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► **To cite this version:**

Marlène Damin-Pernik, Bernadette Espana, Sébastien Lefebvre, Isabelle Fourel, Hervé Caruel, et al.. Management of Rodent Populations by Anticoagulant Rodenticides: Toward Third-Generation Anticoagulant Rodenticides. *Drug Metabolism and Disposition*, 2017, 45 (2), pp.160-165. 10.1124/dmd.116.073791 . hal-01564909

HAL Id: hal-01564909

<https://hal.science/hal-01564909>

Submitted on 19 Jul 2017

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DMD # 73791

**Management of rodent populations by anticoagulant rodenticides: Toward
third-generation anticoagulant rodenticides**

MARLÈNE DAMIN-PERNIK, BERNADETTE ESPANA, SEBASTIEN LEFEBVRE, ISABELLE
FOUREL, HERVÉ CARUEL, ETIENNE BENOIT, VIRGINIE LATTARD

USC 1233 RS2GP, Univ Lyon, VetAgro Sup, INRA, F-69280, MARCY L'ETOILE, France
(M.D.P, B.E, S.L, I.F, E.B, V.L)

Liphatech, Bonnel, 47480 Pont du Casse, France (M.D.P, H.C)

Running Title : Toward third-generation anticoagulant rodenticides

*Corresponding author: Virginie Lattard

USC 1233 INRA-VetAgro Sup 69280 Marcy l'Etoile, France

Email: Virginie.lattard@vetagro-sup.fr; Phone: +33(0)4 78 87 27 27; Fax: +33(0)4 78 87 05

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The number of text pages: 26

Number of tables: 3

Number of figures: 6

Number of references: 40

Number of words in the

- Abstract: 200

- Introduction: 740

- Discussion: 1163

List of nonstandard abbreviations:

AR, anticoagulant rodenticides

FGAR, first-generation anticoagulant rodenticides

SGAR, second-generation anticoagulant rodenticides

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LOQ, limit of quantification

VKORC1, vitamin K epoxide reductase enzyme

ABSTRACT

Second-generation anticoagulant rodenticides (SGARs) have been used since the 1980s for pest management. They are highly efficient even in warfarin-resistant rodents. Nevertheless, because of their tissue persistence, non-target poisoning by SGARs is commonly described in wildlife. Due to this major problem, a new generation of anticoagulants must be developed to limit this risk. This study proposes a method of developing a new generation of anticoagulant rodenticides by re-visiting the old SGARs based on the concept of stereochemistry. Each current SGAR is a mixture of diastereomers. Diastereomers of each compound were purified, and their biological properties were compared by determining their ability to inhibit vitamin K epoxide reductase (VKOR) activity involved in the activation of vitamin K-dependent clotting factors and their toxicokinetic properties. Systematically, for each SGAR, both diastereomers are as effective in inhibiting VKOR activity. However, their toxicokinetic properties are very different, with one of the two diastereomers always more rapidly cleared than the other one. For all SGARs except flocoumafen, the less persistent diastereomer is always the less predominant isomer present in the current mixture. Therefore, the development of baits containing only the less persistent diastereomer would avoid the ecotoxicological risk associated with their use without decreasing their efficacy.

INTRODUCTION

Managing populations of commensal rodents is essential. By sharing our environment, they can transmit to humans, directly or indirectly, more than 40 zoonotic pathogens, such as *Yersinia pestis*, Hantavirus, and *Leptospirosis* sp. (Buckle and Smith 2015). They can cause the destruction or degradation of cereals, accounting for nearly 10% of the grain crops in the world, with considerable variation depending on the country (Buckle and Smith 2015) (Battersby 2004). Rodents also damage substructures and electronic networks.

The management of rodent populations should be based on sanitary and architectural approaches. However, these approaches are often widely associated with the use of rodenticides. The sophisticated social organization of the populations of rats and neophobic behavior of these animals make the action of toxicity with an immediate effect completely ineffective. The only really effective molecules that can be used to manage such populations are anticoagulant rodenticides (ARs). Their delayed action, with mortality occurring several days after consumption, makes them effective in controlling neophobic species. Indeed, ARs, by inhibiting the VKORC1-dependent vitamin K epoxide reductase activity, lead to the progressive reduction of the pool of vitamin K necessary for the activation of clotting factors II, VII, IX, and X (Suttie 1985; Furie and Furie 1988). Therefore, prolonged or repeated exposure to ARs leads to rodent death by hemorrhage within 3 to 7 days. ARs are usually classified into two generations. The first generation of ARs includes diphacinone, warfarin, coumatetralyl, and chlorophacinone. The second generation of ARs includes bromadiolone, difenacoum, brodifacoum, flocoumafen, and difethialone. All ARs are 4-hydroxycoumarin derivatives except chlorophacinone and diphacinone, which is a derivative of indane-1,3-dione (Hadler and Buckle 1992).

First-generation ARs (FGARs) generally require several days to cause rodent death. The activity of these FGARs is now largely limited by the genetic resistance phenomenon. The

first case of resistance to FGARs in brown rats was observed in 1958 in the United Kingdom (Boyle 1960). In the following years, resistance was reported everywhere in rats and mice in Europe (Dodsworth 1961), the United States (Jackson and Kaukeinen 1972), Canada (Siddiq and Blaine 1982), Australia (Saunders 1978), and Japan. The most common resistance mechanism is a result of single-nucleotide polymorphisms (SNPs) in the *Vkorc1* gene, leading to an enzyme that is less sensitive to the action of ARs (Li et al. 2004; Rost et al. 2004).

The development of second-generation ARs (SGARs) resulted from the emergence of resistance to FGARs in rodent populations. SGARs have been registered from 1975 to 1984, with difenacoum being used first, followed by brodifacoum, bromadiolone, difethialone, and flocoumafen. They are more potent and active after only one feeding due to their longer half-life (Vandenbroucke et al. 2008). Nevertheless, due to their increased tissue persistency, non-target poisoning by SGARs is commonly described in wildlife. Different species are affected by this exposition, such as stoats, weasels (Elmeros et al. 2011), red foxes (Sage et al. 2010; Geduhn et al. 2015), European minks (Fournier-Chambrillon et al. 2004), bobcats (Serieys et al. 2015), various raptors such as red kite, buzzards, kestrels, peregrine falcons and eagle owls (Thomas et al. 2011; Christensen et al. 2012; Hughes et al. 2013; Langford et al. 2013; Ruiz-Suárez et al. 2014). ARs were thus identified by the European Union as candidates for future comparative risk assessment and substitution. However, in the absence of an alternative, AR molecules were included in Annex 1 of the EU Biocidal Products Directive 98/8/EC, and their use is still tolerated until a more appropriate solution is found.

This study proposes a method of developing a new generation of ARs by improving the old SGARs based on the concept of stereochemistry. A previous study (Damin-Pernik et al. 2016) demonstrated that difenacoum, the first developed SGAR, is a mixture of two diastereomeric forms, namely, 43% of the composition is trans-isomers, with a short half-life and 57% of the composition is cis-isomers, with a long half-life. The production of a new difenacoum

containing exclusively trans-isomers could significantly reduce its tissue persistency and thus avoid secondary poisoning associated with its use. This method of producing an ecofriendly difenacoum may be applicable to all SGARs. Indeed, all SGARs have two asymmetric carbons (Kelly et al. 1993; Buckle and Smith 2015). Therefore, all SGARs are a mixture of two diastereomeric forms that could have different biological properties. This study aimed to explore the differences in the biological properties of the diastereomers of SGARs and to propose the best diastereomer of each SGAR mixture to use as a rodenticide.

MATERIALS AND METHODS

Chemicals

Bromadiolone (3-[3-[4-(4-bromophenyl)phenyl]-3-hydroxy-1-phenylpropyl]-4-hydroxychromen-2-one) and difethialone (3-[3-[4-(4-bromophenyl)phenyl]-1,2,3,4-tetrahydronaphthalen-1-yl]-2-hydroxythiochromen-4-one) were supplied by Liphatech (Agen, France). Difenacoum (2-hydroxy-3-[3-(4-phenylphenyl)-1,2,3,4-tetrahydronaphthalen-1-yl]chromen-4-one), brodifacoum (3-[3-[4-(4-bromophenyl)phenyl]-1,2,3,4-tetrahydronaphthalen-1-yl]-4-hydroxychromen-2-one), and flocoumafen (4-hydroxy-3-[3-[4-[[4-(trifluoromethyl)phenyl]methoxy]phenyl]-1,2,3,4-tetrahydronaphthalen-1-yl]chromen-2-one) were purchased from Hangzhou Ich Biofarm Co. (China).

(1R,3R)(1S,3S)-isomers (called trans-isomers) and (1R,3S)(1S,3R)-isomers (called cis-isomers) of difenacoum, difethialone, flocoumafen, and brodifacoum were separated and purified in a silica gel column in our laboratory and assigned according to information available in published articles (Damin-Pernik et al, 2016), regulatory documents (European Parliament, 2007, 2009) and patents (Caruel et al, 2015). (1R,3S)(1S,3R)-isomers (called trans-isomers) and (1R,3R)(1S,3S)-isomers (called cis-isomers) of bromadiolone were also separated and purified in a silica gel column in our laboratory. Dimethyl sulfoxide (DMSO), acetonitrile, methanol, acetone, diethyl ether, hexane, and orthophosphoric acid were obtained from VWR International (Fontenay sous bois, France), and Vetflurane[®] and vitamin K1 were obtained from Alcyon (Miribel, France). Vitamin K1 was converted to vitamin K epoxide according to the method described by Tishler et al. (Tishler et al. 1940). Purity was estimated by LC/MS and was higher than 99%. High-performance liquid chromatography (HPLC)-grade water, which was prepared using a milli-Q plus system (Millipore, Saint-Quentin en Yvelines, France), was used for the preparation of the HPLC eluents.

Animals

Eight-week-old male OFA Sprague-Dawley rats (each weighing 175-200 g) were obtained from a commercial breeder (Charles Rivers, l'Arbresle, France) and were acclimated for a minimum period of 5 days. The rats were housed four per cage under a constant photoperiod and ambient temperature. The animals were kept in standard cages (Eurostandard, Type IV, Tecniplast, Limonest, France) and received standard feed (Scientific Animal Food and Engineering, reference A04) and water *ad libitum*. Experimental research on the rats was performed according to an experimental protocol following international guidelines and with approval from the ethics committee of the Veterinary School of Lyon.

Pharmacokinetic study

Male OFA Sprague-Dawley rats received, by *per os* administration, cis- or trans-isomers of difenacoum, bromadiolone, flocoumafen, brodifacoum, and difethialone. Anticoagulants were dissolved in 10% DMSO and 90% vegetable oil and were administered by force feeding. The animals were given a daily subcutaneous injection of vitamin K1 (5 mg/kg). Finally, the rats were anesthetized with Vetflurane[®]. Blood samples were obtained via terminal blood collection by cardiac puncture in citrated tubes. The rats were euthanized with CO₂, and the livers were then collected and stored at -20°C until analysis.

No-choice feeding test with cis- or trans-isomers of bromadiolone and difethialone

Before testing, all of the animals were maintained on diet A04 (Scientific Animal Food and Engineering) and water *ad libitum*. Individuals were weighed before the start of the test. No-choice feeding tests were performed on groups of 10 rats. The rats were fed bait containing 0.005% cis- or trans-isomers of bromadiolone or 0.0015% cis- or trans-isomers of difethialone. Cis- and trans-isomers of bromadiolone and of difethialone are pure at 93, 98,

99, and 94%, respectively (the remaining part is the other stereoisomer). Feeding periods of 4 days were used. The contents of food pots were weighed daily, and the intake of bait was recorded. The mortality of rats was also observed daily. At the end of the feeding period with toxic baits, the animals were given diet A04 again and were observed for an additional period of 17 days.

Determination of AR concentration in the liver

Liver tissue (1 g) was extracted with acetone using an Ultra Turrax tissue disperser from IKA Labortechnik® (VWR International, Strasbourg, France) according to the method previously published by Damin-Pernik et al (2016) and validated according to the guideline on Bioanalytical Method Validation published by the European Medicines Agency (2011). The extract was centrifuged at 3000 rpm for 10 min, and the supernatant was evaporated at 60°C under a gentle nitrogen flow. Dry extract was resuspended in acetonitrile/hexane (50%/50%). The hexane layer was eliminated, and the remainder was dried at 60°C under a gentle nitrogen flow. The final dry extract was dissolved in methanol, and the AR concentrations were analyzed by HPLC on a reverse phase C-18 column (4.6×150 mm, 5 µm; Waters, Milford, MA, USA) at detection wavelengths of 258, 260, 265, 267, and 272 nm for difenacoum, difethialone, bromadiolone, brodifacoum, and flocoumafen, respectively. The C-18 column was heated at 40°C for difenacoum, bromadiolone, flocoumafen, and brodifacoum and at 30°C for difethialone. A gradient elution system was used with a flow rate of 1 mL/min as follows: from 30% methanol/70% water (acidified with 0.2% H₃PO₄) to 70% methanol/30% water at 10 min for difenacoum; from 30% acetonitrile/70% water (acidified with 0.1% H₃PO₄) to 90% acetonitrile/10% water at 14 min for brodifacoum; from 60% methanol/40% water (acidified with 0.2% H₃PO₄) to 80% methanol/20% water at 20 min for bromadiolone; from 60% methanol/40% water (acidified with 0.2% H₃PO₄) to 90% methanol/10% water at

22 min for flocoumafen; and from 60% acetonitrile/40% water (acidified with 0.2% H₃PO₄) to 75% acetonitrile/25% water at 10 min for difethialone. The recovery rate of diastereomers of SGAR from tissues were between 75 to 95 % in liver and the CV% values for precision of the different ARs were less than 15%.

VKOR activity assay and kinetics

Liver microsomes were prepared from fresh livers by differential centrifugation according to the protocol described by Hodroge et al. (2011). Microsomal vitamin K epoxide reductase (VKOR) activity was assayed according to the protocol described by Hodroge et al. (2011, 2012). The inhibiting effect of the silica gel column-purified cis- or trans-isomers of ARs was evaluated by the determination of K_i after the addition of various concentrations of the anticoagulant to the standard reaction in the presence of increasing amounts of vitamin K epoxide (from 0.001 to 0.2 mM) using anticoagulant concentrations from approximately 0.05 to 20 × K_i.

Data analysis

Pharmacokinetic calculations were performed using the noncompartmental approach on the mean results per group. The elimination half-life ($t_{1/2(e)}$) was calculated using linear regression in GraphPad Prism 6 software (California, USA). The total area under the curve (AUC) was calculated using the linear trapezoidal method and adding the estimated terminal portion of the curve (AUC 0→∞). Statistical analysis of pharmacokinetic parameters were done using GraphPad Prism 6 software (CA, USA). A Mann-Withney test was used with $\alpha < 0.05$ in order to compare statistically the results between the two groups.

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For kinetic analysis of the VKOR activity, data were fitted by non-linear regression to the non-competitive or competitive inhibition model using GraphPad Prism 6. The choice of the best model was based on the *Corrected Akaike Information Criterion*.

RESULTS

Separation of the diastereoisomers of SGARs

The analysis of SGARs by HPLC on a C18 reverse phase column as described in the experimental procedure showed, for each compound, two different peaks corresponding to the cis- and trans-diastereomer forms. The structures of the cis- and trans-isomers of flocoumafen, difethialone, brodifacoum, bromadiolone, and difenacoum are shown in Figs. 1A, 2A, 3A, 4A, and 5A, respectively. The cis- and trans-isomers are shown in only one alternative orientation of substituents. Both peaks of each compound presented the same absorption spectrum, with maximum absorbances at 258, 265, 272, 267, and 260 nm for difenacoum, bromadiolone, flocoumafen, brodifacoum, and difethialone, respectively. The retention times, respectively, were 11.3 and 12.5 min for the cis- and trans-isomers of difenacoum, 15.0 and 16.2 min for the cis- and trans-isomers of brodifacoum, 20.1 and 20.7 min for the cis- and trans-isomers of flocoumafen, 17.1 and 17.9 min for the cis- and trans-isomers of bromadiolone, and 20.2 and 21.3 min for the cis- and trans-isomers of difethialone.

Comparative analysis of the tissue persistence of the trans- and cis- isomers of the five SGARs in the liver

To study the hepatic persistence of the trans- and cis-isomers of SGARs, male OFA Sprague-Dawley rats were divided into groups of 4 rats that received, by *per os* administration, 3.0 mg.kg⁻¹ of trans- or cis-isomers of flocoumafen, difethialone, brodifacoum, bromadiolone, or difenacoum. The rats were killed 24, 72, 168, 336, or 504 hours after the administration, and the concentrations of the trans- and cis-isomers of SGARs were determined in the liver for each rat. The time-dependent evolution of the cis- and trans-isomers in the liver is presented in Fig. 1B for flocoumafen, Fig. 2B for difethialone, Fig. 3B for brodifacoum, Fig. 4B for bromadiolone, and Fig. 5B for difenacoum.

The profile of elimination was the same for the 5 molecules studied; specifically, one isomer was eliminated more quickly than the other, and the decrease followed a clear biphasic profile. For difenacoum, difethialone, and brodifacoum, the cis-isomer was eliminated less rapidly than the trans-isomer. However, for bromadiolone and flocoumafen, the cis-isomer is eliminated more quickly. The half-lives and areas under the curve (AUCs) of the trans- and cis-isomers in the liver were calculated using a linear regression model and are presented in Table 1.

Analysis of the inhibiting effect of the trans- and cis-isomers of SGARs on vitamin K epoxide reductase activity

The ability of the cis- and trans-isomers of each SGAR to inhibit the VKOR activity catalyzed by warfarin-susceptible rat liver microsomes was evaluated by determining K_i constants. The results are presented in Table 2. The cis- and trans-isomers of each SGAR were all able to inhibit VKOR activity. For each SGAR, the K_i constants were similar between the diastereomers.

No-choice feeding test with the cis- or trans-isomers of bromadiolone or difethialone

A no-choice feeding test was performed to confirm the efficiency of baits enriched with the less persistent pair of diastereomers and to determine the benefits in term of liver residues. Assays were performed with bait containing mainly one isomer of bromadiolone or difethialone at the concentrations of 50 and 15 ppm for bromadiolone and difethialone, respectively (Table 3). Bromadiolone was chosen because it is the SGAR with the greatest difference of half-life between cis- and trans-isomers. Difethialone was chosen because it is the SGAR with the smallest difference of half-life between cis- and trans- isomers. The baits were composed of 93% cis-isomers with 7% trans-isomers or 2% cis-isomers with 98% trans-isomers for bromadiolone and 6% cis-isomers with 94% trans-isomers or 99% cis-isomers

with 1% trans-isomers for difethialone. The daily consumption of baits was similar for the first 3 days between treatments, with an important decrease on day four. A 100% mortality rate was observed for all of the baits used. Regardless of the bait used, the rats died in an average of 5 days, with mortality observed between days 3 and 9. The concentrations of the AR residues were determined after the death of the rats. The residues of bromadiolone in the liver following the death of the rats were 4-fold reduced using the bait containing principally the cis-isomers compared with the bait containing the trans-isomers of bromadiolone. The residues of difethialone in the liver were only 1.5-fold reduced using the trans-isomers compared with the cis-isomers.

DISCUSSION

ARs are crucial for controlling rodent populations. Indeed, trapping can be effective if there is a low density of rodents; however, in the presence of a large population, trapping is time-consuming and ineffective (Buckle and Smith 2015). Efficient repellents/attractants and ultrasound seem limited because of the habituation of rodents (Berny et al. 2014). Other chemical products (alphachloralose, cholecalciferol, zinc phosphide, and bromethalin) present major drawbacks, making their use or their approval difficult (RRAC 2015). Nevertheless, despite the importance of ARs in the management of rodent populations, their use must take into account different issues: the resistance problem associated with the use of FGARs (Hadler and Buckle 1992; Rost et al. 2009) and the secondary poisoning of wildlife essentially associated with the use of SGARs (Rattner et al. 2014). Therefore, third-generation ARs are urgently needed. The stereochemistry-based solution proposed recently to produce a new and eco-friendly difenacoum (Damin et al. 2016) seems to be a method of rapidly generating this third-generation of ARs at a reduced cost. Indeed, the 5 commercially available SGARs are a mixture of diastereomeric forms (Hunter et al. 1988; Huckle et al. 1988; Kelly et al. 1993; European Parliament 2007; Cort et al. 2012), which are generally easy to separate and purify. To rapidly generate the TGARs, it is necessary to revisit the SGARs to identify the diastereomer of each SGAR with the smallest half-life and increase its amount in each mixture.

The data found in the scientific literature, in the regulatory documents, allowed the systematic identification of the major and minor diastereomers in the commercial preparation of each SGAR. Flocoumafen, difenacoum, and brodifacoum are a mixture of 50 to 80% cis-isomers and 20 to 50% trans-isomers (Swaine 1985; Kelly et al. 1993; European Parliament 2009; Damin-Pernik et al. 2016); bromadiolone is a mixture of more than 70 of trans-isomers and less than 30% of cis-isomers; and difethialone is a mixture of more than 70 of cis-isomers and

less than 30% of trans-isomers (Caruel et al. 2015). The original reasons for the development of SGARs with such ratios between diastereomers have never been mentioned previously in the literature. We could assume that such ratios resulted from the need to kill the rodent after a single administration when these SGAR were developed (Hadler and Buckle 1992) by enriching the mix with either the most potent inhibitor or the most persistent pair of diastereomer. Another possibility is that the SGAR synthesis process does not give a racemate but a mixture of isomers enriched in one of them.

To characterize the inhibiting properties, ability to inhibit VKOR activity of cis- or trans-isomers of SGAR was determined. K_i results obtained in this study demonstrated that cis- and trans-isomers of the same SGAR could similarly inhibit VKOR activity (Table 2 and Fig. 6). Therefore, at identical hepatic concentrations, diastereomers of the same SGAR have the same anticoagulant properties when used in warfarin-susceptible rats.

To characterize the tissue persistence, toxicokinetic analyses were done. These toxicokinetic studies were conducted in rats after a single oral administration of the AR. This administration was performed by force feeding to better control the dose and time of ingestion. The same dose was used between SGARs regardless of the LD50 to be able to compare the molecules. Only the concentrations of ARs in the liver could be followed for 21 days because plasma and other tissue concentrations rapidly decrease below the LOQ. Two toxicokinetic parameters were calculated in this study, the AUC and half-life (Table 1 and Fig. 6). The AUC is the integrated area under the hepatic AR concentration versus time curve. The more the AUC is elevated, the greater the importance of the risk of secondary poisoning of wild predators or scavengers. The results obtained herein demonstrated that, in each mixture of SGAR, there is systematically one diastereomeric form with a lower AUC. The ratios of the AUCs between diastereomers of the same SGAR were 1.5 for difethialone and 3-3.5 for difenacoum, bromadiolone, and brodifacoum. The lowest AUC for all of them was systematically observed

for the minor diastereoisomer, except for flocoumafen, for which the lowest AUC was observed for the major diastereomer of the current commercial preparation. Because AUCs could be affected by absorption or clearance, the half-life of diastereomers in the liver was also calculated. Interestingly, the diastereomer with the lowest AUC systematically corresponded to the diastereomer with the lowest half-life in the liver, suggesting a faster elimination of this diastereomer than of the other one. This result was already demonstrated for difenacoum (Damin-Pernik et al. 2016).

These AUC and half-life differences between diastereomers of the same SGAR could result in major practical consequences. The composition of each SGAR could thus be systematically modified to enrich the mixture with the less persistent diastereomer to obtain a new AR with the same anticoagulant properties but with decreased tissue persistence. Thus, a new bromadiolone containing more than 70% cis-isomers and a new brodifacoum, difenacoum, or difethialone containing more than 70% trans-isomers may be developed to create the new generation of anticoagulants.

The benefits will be greater for the current ARs i) containing essentially the most persistent diastereomer and ii) with the most significant differences in half-life between the diastereoisomers. Bromadiolone and difethialone were chosen because bromadiolone is the most favorable example to illustrate the concept (highest difference in AUC and half-life and high percentage of the persistent diastereomer in the current mixture), while difethialone is the most unfavorable example (almost no difference in the AUC between the diastereomers). To illustrate this benefit, the efficiency and persistence of 4 different types of baits were evaluated: a bait containing the current bromadiolone mixture was compared with a bait containing a new bromadiolone mixture containing 93% cis-isomers, and a bait containing the current difethialone mixture was compared with a bait containing a new difethialone mixture containing 94% trans-isomers. The baits were all effective, with 100% mortality obtained

after 4 days of exposure at the current commercial concentration for bromadiolone baits or even below for difethialone baits (concentrations of commercial baits containing bromadiolone or difethialone is currently 50 ppm and 25 ppm, respectively). However, the baits enriched with the lowest persistent diastereomer systematically showed a decrease in the quantity of residues in the liver at the time of death of the animals. The reduction of the residues in the liver was greater for bromadiolone enriched in cis-isomers with a 4-fold reduction of the residues compared with the current bromadiolone. For difethialone, the use of trans-isomers reduced the liver residues by a factor of approximately 2 compared with the current difethialone.

The development of such baits enriched in the less persistent diastereomer could avoid the ecotoxicological risk associated with their use, especially regarding plant protection. These baits would reduce the amount of residues at the time of death of the rodents. Nevertheless, if these dying rodents with reduced residues are eaten by scavengers or predators, the elimination of such an AR could also be accelerated in the liver of scavengers or predators. Therefore, the ecotoxicological gain could be at both levels, and these TGARs would be a good solution to avoid environmental problems associated with pest management.

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: EB, VL

Conducted experiments: BE, EB, IF, MDP, VL

Contributed new reagents or analytic tools: BE, HC, MDP

Performed data analysis: BE, EB, MDP, SL, VL

Wrote or contributed to the writing of the manuscript: EB, IF, MDP, SL, VL

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DMD # 73791

FOOTNOTES

This work was supported by Bpi France [Grants ISI n°I1301001W “NEORAMUS”].

FIGURES LEGENDS

Fig. 1. Structures of the cis- and trans-isomers of flocoumafen (A). The cis- and trans-isomers referenced in the text arise from the alternative orientations of the substituents on the tetralin ring system. Time-dependent concentrations of the cis- and trans-isomers of flocoumafen after the *per os* administration of, respectively, 3 mg.kg⁻¹ of silica gel column-purified cis- or trans-isomers of flocoumafen to susceptible rats. The results are the mean ± SD of four rats. The time points are 24, 72, 168, 336, and 504 hours, which correspond, respectively, to 1, 3, 7, 14, and 21 days (B).

Fig. 2. Structures of the cis- and trans-isomers of difethialone (A). The cis- and trans-isomers referenced in the text arise from the alternative orientations of the substituents on the tetralin ring system. Time-dependent concentrations of cis- and trans-isomers of difethialone after the *per os* administration of, respectively, 3 mg.kg⁻¹ of purified cis- or trans-isomers of difethialone to susceptible rats. The results are the mean ± SD of four rats. The time points are 24, 72, 168, 336, and 504 hours, which correspond, respectively, to 1, 3, 7, 14, and 21 days (B).

Fig. 3. Structures of the cis- and trans-isomers of brodifacoum (A). The cis- and trans-isomers referenced in the text arise from the alternative orientations of the substituents on the tetralin ring system. Time-dependent concentrations of cis- and trans-isomers of brodifacoum after the *per os* administration of, respectively, 3 mg.kg⁻¹ of silica gel column-purified cis- or trans-isomers of brodifacoum to susceptible rats. The results are the mean ± SD of four rats. The time points are 24, 72, 168, 336, and 504 hours, which correspond, respectively, to 1, 3, 7, 14, and 21 days (B).

Fig. 4. Structures of the cis- and trans-isomers of bromadiolone (A). The cis- and trans-isomers referenced in the text arise from the alternative orientations of the substituents on the tetralin ring system. Time-dependent concentrations of cis- and trans-isomers of bromadiolone after the *per os* administration of, respectively, 3 mg.kg⁻¹ of purified cis- or trans-isomers of bromadiolone to susceptible rats. The results are the mean ± SD of four rats. The time points are 24, 72, 168, 336, and 504 hours, which correspond, respectively, to 1, 3, 7, 14, and 21 days (B).

Fig. 5. Structures of the cis- and trans-isomers of difenacoum (A). The cis- and trans-isomers referenced in the text arise from the alternative orientations of the substituents on the tetralin ring system. The time-dependent concentrations of the cis- and trans-isomers of difenacoum after the *per os* administration of, respectively, 3 mg.kg⁻¹ of purified cis- or trans-isomers of difenacoum to susceptible rats. The results are the mean ± SD of four rats. The time points are 24, 72, 168, 336, and 504 hours, which correspond, respectively, to 1, 3, 7, 14, and 21 days (B).

Fig. 6. 2D-representation of the biological properties of cis- and trans-of SGAR

TABLES

Table 1. Pharmacokinetic parameters in the liver after the single oral administration of the cis- or trans-isomers of anticoagulant rodenticides in susceptible rats at a dose of 3 mg.kg⁻¹.

Molecule	AUC (µg.h.g ⁻¹)	t _{1/2} (h) [95% CI]	
DFC cis-isomers	709,4 ± 206,10 *	78.33 [64.66-99.33]	p=0.0044
DFC trans-isomers	236,8 ± 87,37 *	24.18 [14.67-68.56]	
BDF cis-isomers	4827 ± 830,40 *	120.82 [110.74-732.89]	p<0.0001
BDF trans-isomers	1665 ± 242,80 *	68.73 [57.28-85.84]	
FLO cis-isomers	1247 ± 176,00	76.73 [60.48-104.91]	p<0.0001
FLO trans-isomers	2713 ± 446,00	177.45 [145.13-228.31]	
BDL cis-isomers	822,7 ± 131,40 *	26.89 [19.65-42.55]	p<0.0001
BDL trans-isomers	2733 ± 379,90 *	75.62 [64.72-90.96]	
DFT cis-isomers	2410 ± 308,00 *	71.60 [44.52-61.78]	p=0.0310
DFT trans-isomers	1894 ± 459,00 *	52.93 [46.36-61.67]	

* denotes a significant difference between two groups (AUC of cis- or trans-isomers in isolated administration) with $\alpha < 0.05$ by using a Mann-Whitney test. (DFC, difenacoum; BDF, brodifacoum; FLO, flocoumafen; BDL, bromadiolone; DFT, difethialone)

Table 2. K_i values of the VKOR activity catalyzed by warfarin-susceptible rat liver microsomes for the cis- and trans-isomers of each SGAR.

(nM)	K _i of VKOR activity toward:	
	cis-isomers	trans-isomers
Brodifacoum	18 ± 2	22 ± 1
Bromadiolone	31 ± 2	26 ± 2
Difenacoum	17 ± 1	21 ± 2
Difethialone	27 ± 1	22 ± 1
Flocoumafen	47 ± 2	55 ± 5

To determine the VKOR activity, standard reactions were performed in 200 mM Hepes buffer (pH 7.4) containing 150 mM KCl and 0.5 g.L⁻¹ of microsomal proteins. Each data point represents the mean ± SD of three individual analyses. * Comparison between two groups was performed using the Mann-Whitney test with p<0.05

Table 3. No-choice feeding test with the cis- or trans-isomers of bromadiolone and difethialone.

Bait containing	Number of death	Day of death	Consumption of			Residues ($\mu\text{g/liver}$)			% residues / total AR ingested
			Bait (g)	cis-isomers (μg)	trans-isomers (μg)	cis-isomers (μg)	trans-isomers (μg)	Total residues (μg)	
BDL, 50 ppm 93% cis /7% trans	6/6	6	44	2025	152	25	32	57	2.6
BDL, 50 ppm 2% cis /98% trans	6/6	5	47	47	2292	236	0	236	10.1
DFT, 15 ppm 94% cis /6% trans	5/5	5	49	44	691	43	4	47	6.4
DFT, 15 ppm 1% cis /99% trans	5/5	5	47	695	7	0	71	71	10.2

The daily consumption of bait was measured, and the liver of each rat was collected after death. The concentrations of the different isomers were measured in the liver. The results are the mean of 10 rats.

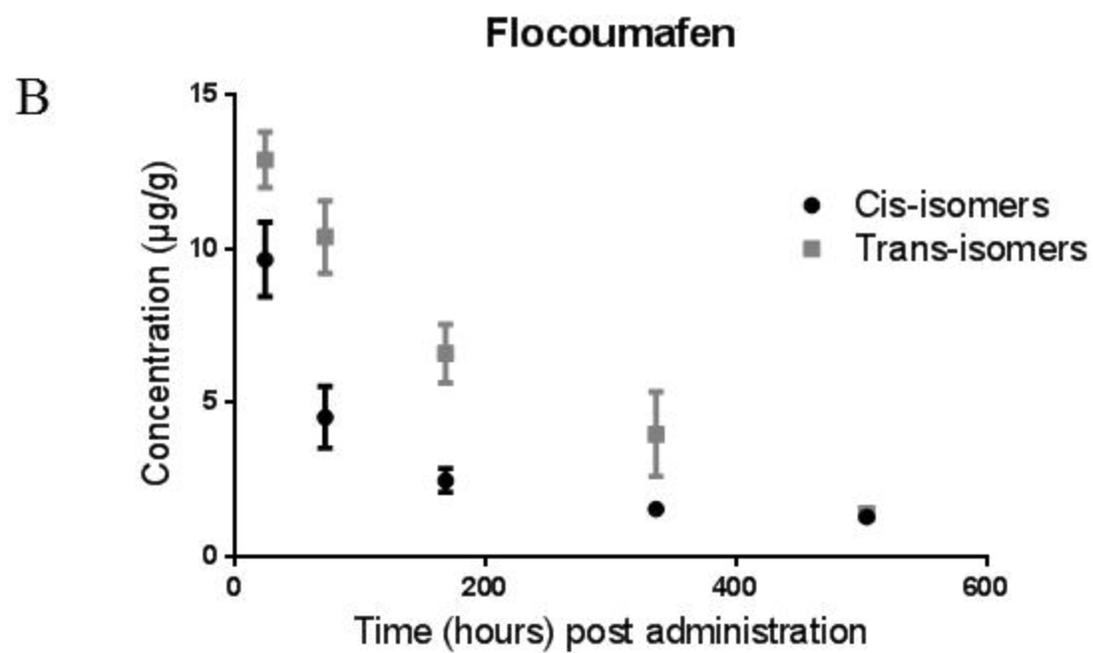
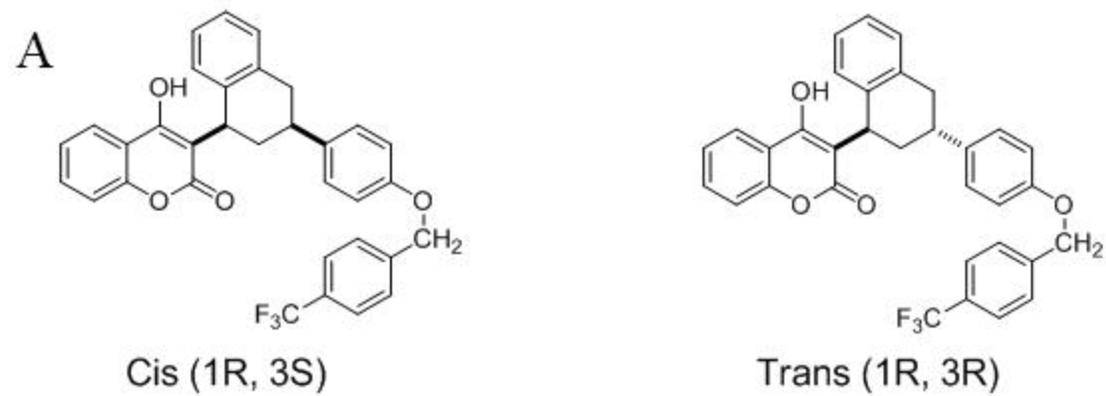


Fig. 1

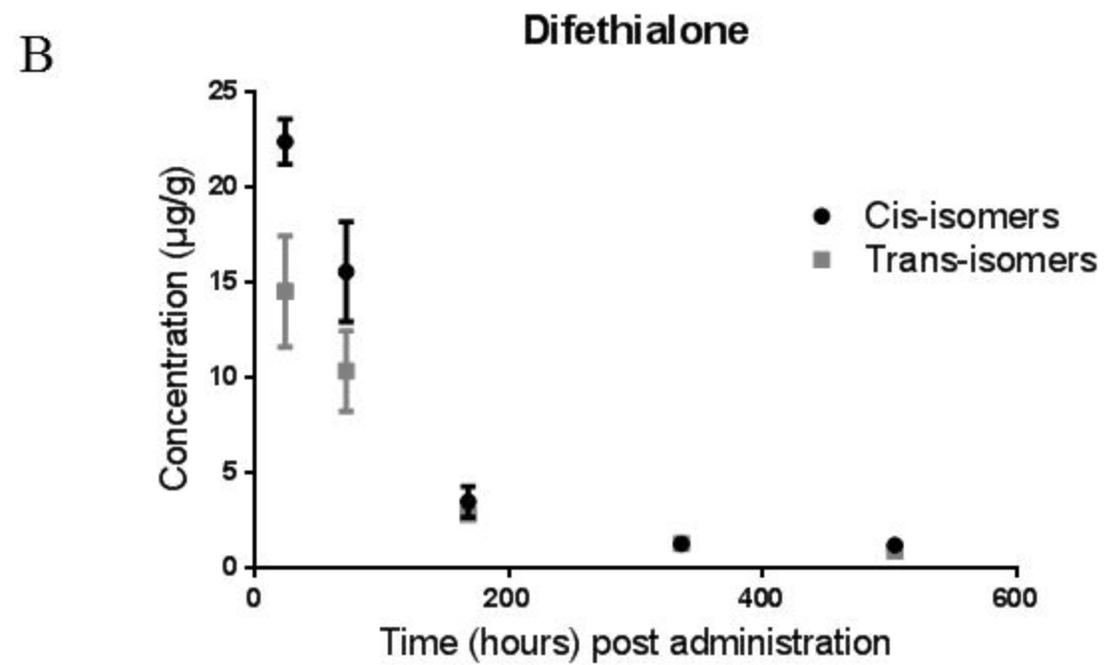
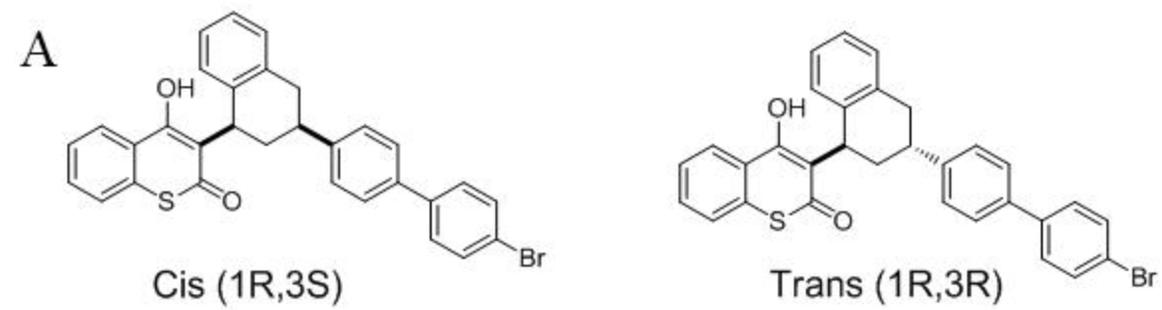


Fig. 2

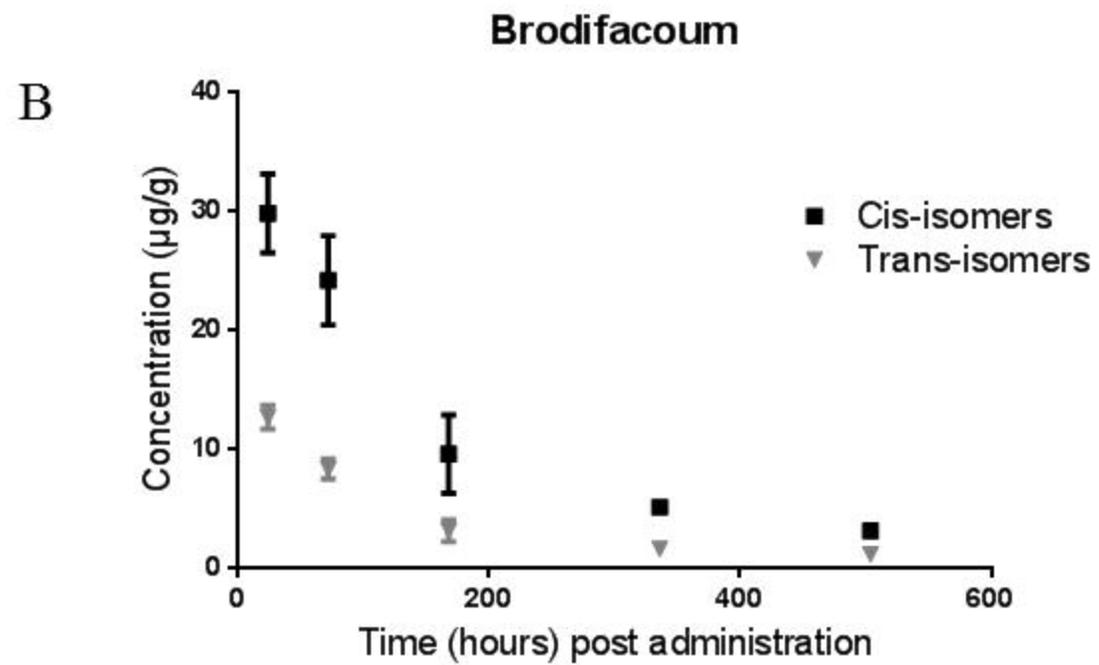
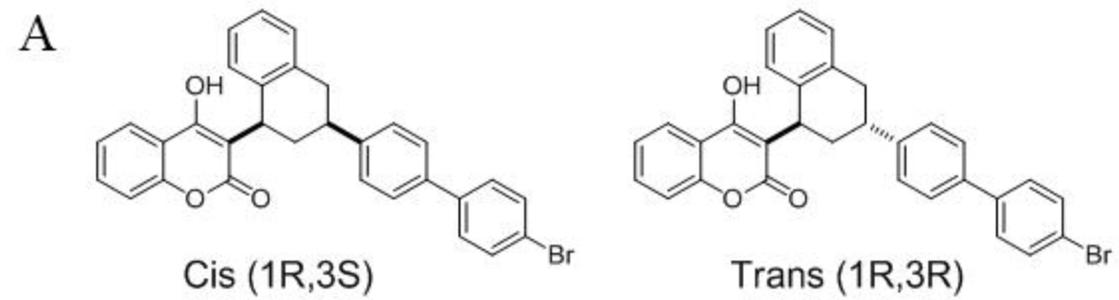


Fig. 3

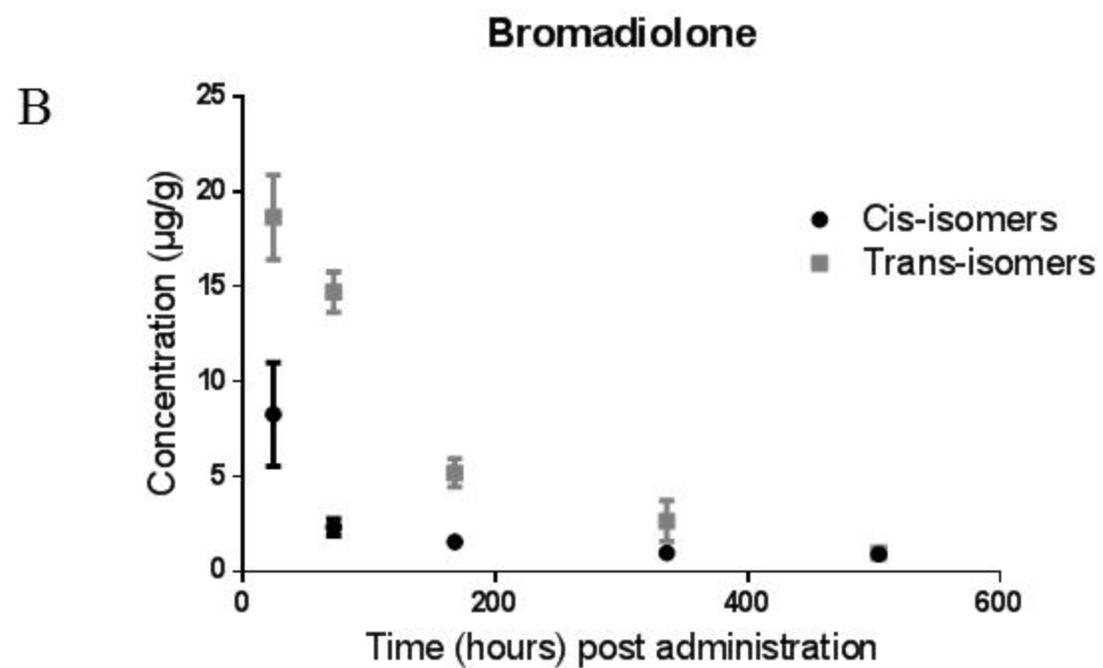
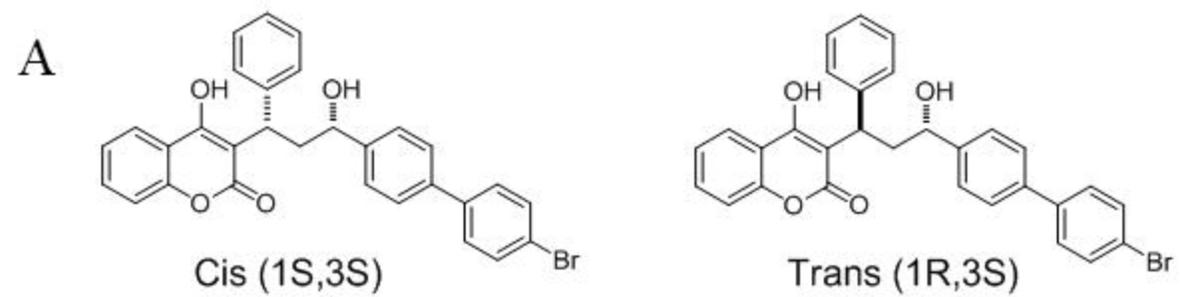
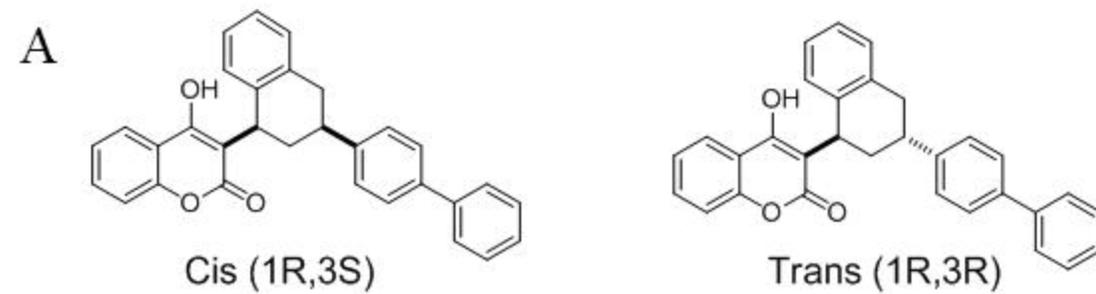


Fig. 4



Difenacoum

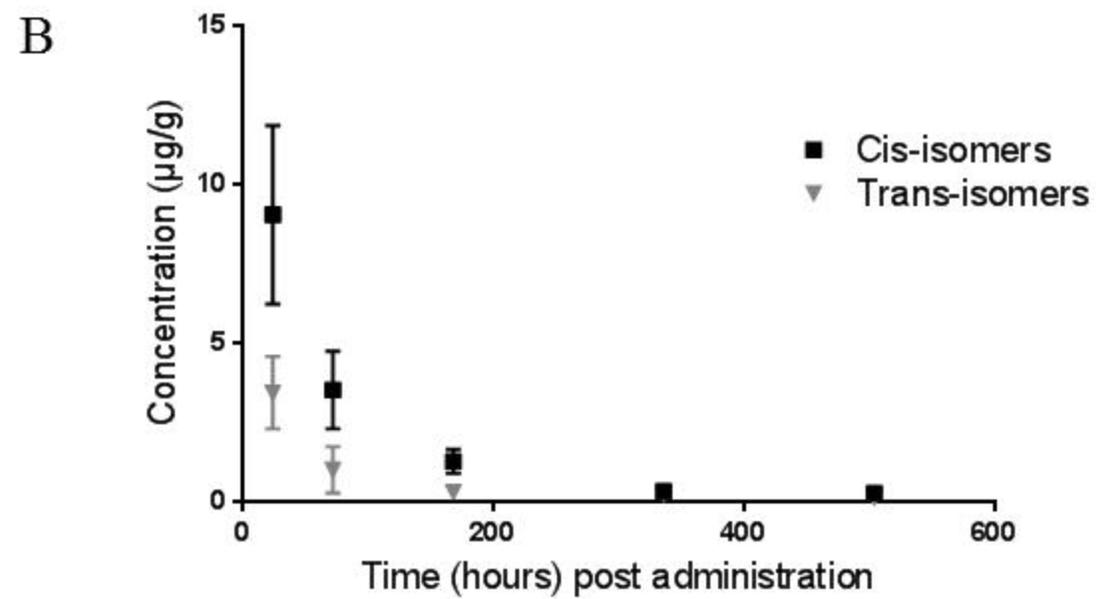


Fig. 5

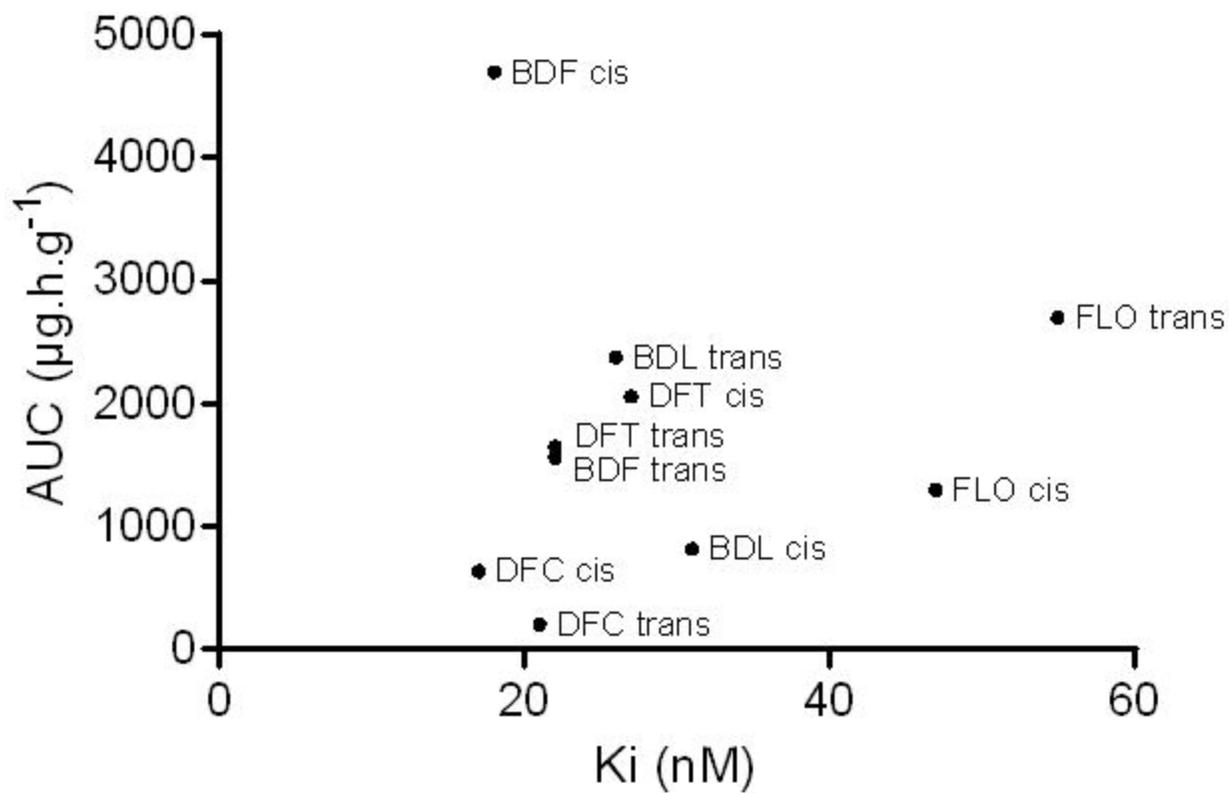


Fig. 6