calculated energy barriers was observed (Figure 7B). It was then extended to the detection of atropisomers in databases of drugs and drug candidates. By use of a threshold of the calculated rotational energy barrier $\Delta E_{rot} \geq 20$ kcal/mol to separate atropisomers from non-atropisomers, an atropisomer prediction accuracy of 86% was reported (25 out of 29 predicted atropisomers were confirmed).

The use of this computational tool for predicting atropisomers has significant potential. When coupled with the classification scheme mentioned above (Figure 4), practical medicinal chemistry options can be directed and development strategies can be envisaged. The most valuable utility of calculating barriers to rotation for in-house purposes has been to predict the existence of atropisomerism along the drug discovery pathway, especially when used in concert with our compound classification scheme described earlier or when physical detection or separation of atropisomers proves to be challenging. Efforts can be made during the early discovery stages such as "hit-to-lead" and "lead optimization" to predict and validate the existence of atropisomers and to exercise options for eliminating, further stabilizing, or rendering the chiral axis of interest more freely rotating via structure−activity relationship (SAR) design, thereby reducing this potential liability within a compound series. The strategy can also improve drug development plans, such as determining whether a drug or series should be developed as a racemic mixture or as an isolated single isomer.

Because of potential liabilities associated with class 2 atropisomers, one important application of the prediction of rotational energy barriers is to identify compounds that fall into the intermediate range $\sim$20 kcal/mol $\leq \Delta E_{rot} \lesssim$ 30 kcal/mol. This is preferably done at, or before, the lead optimization stage. If possible, efforts could be made to synthesize a related compound that has symmetry about the hindered axis, thus nullifying this

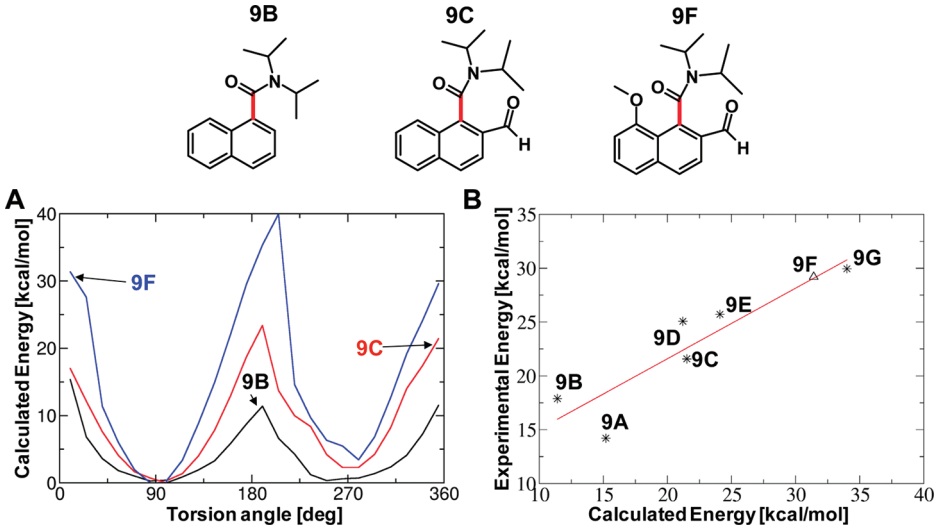


Figure 7. (A) Quantum mechanics torsion (axial) profiles for the red-colored bonds of the compounds shown above and in Figure 3.[11] (B) Comparison of experimental versus calculated lowest-energy barrier to rotation.[11]

chirality (e.g., see Scheme 3 and discussion thereof).[11] Otherwise, in silico testing of structural modifications that aim to shift ΔE_{rot} into the preferred ranges above ~30 kcal/mol or below ~20 kcal/mol could be conducted. Successful modifications of ΔE_{rot} is usually achieved through synthetically increasing or decreasing steric requirements around the rotational axis in question, respectively. See Scheme 4 as an example of how decreasing steric hindrance can reduce the liability arising from a chiral axis.

Another very interesting application of the computational tool was to flag potential atropisomeric compounds from drug databases. This study not only identified known atropisomeric drugs but it also revealed drugs with unreported atropisomeric properties. Figure 8 provides the structures and predicted energy barriers to rotation. It is noted that complex natural products and compounds containing medium-sized rings were not considered in this exercise. Energy barriers for hindered "ring-flipping" of medium-sized rings (e.g., seven-membered rings) are more complex, and a subsection below is devoted to atropisomers involving medium-sized rings.

Some of the drugs shown in Figure 8 are noteworthy. Dicoumarol **34** is used as an anticoagulant, and a subsequent literature search found a report that detected the existence of atropisomer chiral properties for this compound.[27] Laquinimod **21** is a drug currently in phase III clinical trials for the treatment of multiple sclerosis and other diseases, such as Crohn's disease. However, we found no reports of atropisomers for laquinimod **21**.[28] It is likely that if no analytical methods with chiral resolution were applied, then the existence of enantiomers would have been easily overlooked. In our hands, laquinimod displayed a single set of NMR resonances (Figure 6), consistent with either a nonchiral compound or a mixture of enantiomers. Unfortunately, appropriate chiral HPLC conditions could not be found for separating the enantiomers. To clarify the situation, the related diastereoisomeric derivative **22** was designed and synthesized (shown in Figure 6B) and it gives rise to two sets of NMR resonances, as would be expected if this compound existed as diastereoisomers because of atropisomeric chirality. Figure 6B confirmed that in fact two sets of NMR methyl resonances were observed for the diastereoisomeric derivative in DMSO and other solvents. Raising the temperature helped with visualizing the distinct resonances.

Further, calculations also predicted and flagged drugs that could have class 3 atropisomeric properties, with rotational energy barriers of ΔE_{rot} > 30 kcal/mol (Figure 8). Iomeprol **28** belongs to a family of X-ray contrast reagents[29] that have been found to exist as mixtures of diastereoisomers based on the chiral centers and axis that exist in these compounds.[30] Afloqualone **29** belongs to a large family of older drugs, often referred to as "qualudes" that have been used extensively for sedative and illicit recreational purposes.[31] These early "designer drugs" were likely taken as racemic mixtures and only much later were they found to have atropisomer properties. SY-801 **31** and its related drug DDB are currently administered as a mixture of atropisomers for the treatment of hepatitis B and other diseases, and their existence as atropisomers was only recently revealed. Clinical trials are also currently underway, and a patent application has been recently submitted for the purified enantiomer.[32]

Literature searches revealed that most of the other compounds in Figure 8 also had atropisomeric properties, with the exception of compounds **23**−**25** and **33** for which no direct evidence of atropisomeric properties was reported. Overall, this work reinforces the powerful utility of combining prediction and detection strategies.

■ SPECIAL CASE OF ATROPISOMER PROPERTIES INVOLVING MEDIUM-SIZED RING FLIPPING

Atropisomers may also exist for compounds having asymmetric medium-size rings within their structures.[33] In these cases, it is not a single bond rotation involved in atropisomer interconversion. Rather, a "ring-flip" is required to bring about the change from one enantiomer to the other. A further complication is the fact that medium-size rings have many possible local energetic minima, which themselves will be influenced by substitution pattern and the number of degrees of unsaturation. Within the context of drug discovery, medium-sized rings have occupied privileged chemical space for decades and there are a plethora of examples of drug leads, clinical candidates, and marketed drugs that display atropisomeric properties.

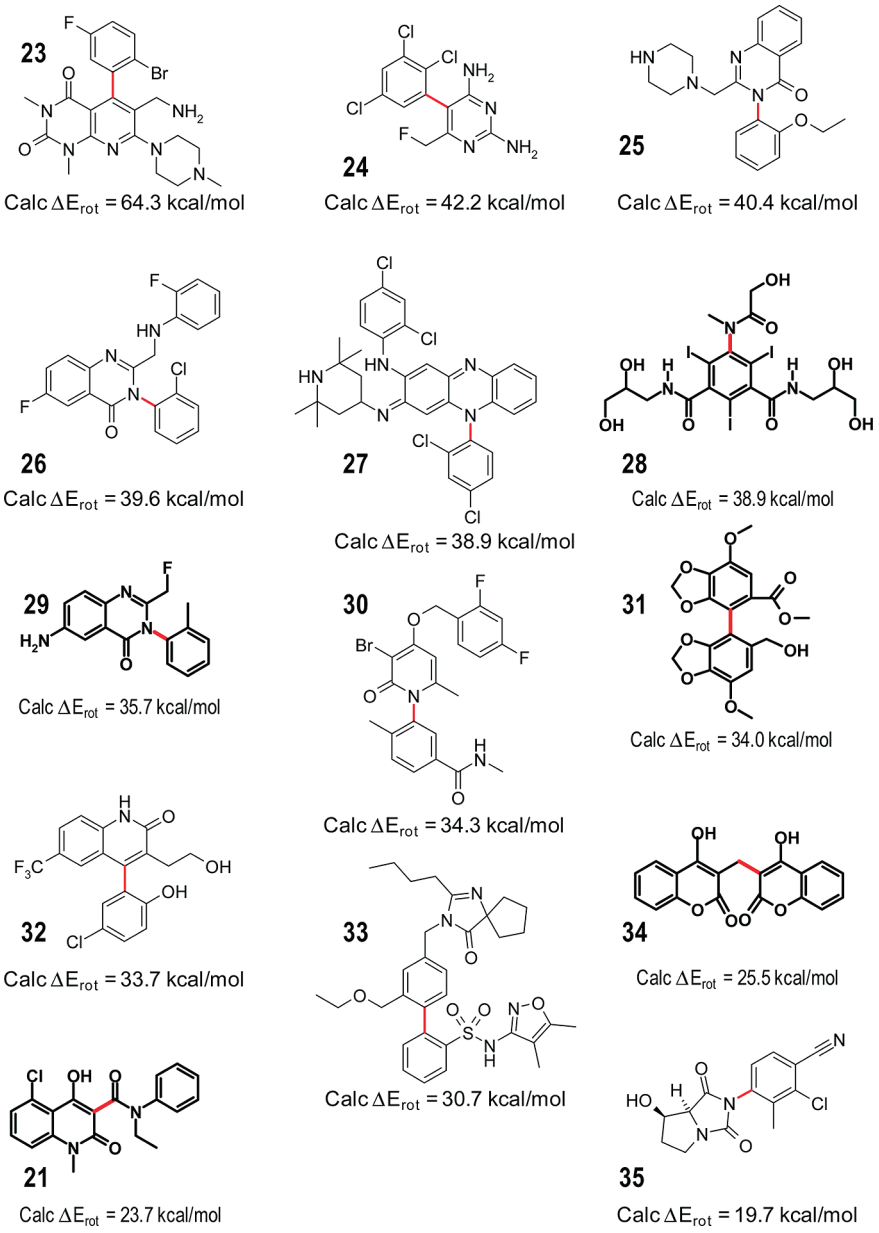


Figure 8. Drugs/candidates identified from our first search of a drug datebase that employed the quantum mechanics prediction strategy. Further information and references for the above compounds can be found in the text and Supporting Information of ref 11.

Compound **10** is a well-known example of a marketed drug from the medium-sized ring family (Scheme 7). In this case, its atropisomers are stable, having a $t_{1/2}$ of racemization of 1000 years at 20 °C, and so atropisomers could be separated and studied independently.

At the other end of the ring-flipping spectrum, nevirapine (**37**)[34] and diazepam (**38**)[6,33] were examples of approved drugs that existed as mixtures of chiral conformations that were not reasonably separable at room temperature. Their barriers to interconversion were sufficiently low that they could be advanced along a traditional development path as if they were single compounds. In these cases, their development situation was simplified by virtue of the enantiomeric relationship between the chiral conformations. However, cases also exist where the chiral conformations were diasteroisomeric (e.g., compounds **36**, **39**, **41**), and they could be observed by commonly employed analytical methods. In such cases, partial resolution was often possible. Successful drug candidates from this class of atropisomer usually rely on a favorable shift of the conformational equilibrium brought on by the second chiral element of the molecule. As with other types of atropisomers, the situation is most problematic for medium rings that fall into class 2 (vide supra). The difference for medium rings is that substitution around the (medium) rings must impose severe steric restrictions to bond rotation before these molecules become class 3 atropisomers. The prototypical example of this phenomenon is found with the recent structural modifications of diazepams (Scheme 8).[6,33] With small nitrogen substituents such as hydrogen and methyl, the diazepam scaffold already has hindered rotation and more readily interconverts between enantiomers. Increasing the size from a proton to larger groups increases the barrier to interconversion. In the case of the *tert*-butyl group, the atropisomers are stable and separation

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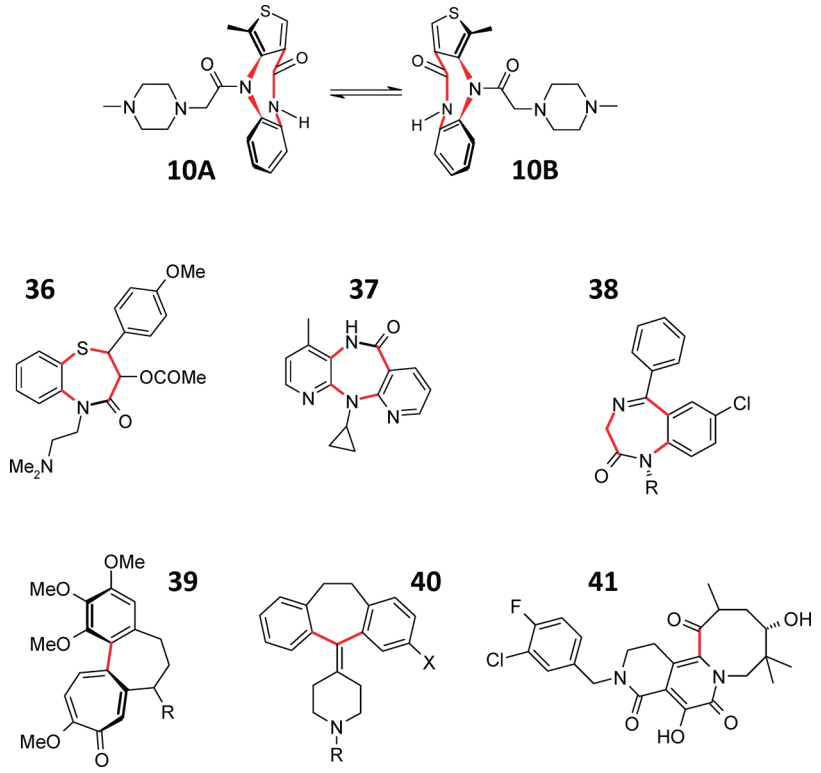
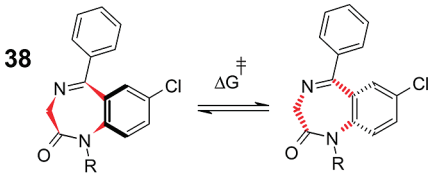
Scheme 7. Examples of Compounds That Have Medium-Sized Rings[a]



[a] The ring flipping can be visualized as shown by the atropisomeric puckers of **10A** and **10B** telenzepine. Further information and references for the above compounds can be found in the text and Supporting Information of ref 11.

Scheme 8. Structural Modification of Diazepam 38



R	$\Delta G^{\ddagger}$ kcal/mol
H	12.3
Me	18.0
i-Pr	21.1
t-Bu	>24

allowed for the determination of affinity for the benzodiazepine receptor of each enantiomer. Although one atropisomer is more potent than the other, which is the *N*-methyl analogue (R = Me) shown in Scheme 8, both enantiomers are at least 100-fold less active than diazepam itself. This illustrates the central problem of balancing stereoisomer stability and intrinsic potency in the case of medium-size ring atropisomers.

■ ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION, AND TOXICITY (ENVIRONMENT) CONSIDERATIONS

One should also keep in mind that atropisomerism chirality is time-dependent, which gives rise to its distinct feature compared to relatively more stable atom-centered chirality. Atropisomer racemization occurs by simple rotation along a bond axis, which results in compound instability. One consequence of axial chirality is that additional equilibria rates (Figure 9) and biological properties need to be considered,[35] as atropisomeric drugs pass through the body, into the environment, and potentially back into the food chain and/or body. Even though a drug is administered to a patient as a single enantiomer, over time racemization will occur. The latter phenomenon is a current concern from drug and agrochemical residues (e.g., gossypol) found as contaminants in drinking water[36] and other environmental sources.

The display of the complex ensemble of equilibria shown in Figure 9 is not meant to discourage the development of atropisomeric compounds. Rather, researchers should be aware of the potential equilibria and determine which, if any, are significant to their compound(s) of interest. Those that are critical should, in turn, be monitored closely while keeping in mind the goal of dosing patients with a safe and stable medication.

Class 3 atropisomers are theoretically unlikely to rotate and racemize and should be considered and developed as a single, purified compound.[37] However, the appearance of the atropisomeric distamer should be monitored for the sake of safety. One should be especially prudent for compounds that approach the class 2 rates. Regarding the stability of drug substance, a desirable property would be that the compound of interest maintains 99.5% homogeneity over the course of 24 h or so in vivo, which corresponds to a half-life of 138 days or greater and $\Delta E_{rot} \geq 27.3$ kcal/mol. Two points are worth noting. Apparently small increases in ΔE_{rot}, for example, up to 29.4 kcal/mol, correspond to a half-life of 4609 days. Also, one must be aware that ΔE_{rot} can be significantly influenced by many in vivo influences (protein binding, etc.).

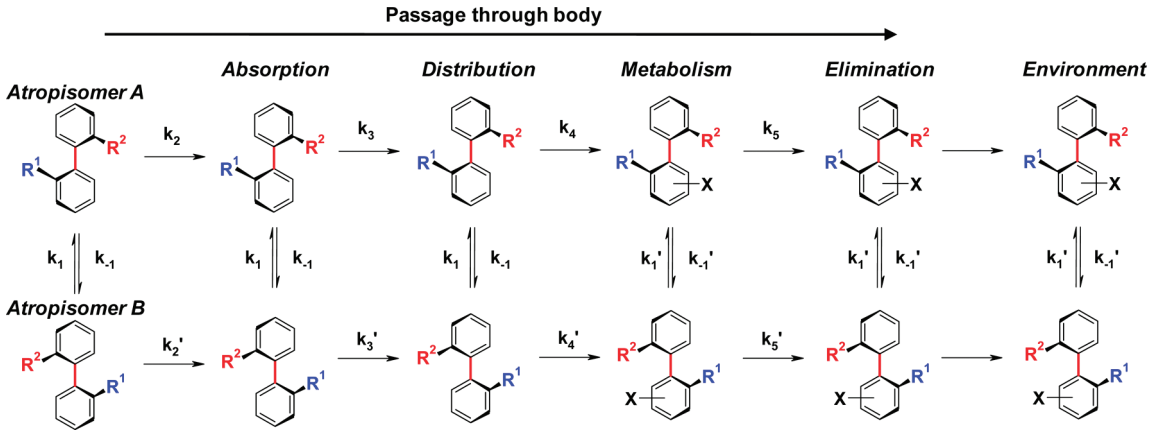
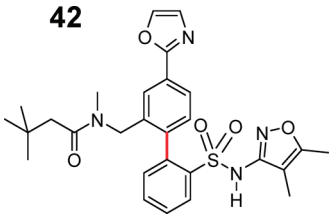


Figure 9. Atropisomer compounds should have addition equilibria and rotation rates that may need to be considered for dosing patients with stable and safe drug substances. The chiral axes are colored red.

Scheme 9. Compound 42 Atropisomer[a]



[a] The hindered axis of rotation is colored as a red bond.

Compounds that have slower interconversion rates within class 2 (red zone, Figure 4) should be monitored more carefully, as such compounds may be more problematic. The dosed mixture may not remain constant, as ADMET could result in predominating k_{2-5} equilibria compared to $k_{1,-1}$. The isomer ratio of the dosed mixture may not remain constant, as the k_1 and k_{-1} rate constants will likely be affected by the formation of metabolites and possibly influenced by membrane transport, protein binding, and other in vivo effects. This being stated, class 2 atropisomeric compounds can be developed, but one should consider racemization rates, as this influences the goal of delivering stable drug substances (vide supra).

Because of the rapid interconversion of class 2 atropisomers, dosing as a mixture is justifiable, as they are expected to maintain a relatively consistent enantiomer/diastereoisomer ratio. An exemplary study was reported by Zhou et al. for the development of the class 2 atropisomeric compound BMS-207940 (**42**) (Scheme 9),[38] which is a potent endothelin receptor antagonist and was a development candidate for the treatment of congestive heart failure. Zhou and co-workers found that the $t_{1/2}$ for interconversion of compound **39** atropisomers varied depending on the medium, noting a 30-fold decrease in the half-life in going from aqueous medium to human plasma, and even detected racemization in the crystalline form.[38] The BMS team monitored the atropisomer racemization rates for **42** and found that the half-life in aqueous medium was 15.8 h. However, the half-life of ~2.5 h for interconversion in human plasma was surprisingly different at 400 μg/mL and dropped to <0.1 h at 20 μg/mL. This medium and concentration dependent atropisomer stability made it extremely difficult to conduct pharmacokinetic studies of the individual atropisomers. Conversely, the pharmacokinetics of racemic **42** in humans were reasonably described by a one-compartmental model with an apparent terminal elimination $t_{1/2}$ of 15 h. On the basis of these data, the plasma exposure of each atropisomer was simulated in order to explore the differences in the rate of elimination of the individual enantiomers relative to the racemate. Examination of a range of scenarios revealed that the greatest difference possible was less than a 20% difference in atropisomer ratio. This finding suggested that it was appropriate to develop **42** in analogy to a classical racemic mixture, since in vivo this compound behaved as a mixture of interconverting atropisomers. Moreover, these results helped to further clarify the boundary between rates of interconversion of conformers versus quasi-stable atropisomers by establishing a dependence on both the intrinsic barrier to interconversion in vivo and pharmacological elimination of each conformational isomer.

Another consideration when developing atropisomers is the numerous reports that have demonstrated differences in target binding specificities for atropisomeric pairs. An interesting example of this was reported by scientists from GlaxoSmithKline.[39] A relatively new area of HIV therapy centered on inhibiting the C−C motif chemokine type 5 receptor (CCR5) antagonists, which helps prevent entry of HIV virions into host cells. However, antagonism of host CCR5 function has been implicated in the literature as a possible cause of adverse events in clinical studies involving CCR5 antagonistic antiretrovirals.[40] Kenakin and co-workers introduced the concept of "function sparing" in this context by showing that current CCR5 antagonists in development, or on the market, show a striking range of selectivity for antiviral potency over CCL3L1 induced CCR5 internalization.[10] A number of these molecules possess a chiral axis exemplified by the red bond, shown on compound **8**, GSK214096 (Figure 2). From their medicinal chemistry program, Kazmierski and co-workers described a compound related to those in development or on the market whose atropisomers were separable and stable owing to a high barrier of interconversion.[41] The authors demonstrated that one atropisomer was 67-fold more potent in an HIV antiviral assay (HOS cells) relative to CCL3L1-mediated chemotaxis while the other showed little selectivity for CCR5 antagonism over chemotaxis, suggesting a clear path toward improved clinical safety for this class of antiretrovirals. Given the link between CCR5 antagonism to adverse drug events, this example underscores the need to evaluate atropisomerism at an early stage of their discovery, especially since the

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distomeric atropisomer of a pair can increase the potential for toxicity or a reduced saftey profile.

Finally, atropisomer pairs are also likely to have distinct physicochemical properties (e.g., solubility, protein binding, etc.).[42] This, in turn, could affect preclinical studies that require compounds to achieve sufficiently high exposure levels of the drug substance to demonstrate upper toxicity limits. The issue of polychlorobiphenyls (PCB),[43,44] a class of persistent environmental pollutants that has been intensively studied for nearly 40 years, continues to provide toxicity data regarding atropisomer properties in vivo. A number of toxicokinetic studies have been published for PCBs that qualify as class 3 atropisomers (vide infra). These studies have clearly demonstrated that stable atropisomeric PCBs, which have been manufactured as racemic mixtures, are metabolized and eliminated enantioselectively and have been enriched enantioselectively in a variety of biological matrices including soil, aquatic environments, and a wide variety of marine and terrestrial animal species and organs. The knowledge of this atroposelective bioconversion is relevant to human pharmacokinetic study of atropisomeric drugs.

This highlights that the environmental impact of atropisomers should also be considered. Besides the usual ADMET concerns, which focus on the unidirectional flow of drug substance from administration to excretion, there is a serious potential for the environment and subsequent human exposure. For example, a wealth of information has been reported regarding the environmental persistence and toxicology of PCBs.[43] Because of their remarkable stability in the environment, these xenobiotics have accumulated in a wide variety of animal species because of their propensity to be distributed in adipose tissue. This class of molecules has also been the subject of a variety of toxicological studies and is considered a major risk to human health.[44] Among other findings, it was observed that atropisomers of a pair can separately accumulate in different tissues and organs.

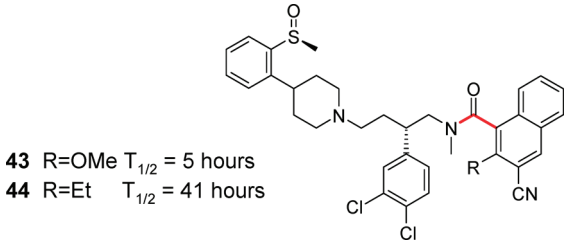
The issues of atropisomers and metabolites with the environment are not limited to the PCBs. For example, compound **28** is a nonionic iodinated contrast medium used in diagnostic imaging procedures.[29] We computationally flagged this compound in a database search as having atropisomer properties (vide supra), and atropisomers have been observed for it and related drugs.[30] Surprisingly, it exists as a drinking water contaminant from patient elimination and excretion[45] and represents environmental contamination issues as noted for other drugs and metabolites (e.g., estrogen, etc.).

■ ATROPISOMER DRUG SUBSTANCE PRODUCTION, CRYSTALLIZATION, AND FORMULATION

Once a final compound of interest is identified, it must then advance through chemical and clinical development to market approval. The stage of chemical development serves a critical role as a bridge between discovery of the pharmaceutically active molecule to the ultimate manufacture and launch of a drug substance. In this capacity, chemical development is responsible for defining and developing a safe, robust, and economical synthetic process for the production of high-quality drug substances to meet preclinical and clinical needs, as well as the requirements of the NDA/FDA filings and launch.

Given the significant time and costs of clinical trials, it is critical that a reliable and sustainable decision be made during the drug discovery stage to develop an atropisomeric drug either as a single compound or as a mixture. The cross-discipline guide in Figure 4 can help communicate the intended decision and

Scheme 10. Structures and Atropisomer Half-Lives for 43 and 44



reasoning to colleagues in chemical development and in medicine, keeping in mind the appropriate precautions. In addition, with the added information on the kinetics of isomerization of the atropisomeric chiral element, appropriate design of the manufacturing processes can be initiated to avoid costly delays in supplying drug substance for clinical and market needs. As some processes, such as high temperature crystallization, can erode the atropisomeric purity, a batch could then become "out of specification" if this issue is not anticipated early.

Class 3 compounds ($\Delta E_{rot} \gg 30$ kcal/mol) experience very slow torsion−rotation rates ($t_{1/2}$) on the order of years (Figure 4). These chiral compounds are stable over time and can be isolated optically pure and stored in a pharmaceutically relevant formulation for a period of time consistent with a required shelf life. One can envisage developing a class 3 atropisomeric drug as a single, pure, stereochemically stable isomer, such as a single enantiomer or diastereoisomer. The issue of atropisomerism can be considered as another stereochemical element of these compounds in which the single atropisomer can be produced by asymmetric synthesis,[46] resolved by preparative scale chromatography (SMB[47]) or crystallographic separation with the use of chiral auxiliaries[48] or the formation of diastereoisomeric salts.[49] If an additional stereochemical element is present, it is also possible to selectively crystallize one diastereoisomer.[50] Depending on the barrier of rotation of the atropisomer and the level of thermal equilibration, a dynamic resolution[51] may be feasible. Overall, the issue of atropisomerism can be treated like a specification issue similar to diastereoisomers or enantiomers of chiral centers such that the required drug substance is supplied with the requisite atropisomeric purity and stability. It is noted that procedures requiring high temperatures (e.g., crystallization) may result in some racemization. Also, it would be prudent to monitor the ADMET properties on sample animal models and/or clinical patients to verify the absence of unanticipated racemization.

Class 2 atropisomeric compounds are those that experience moderate rates of axial interconversion. In general, they have $t_{1/2}$ in the range of minutes, days, or months, which qualitatively corresponds to rotational energy barriers (ΔE_{rot}) between 20 and 30 kcal/mol at ambient temperature (Figure 4). The interconversion properties of class 2 atropisomeric compounds make them challenging to develop as drug substances, which should ideally be consistent and stable both for production purposes and to elicit only the desired effect in the human body. Racemization can occur during production, assays, analytical characterization, storage, and in vivo. Given that this can lead to inconsistencies in the safety and efficacy of the drug, it would be prudent to develop class 2 atropisomers as stereoisomer mixtures, as long as the ratio of atropisomers is constant (at equilibrium state) and the toxicological profile is acceptable.

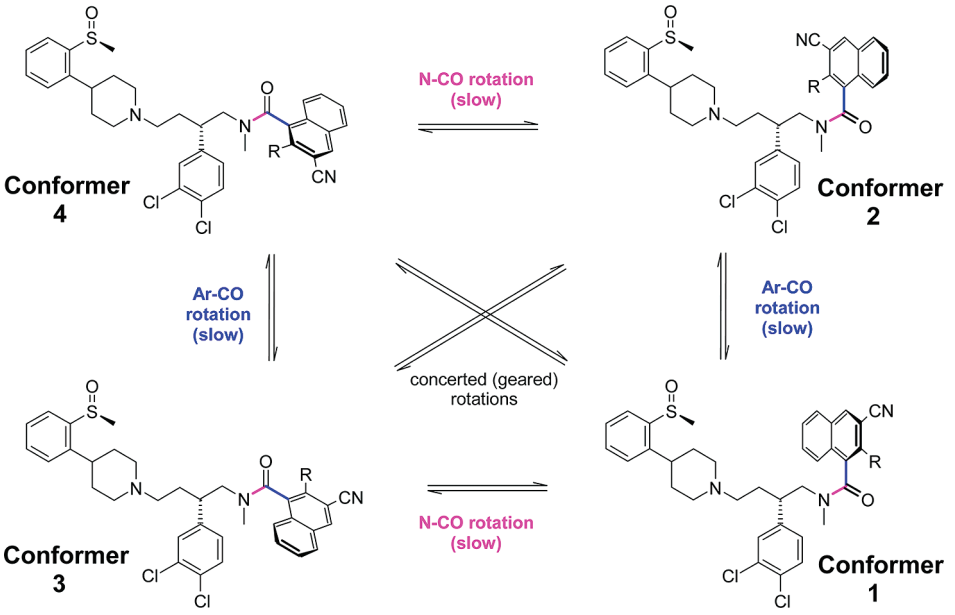


Figure 10. Conformers and atropisomers of **43** and **44**. Bonds are colored in blue for Ar−CO and magenta for N−CO bonds.

It is important to note that class 2 compounds can be further separated into two subclasses. Class 2 compounds with lower barriers to rotation (e.g., the lower yellow and green regions of Figure 4) can be expected to racemize rapidly in vivo relative to the elimination rates, so one can expect a consistent stereoisomeric mixture in vivo. The other subclass can be defined as compounds for which $t_{1/2}$ of rotamer interconversion is of the same order of magnitude as, or less than, the half-life of in vivo elimination. Differential elimination of the atropisomers can lead to an undesirable imbalance of the concentrations of the atropisomers. Thus, experimental measures should be in place to detect any potential imbalance. It is also noted that there are reports where $t_{1/2}$ for interconversion of the atropisomers varied depending on the medium; e.g., a 30-fold decrease in the half-life was observed in going from aqueous medium to human plasma.[38]

With regard to chemical development and the first subclass (faster interconversion), the crystallographic consequences are negligible, as the effect of atropisomers is reduced to another low energy barrier conformational element of the compound. The other class 2 subclass denoted by the red region in Figure 4 (e.g., half-lives on the order of days and longer) poses an important challenge for drug development. From a physiological standpoint, these compounds are developed as a mixture of both atropisomeric elements (either enantiomeric or diastereoisomeric). Additionally, efforts for the independent synthesis or separation of atropisomers are not appropriate, as preservation of the single or enriched atropisomer is difficult and interconversion would occur at ambient conditions. Consequences for the higher barrier of rotation for these compounds can be found in both the analytics and the crystal/polymorph forms of the substances.[52] Due care needs to be taken to control the crystallization of these substances in order to consistently produce the desired polymorph,[53] which in turn is dependent on the atropisomeric ratios. Also, chiral formulations may perturb atropisomeric ratios.

A notable example of a class 2 compound in clinical development was reported by Parker and co-workers in the Department of Process Research and Development of AstraZeneca, during the initial development work on the neurokinin antagonist

Table 1. Salts and Corresponding Conformer Distribution for ZD4974 (43)

salt	% conformer 1	% conformer 2	% conformer 3	% conformer 4
citrate (amorphous)	6.0	13.0	53.0	28.0
citrate (crystalline)	1.1	1.5	89.2	8.3
malonate	0.9	5.8	93.3	0

ZM374979 (**44**).[54] Two structurally related derivatives (Scheme 10) differ with respect to the substitution on the appended amide aryl fragment, in which the methoxy substituent ZD4974 (**43**) has a rotational half-life of ∼5 h while the corresponding ethyl derivative **44** has a half-life of ∼41 h. Because of the high rotation barrier for the amide bond, as well as the amide−aryl axis (atropisomers), four distinct conformers are present and for **44** these conformers are separable by HPLC (Figure 10).

As the rotation barrier and thus rotation half-life are relatively low for compound **43**, the atropisomeric composition is irrelevant, as interconversion would occur in vivo and revert to the solution equilibrium composition. As such, the development work for **43** was focused on providing material with acceptable filtration properties. Initial formation of the citrate salt of **43** provided an amorphous material with a conformer distribution similar to the solution distribution (Table 1). However, the corresponding crystalline citrate, malonate, and fumarate salts were produced which enriched conformer 3. The citrate salt was selected for initial delivery, and 1.77 kg of **43** citrate salt was produced in 66% yield. As demonstrated in this example for the filtration rate of **43**, the criteria for favorable solid state properties (e.g., filtration rate, solubility, and polymorphism) are the sole drivers for development of material with a specified atropisomeric ratio for low barrier atropisomers (faster class 2 compounds). The solid state properties can then be attributed to atropisomeric ratios, which then would define conformations within in the crystal lattice and thus the polymorph of the drug substance and/or intermediate.

Compound **44** possess a higher barrier of rotation, and as the half-life is ~41 h, it would be considered as a class 2 compound (as defined previously). However, conformer 4 of **44** was determined as the active atropisomer, and as such, development efforts were extended by Parker and co-workers in developing a scalable process that did not rely on chromatography. The issue of atropisomerism presented two challenges for the development of this compound: first, consistent ratios of the conformers were required for the screening/development phase of the crystallization, in order to acquire meaningful data, and second, a robust and consistent process needed to be developed for the crystallization. As the compound is thermally equilibrated to a 1:1:4:4 ratio (conformer 1/conformer 2/conformer 3/conformer 4), heating the compound at 60 °C for 48 h accomplished the goal of providing material with consistent conformer ratios for screening, and subsequently the maleate salt was chosen for development. In order to develop a robust process, seeding was found to be important, which allowed the formation of smaller crystals and improved atropisomeric ratios. Additionally, Parker and co-workers also found that the amount of maleic acid, the isolation temperature, and antisolvent amounts were important parameters in order to isolate material with high selectivity for the desired conformer. From these efforts, they were able to produce a 23% yield (58% recovery of the available conformer 4 in solution at equilibrium) of **44** maleate rotamer 4 in >97% purity. Parker and co-workers demonstrated this process on 830 g of **44** and isolated 237 g of **44** maleate rotamer 4 with >97% purity, thus supplying the required drug substance for delivery. They also demonstrated that recycling the **44** maleate from the mother liquid is possible by first heating the material at 60 °C for 72 h, to form the equilibrium ratio of conformers. After adjusting the solvent volume and crystallization, they were able to obtain a 23% yield of **44** maleate conformer 4 with >96% purity. The combined processes represent a 40% yield overall. No further work was presented by the authors because the project was put on hold. The further development of this isolation process into a crystallographic thermal dynamic resolution would be the ideal process for compounds of this nature, with thermally assessible interconversion barriers.

CONCLUSIONS

It is apparent that atropisomer chirality can have a significant impact on drug discovery and so must be managed appropriately. Here, we illustrate some interesting features and properties of atropisomers to point out that conformational chirality should be treated as seriously as atom-centered chirality, with additional considerations as proposed herein. The first step in dealing with this often-overlooked phenomenon would be to recognize its existence for compounds of interest. To help with this process, a variety of chemotypes are displayed in the main text. Furthermore, strategies for detecting and computationally revealing atropisomers are discussed. Once identified, medicinal chemists have multiple options that range from avoiding atropisomerism to designing compounds that rotate more quickly or more slowly, with the goal of preparing for development options. A categorization scheme is proposed as a guide to help bridge the efforts of chemists at the early drug discovery stages with later efforts of development scientists. Overall, this Perspective assesses atropisomerism on drug discovery, development, and delivery and espouses the view that atropisomeric compounds can be successfully developed with caution and proper management.

AUTHOR INFORMATION

Corresponding Author

*For S.R.L.: phone, 1-450-682-4640; fax, 1-450-682-8434; e-mail, steven.laplante@boehringer-ingelheim.com. For P.J.E.: phone, 1-450-682-4640; e-mail, paul.edwards@boehringer-ingelheim.com.

BIOGRAPHIES

Steven R. LaPlante obtained his Ph.D. degree (1988) in Biophysics at Syracuse University, NY, under the direction of Prof. Philip Borer and Prof. George Levy, where he developed NMR methods to monitor dynamic and structural properties of DNA oligonucleotides. He then pursued postdoctoral studies at the Ecole Polytechnique and the CNRS in France under Prof. Jean-Yves Lallemand, where he solved protein solution structures of cobra and scorpion toxins. Since 1991, he has been at Boehringer Ingelheim (Canada) Ltd., where his research has focused on developing and applying practical tools for drug discovery and design. His interdisciplinary approach exploits NMR, X-ray, and computational information for drug design purposes. Other areas of interest include the characterization of compound atropisomerism and aggregation.

Lee D. Fader obtained his B.Sc. in Chemistry from Concordia University, Canada. Following his undergraduate studies, he moved to McGill University, Canada, to pursue his Ph.D. degree under the supervision of Youla Tsantrizos, where he worked on the design, synthesis, and biophysical characterization of aromatic peptide nucleic acids. He then moved to the Swiss Federal Institute of Technology, Switzerland, as an NSERC Postdoctoral Fellow with Erick Carreira, where he focused on the application of nitrile oxide cycloadditions to natural product synthesis. In 2004 he joined Boehringer Ingelheim (Canada) Ltd. in the Department of Medicinal Chemistry, where he continues to focus on the discovery of novel small molecule antiviral agents.

Keith R. Fandrick obtained a B.S. degree in Chemistry from the University of California at San Diego. During his undergraduate studies at UCSD, he worked at the Human BioMolecular Research Institute, CA, under Dr. John Cashman, synthesizing and studying pharmacologically active amine serotonin reuptake inhibitors. Keith then went to Harvard University, MA, to pursue his doctoral degree under the guidance of Prof. David Evans, where he developed asymmetric catalytic Friedel–Crafts reactions catalyzed by scandium–PyBox complexes. After obtaining his Ph.D. degree, he joined the process development group at Boehringer-Ingelheim Pharmaceutical Inc. in Ridgefield, CT, where he developed safe, robust, and scalable processes to supply necessary drug substance. His interest includes developing asymmetric catalytic methodologies and total synthesis.

Daniel R. Fandrick earned his B.S. degree from the University of California at San Diego. His interest in organic chemistry originated in the laboratory of Joseph O'Conner, where he contributed to the synthesis of a ferrocenylendiyne complex. During his undergraduate studies, Daniel was employed at the Torrey Pines Institute for Molecular Studies, CA, and later at Corvas International, CA. Daniel Fandrick then joined the Trost Group at Stanford University, CA. His work focused on the total synthesis of lasonolide A and development of novel dynamic kinetic asymmetric transformations of allenes and vinylaziridines. After obtaining his Ph.D. degree in 2006, Daniel Fandrick assumed a Senior Scientist position in the Department of Chemical Development at Boehringer Ingelheim Pharmaceutical Inc. (Ridgefield, CT).

Oliver Hucke received his Ph.D. from the Institute of Physical Chemistry of the University of Freiburg, Germany (2002), where he worked on membrane proteins of photosynthesis (reaction centers) using molecular modeling in combination with transient spectroscopy. As a fellow of the Leopoldina (German National Academy of Sciences), he then joined the Biomolecular Structure Center of the University of Washington in Seattle, U.S., to take on molecular modeling responsibilities in a drug discovery project focused on tropical diseases. In 2005 he moved to Boehringer Ingelheim (Canada) Ltd., where he now holds a position as Senior Research Scientist. His work covers various computational aspects of drug discovery, encompassing structure- and ligand-based approaches, as well as QSAR and chemoinformatics.

Ray Kemper received his doctorate in Pharmacology and Toxicology from the University of Louisville, KY, in 1995 and undertook postdoctoral research at the University of Wisconsin. Ray currently heads the Cross Project Screening and Computational Toxicology lab in the Integrative Toxicology section at Boehringer Ingelheim Pharmaceutical Inc. in Ridgefield, CT, where he is responsible for project-independent toxicity screening and other early safety support for projects in the Discovery pipeline. Prior to joining Boehringer Ingelheim Pharmaceutical Inc. in 2005, Ray led the Discovery Toxicology Group at DuPont Haskell Laboratory from 2003 to 2005 and held a position in the Biochemical Toxicology Group at DuPont from 1999 to 2003. Ray earned his certification in General Toxicology from the American Board of Toxicology in 2006.

Stephen P. F. Miller received his Ph.D. in Organic Chemistry from the University of Wisconsin at Madison (1983). After a postdoctoral appointment at the Department of Pharmaceutical Chemistry in Madison (1984), he joined the National Institutes of Health (1985−1993). Stephen joined the FDA in 1994, and in his current position with the FDA's Office of New Drug Quality Assessment, he focuses on the chemistry, manufacturing, and controls (CMC) data for antiviral drugs that are entering clinical trials in the U.S. (IND applications) or that have reached the marketing application (NDA) stage. His current professional interests include the regulatory aspects of drug substance manufacture and quality control, with particular emphasis on issues related to polymorphism, stereochemistry, and mutagenic impurities.

Paul J. Edwards obtained his Ph.D. degree from the University of Leicester in the United Kingdom (1993), working with Dr. Robert S. Atkinson. He then pursued postdoctoral studies, first as a SmithKline Beecham Postdoctoral Fellow with Regents' Professor Paul G. Gassman at the University of Minnesota, U.S., then as a Postdoctoral Fellow with Professor Steven V. Ley, FRS, at the University of Cambridge in the United Kingdom. His industrial employment includes working at Oxford Diversity and then Pfizer in the United Kingdom, followed by employment as Director of Medicinal Chemistry for Santhera Pharmaceuticals (Heidelberg, Germany) and then as Director and Head of Chemistry for Galapagos (Belgium). Currently he is Vice President of Chemistry for Boehringer Ingelheim (Canada) Ltd. and is based in Quebec, Canada.

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■ DISCLOSURE

The views expressed are those of the authors and do not reflect the official views of the FDA.

■ ABBREVIATIONS USED

ADMET, absorption, distribution, metabolism, excretion, and toxicity; ADMET(E), absorption, distribution, metabolism, excretion, and toxicity (environment); Calc ΔE_{rot}, computationally calculated torsion angle rotation energy barrier in kcal/mol; ΔE_{rot}, torsion angle rotation energy barrier in kcal/mol; NCE, new chemical entities; DMSO, dimethyl sulfoxide; EXSY, nuclear magnetic exchange spectroscopy; FDA, U.S. Food and Drug Administration; HIV, human immunodeficiency virus; HPLC, high-pressure liquid chromatography; NDA, new drug application; NMR, nuclear magnetic resonance; PCB, polychlorobiphenyl; ROESY, rotating-frame exchange spectroscopy; *S* and *R* enantiomers, enantiomers resulting from *S* and *R* chiral centers; S_a and R_a enantiomers, *S* and *R* chiral entities resulting from axial chirality; SAR, structure−activity relationship; SMB, preparative scale chromatography; *T*, temperature; $T_{1/2}$ and $t_{1/2}$, half-lives of atropisomer interconversion

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