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Exploration of the SAR of anti-invasive chalcones: Synthesis and biological evaluation of conformationally restricted analogues

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ABSTRACT

In order to get a clearer view on the active geometry of anti-invasive chalcones, we have prepared a number of isoxazoles and related substances as conformationally restrained mimics of 1,3-diarylpropenones, and also of (*Z*)-stilbenes. In vitro anti-invasive activity data for 3,5-isoxazoles and 4,5-isoxazoles, together with an in silico geometrical comparison, point towards an active conformation for chalcones more resembling their s-*trans* geometry than the s-*cis* counterpart.

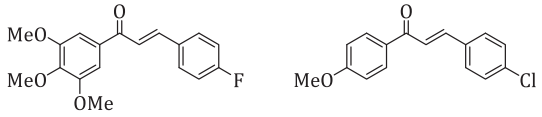
1. Introduction

In the second half of the 20th century, a major leap in the battle against cancer was made due to the advent of cytotoxic anticancer drugs. Most available therapies, however, induce little or no response in advanced stages of the disease, when cancer cells have disseminated throughout the body. This development of so-called metastases is the foremost treat of malignancy and accordingly the major cause of death in cancer patients.[1,2] The process of invasion is a requisite first step in the development of metastases, and can be defined as the protrusion of the basement membrane. In the ensuing steps, cancer cells will move into surrounding tissues, spread throughout the body, and establish colonies in permissive tissues.

In striking contrast to the array of antiproliferative chemotherapeutics targeting primary tumor growth, no effective treatments are available that can tackle the sequelae of metastasis. Our laboratories therefore seek novel agents that can interfere in the first and requisite step of the metastatic cascade: invasion. Drugs tackling this hallmark of malignant disease represent a high unmet need in the management of cancer.

In our quest for such substances, our attention was primarily drawn towards chalcones, which emerged as promising leads from the Indo-Belgian anti-invasive screening program.[3,4] As the molecular target of these substances has not yet been revealed, we developed and validated a predictive quantitative structure-activity relationship (QSAR)-model for chalcones as a rationale behind our work. During these investigations, (*E*)-3-(4-fluorophenyl)-1-(3,4,5-trimethoxyphenyl)propenone **1** and (*E*)-3-(4-chlorophenyl)-1-(4-methoxyphenyl)propenone **2** surfaced as remarkably interesting molecules (Fig. 1). Accordingly, a patent application covering these findings has been filed.[5]

Given the extraordinary in vitro anti-invasive potential of these chalcones, we decided to delve deeper into their structure-activity



1 **2**

in vitro anti-invasive activity at 10 nmol.L^{-1}

Figure 1. Two highly potent anti-invasive chalcones that serve as a starting point for the present SAR-study.

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relationship (SAR) by synthesizing close analogues. We were particularly interested in acquiring more information on the active geometry of these substances, and therefore pointed our attention towards the design, synthesis and assessment of conformationally restrained congeners.

Chalcones can adopt different conformations by rotating around the sigma bond of the enone moiety, leading to two main geometries: the s-*cis* conformer **3a** and the s-*trans* counterpart **3b** (Scheme 1). The antimitotic activity of chalcones is, for example, exerted by their s-*trans* conformers, which bear close resemblance to colchicine and combretastatin and hence bind at the colchicine site of tubulin.[6] It was therefore of fundamental interest to verify whether the molecular target of our anti-invasive chalcones discerns between these two conformers.

For this reason, we decided to prepare isoxazoles **4**, for which the conformation is locked into the s-*cis* form (Scheme 1, **A**). The extra heteroatom introduces an additional hydrogen bond acceptor into these chalcone analogues. Still, these ring systems possess steric and stereoelectronic resemblance to the original enone moiety in chalcones and can accordingly provide information on the conformation-activity relationship of these propenones. Hence, if isoxazoles **4** would fail to exert anti-invasive effects, this would provide an initial indication that the active conformer of the chalcones may be more s-*trans* like.

In addition, we were intrigued to evaluate the potential of 4,5-diarylisoxazoles **5** as well. These substances bear—to some extent—resemblance to s-*trans* chalcones (Scheme 1, **B**). More importantly, they can be considered as stable analogues of *cis*-stilbenes **6** (**C**), a substance class we have recently evaluated as anti-invasive agents (data unpublished). *Cis*-stilbenes tend to isomerize to the thermodynamically more stable *trans*-isomers both during storage and in vivo.[7]

Due to their interest as antitubulin agents, a lot of stable ring-closed analogues have accordingly been proposed (Fig. 2). Examples of evaluated *cis*-1,2-substituted ethylene mimics encompass the cyclopropyl group[8] (**7**) and five-membered heterocyclic rings such as dioxolanes,[9] isoxazoles (**8**),[10] imidazoles (**9**)[11] and thiadiazoles.[12] Hence, given our previous investigations on anti-invasive *cis*-stilbenes, we were interested to evaluate 4,5-diarylisoxazoles **5** as stable analogs of the latter compounds. Furthermore, comparison with the identically decorated 3,5-diarylisoxazole congeners **4** might bring about additional SAR-insights.

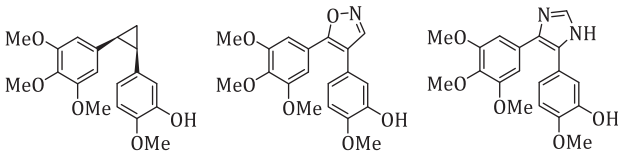


Figure 2. Examples of stable analogues of *cis*-stilbenes (**7**, **8**, **9**).

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of 3,5-diarylisoxazoles

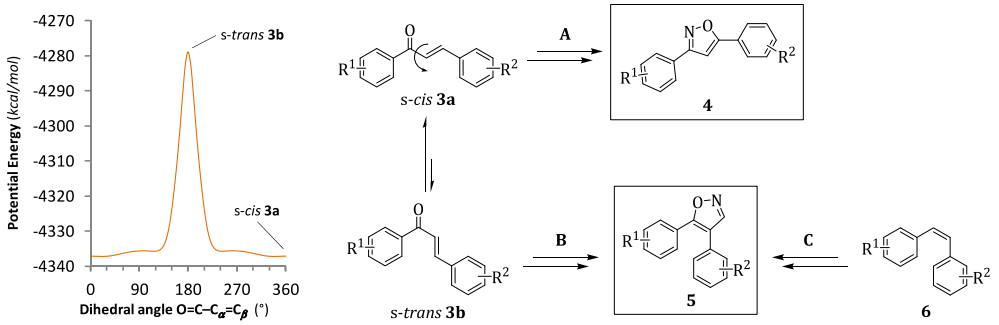
Most strategies for the preparation of 3,5-diarylisoxazoles can be considered as either intermolecular cycloadditions of 1,3-dipoles to alkynes, or condensations of β-diketone equivalents with hydrazine or hydroxylamine. We chose to evaluate two closely related routes belonging to the latter category, as depicted in Scheme 2 (via **A**, or **B**–**C**). These transformations are versatile, straightforward and make use of readily available starting materials. In route **A**, the α-substituent X of chalcone **12** is selected to be a good leaving group, resulting in in situ aromatization by elimination of HX. When proceeding through route B, isoxazolines **13** are isolated at first, and can then be oxidized to the desired isoxazole **4** (**C**).

One problem often encountered when conducting such condensation reactions is the competition between an initial 1, 2- and 1,4-addition of the dinucleophile, thereby resulting in isomeric mixtures of isoxazoles when R^1 differs from R^2. However, Katritzky and co-workers encountered regioselectivity for route A when the benzotriazolyl moiety was chosen as the α-substituent (X = Bt).[13] Hence, we evaluated their protocol in an initial attempt at the synthesis of our desired isoxazoles.

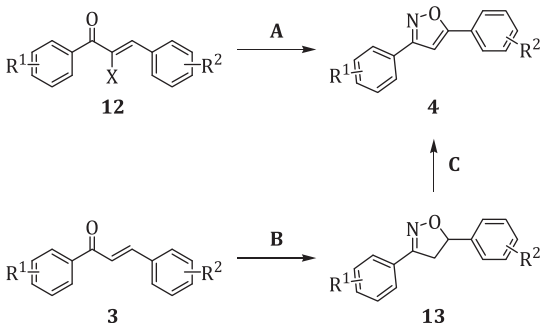
In a first step, 1-(trimethylsilylmethyl)benzotriazole **15** was prepared from benzotriazole **14** and trimethylsilylmethylchloride (Scheme 3**a**).[14] The rather moderate yield for this transformation may be partly explained by the formation of the 2-substituted analogue in a 1:4-ratio to the main product **15**, hence encumbering the isolation of the latter.[15] Next, the benzotriazolylacetophenones **16** were prepared by reaction of **15** with a suitable acid chloride. Although not mentioned in the original protocol,[14] we found the use of a catalytic amount of DMAP in this transformation beneficial as it enhanced chemoselectivity.

Benzotriazolylacetophenone **16a** was subsequently reacted with an appropriately functionalized benzaldehyde in a piperidine-catalyzed Claisen–Schmidt condensation using microwave heating, which furnished (*Z*)-α-benzotriazolylchalcone **12a** as a precipitate. In contrast, despite the evaluation of numerous bases (LiOH, piperidine, NaH, DIPEA, LDA), the analogous reaction of acetophenone **16b** with 4-fluorobenzaldehyde failed.

Subsequently, we tried to convert α-benzotriazolylchalcone **12a** into the corresponding isoxazole via treatment with NaOEt and hydroxylammonium chloride in EtOH (Scheme 4).[13] Although, the desired isoxazole **4a** was indeed present in the reaction mixture, as indicated by LC/MS analysis, an appreciable amount of side



Scheme 1. Left: Potential energy scan along the dihedral angle O=C-C$^{\alpha}$=C$^{\beta}$ of chalcone **3** (AM1 level of theory, HyperChem 8.0). Right: Synthesis of 3,5-diarylisoxasoles **4** as conformationally restrained analogues of s-*cis* chalcones **3a** (**A**); preparation of 4,5-diarylisoxazoles **5** as stable mimics of (*Z*)-stilbenes **6** (**C**), and analogues of s-*trans* chalcones **3b** (**B**).



Scheme 2. Synthesis of isoxazoles **4** from 2-substituted 1,3-diarylpropenones **12** via an annulation-elimination sequence (route **A**), or from chalcones **3** through the corresponding isoxazolines **13** (route **B**–**C**).

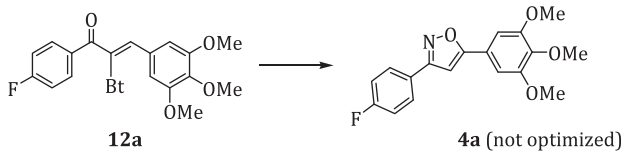
products had also been formed which would render product isolation difficult. Due to this observation, and given the failure of the synthesis of **12b**, we abandoned the benzotriazolyl approach, as this elegant route proved inapplicable to our compounds.

Next, we turned our focus to pathway **b**–**c** from Scheme 2. Synthesis of the isoxazoles through the isoxazolines requires an oxidation step, but starts from readily available chalcones. A further advantage of proceeding via this route is that it enables to evaluate the isoxazolines in our anti-invasive screening program as well. We chose the straightforward protocol of Sinisterra and co-workers for the conversion of substituted chalcones into isoxazolines **13a**–**c**, which were indeed formed using the reported conditions.[16] Nevertheless, we altered the prescribed work-up procedure, as it failed to remove many impurities. In contrast, extraction with EtOAc and recrystallization in absolute EtOH furnished the desired isoxazoles in high purity.

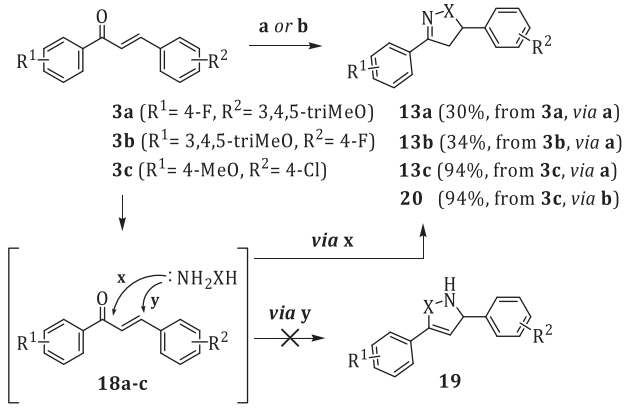
According to Sinisterra, their protocol selectively proceeds through an initial 1,2-addition of hydroxylamine across the enone, thereby allowing for the regiocontrolled formation of a single isoxazoline isomer (Scheme 5, route **x**). We were able to confirm this selectivity by means of NMR analysis of isoxazoline **13a**. The alternative possibility, involving an initial 1,4-addition, would give rise to isoxazoline **19** (route **y**). All NMR-data clearly point towards structure **13a**: (i) HSQC analysis confirmed the presence of a CH2 group. (ii) In the ^{1}H spectrum, chemical shift, signal integration and coupling constants indicate the presence of an H_aH_b-system and a vicinal aliphatic CH (see Scheme 5). (iii) Furthermore, chemical shift values in the ^{13}C spectrum agree with the presence of an imino group and two aliphatic carbon atoms. (iv) Finally, HMBC data indicates a coupling between $H^{2'}$ and $H^{6'}$, which clearly bear a fluorine coupling, on the one hand and an aliphatic CH on the other hand.

In order to increase the molecular diversity in our set, we also prepared an analogous pyrazoline **20** via the same procedure, which was obtained in excellent yield.

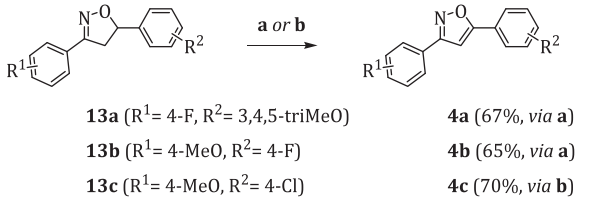
Having the isoxazolines **13** in hand, we proceeded with the aromatization towards the corresponding isoxazoles **4** (Scheme 6). Our first attempts, employing DDQ as an oxidant under fairly classical conditions, resulted in isolation of the starting material for all three substrates. Activated MnO_2, however, proved capable of furnishing the desired isoxazoles **4** at elevated temperatures. For compounds **4a** and **4b**, full conversion was obtained faster in the presence of $MgSO_4$ and under microwave irradiation. It should further be noted that the degree of conversion dropped severely on scale-up (above 1 mmol).



Scheme 4. 10 equiv NaOEt (10 M in EtOH), 2 equiv $NH_2OH.HCl$, rt to Δ, overnight.



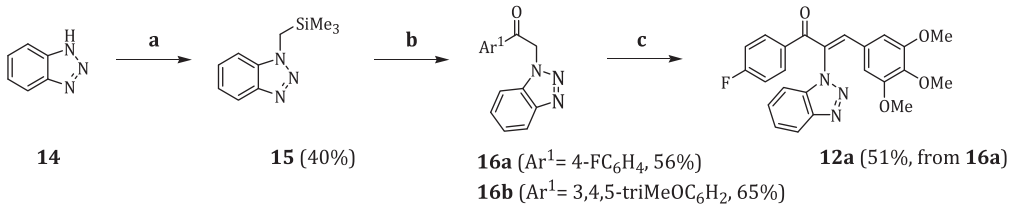
Scheme 5. Regioselective 1,2-addition of hydroxylamine leads to isoxazoline **13** as a sole product (route x); no 1,4-addition was observed (route y). (a) Synthesis of isoxazolines **13a**–**c** (X=O): 6 equiv $NH_2OH.HCl$, 7 equiv KOH, abs EtOH, Δ, 24 h, N_2; (b) Synthesis of pyrazoline **20** (X=NH): 6 equiv $NH_2NH_2.H_2O$, 7 equiv KOH, abs EtOH, Δ, 24 h, N_2.



Scheme 6. Oxidation of isoxazolines **13** towards isoxazoles **4**.

2.1.2. Synthesis of 4,5-diarylisoxazoles

In a second part of the synthetic work, we envisaged preparing 4,5-diarylisoxazoles **5** as stable analogues of *cis*-stilbenes, and to a lesser extent of s-*trans* chalcones. Since we disposed of appropriately decorated benzaldehydes **21**, we opted for a synthetic route involving an initial umpolung of these electrophiles via



Scheme 3. (a) (1) 1 equiv NaOEt (1 M in EtOH); (2) removal of solvent; (3) 50 °C, 16 h, vacuum; (4) 1 equiv $ClCH_2SiMe_3$, DMF, rt, 24 h; (b) 1.2 equiv $Ar^1C(O)Cl$, 0.1 equiv DMAP, dry THF, Δ, 24 h, N_2; (c) 1.02 equiv 3,4,5-trimethoxybenzaldehyde, 1 equiv piperidine, EtOH, 70 °C, 2 h, MW.

transformation to their corresponding *O*-protected α-hydroxyphosphonates **23**, followed by a Horner–Wadsworth–Emmons (HWE)-type reaction, yielding deoxybenzoins **26** (Scheme 7). These intermediates could then furnish the desired isoxazoles upon condensation with dimethylformamide dimethylacetal (DMFDMA) and hydroxylammonium chloride.

In an initial step, we reacted the appropriately decorated benzaldehydes **21** with dimethyl phosphite in the presence of a catalytic amount of *n*-$BuNH_2$ and using Et_2O as a solvent (Scheme 7).[17] These mild reaction conditions provided us with the α-hydroxyphosphonates **22** as a pure precipitate in nearly quantitative yield. Trials employing other base/solvent pairs, such as NaOMe/MeOH[18] or CaO/neat,[19] had an inferior outcome. Next, the hydroxyl group was converted into its THP ether under standard conditions. The protected phosphonate was not isolated, but in situ submitted to a Horner-Wadsworth-Emmons reaction with a suitable second benzaldehyde. The resulting protected enols **24** were immediately hydrolyzed, furnishing the corresponding deoxybenzoins **26**, which were accompanied by homocoupling product **27b** (13–20%).[18]

Subsequently, the deoxybenzoins **26** were condensed with DMFDMA under solventless conditions, yielding β-aminoenones **28**.[20] These molecules were not isolated, but underwent immediate regioselective ring closure upon treatment with hydroxylammonium chloride, furnishing the desired 4,5-diarylisoxazoles **5** in modest yields (Scheme 7).

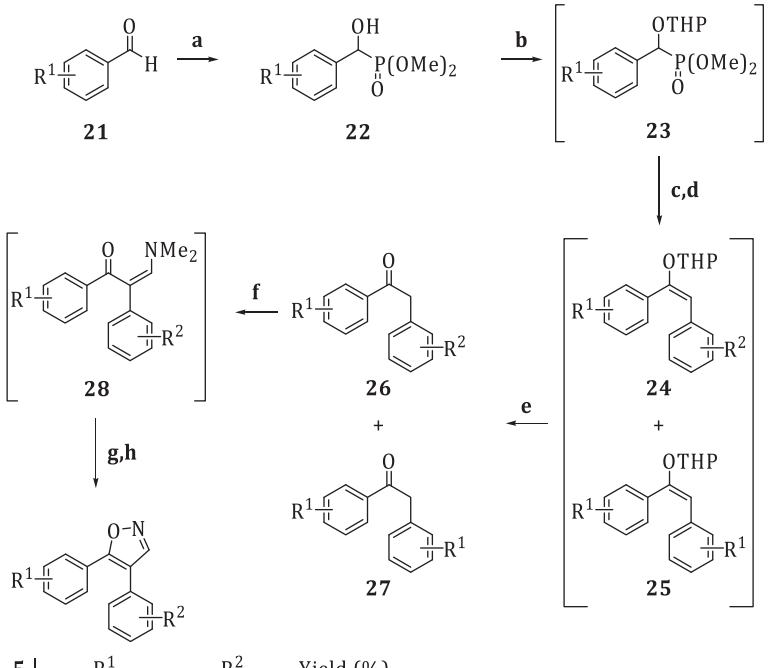
2.2. Biological activity and SAR

In the light of our SAR program, we submitted the newly synthesized, conformationally restrained heterocyclic chalcone mimics to the in vitro chick heart invasion assay.[21] In this screening technique, precultured heart tissue fragments (PHFs), dissected from 9-day-old chick embryos, are confronted with aggregates of human invasive MCF-7/6 mammary carcinoma cells in the presence of a certain concentration of a test compound. After eight days of incubation at 37 °C, the interaction between the cancer cells and the PHF is evaluated histologically and classified along a 5-grades scale (Fig. 3).

Compounds are coined 'active' or 'anti-invasive' when the invasion grade is I or II, and 'inactive' when the grade is III or IV. For substances that exhibit selective toxicity versus MCF-7/6 cells, denoted as invasion grade 0, a judgment on their anti-invasive potency cannot be made. A notable advantage of this type of confronting culture is the involvement of living host tissue. The contributions of such normal tissue to the micro-ecosystem that governs tumor behavior should indeed not be neglected.[3] Moreover, the design of the chick heart assay enables the observer to discern the effect of a substance on the tumor cells from effects on the normal host tissue.

For the compounds depicted in Table 1, the invasion grade in the CHI assay was scored at concentrations ranging from 100 to 0.1 μmol L^{-1} (Table 1). In order to translate the resulting assay data into one anti-invasive potency value per compound, we introduced 'Log c_{min}', the logarithm of the 'lowest active concentration c_{min}' for each compound, being the lowest concentration at which a substance exhibits an anti-invasive effect (invasion grades I or II). Hence, six activity levels were defined, ranging from class-2 for the most active compounds (invasion grade I or II at the 0.01 μmol L^{-1} level) to class 3 (compounds with no apparent effect at 100 μmol L^{-1}.

Unfortunately, all but one of the evaluated molecules completely lacked anti-invasive potency (class ⩾1, Table 1). Isoxazole **4b** inhibited invasion at 10 μmol L^{-1}, which is a rather dissapointing score. 4,5-Diarylisoxazole **5a**, however, proved potent up to 1 μmol L^{-1} (class 0), and thereby distinguished itself clearly from



5	R^1	R^2	Yield (%)
a	3,4,5-triMeO	4-F	28
b	4-F	3,4,5-triMeO	30

Scheme 7. (a) 2 equiv HP(O)$(OMe)_2$, *n*-Bu_2NH, Et_2O, rt, 3 h; (b) 2.2 equiv 3,4-dihydro-2*H*-pyran, 2.6 mol % *para*- TsOH·H_2O, benzene, 50 °C, 2 h; (c) 50 °C to rt; (d) 4 equiv NaH, 1 equiv substituted benzaldehyde, EtOH, rt, 18 h, N_2; (e) 0.95 equiv 10% aq HCl, MeOH, rt, 1 h; (f) dimethylformamide dimethylacetal, Δ, 14 h, N_2; (g) solvent evaporation; (h) 1.96 equiv $NH_2OH.HCl$, 1 equiv Na_2CO_3, MeOH/H_2O (2:1), adjust pH to 4–5 with AcOH, then Δ, 2 h, N_2.

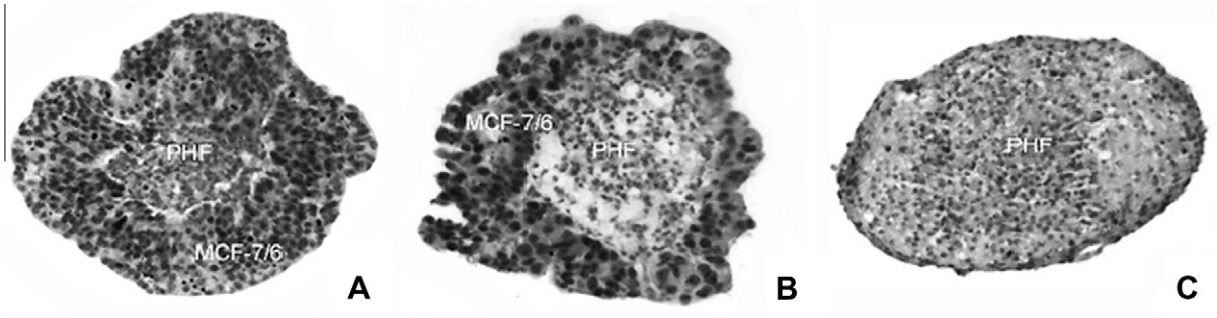
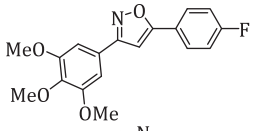
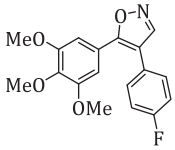
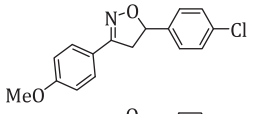
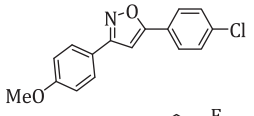
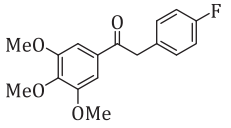


Figure 3. This illustration of the chick heart invasion assay displays 7 μm thick sections from confronting cultures between precultured heart fragments (PHF) and invasive human mammary carcinoma cells (MCF-7/6). Panel A results from an untreated culture, in which the confronting cells have occupied the PHF, and was attributed an invasion grade of III. In Panel B, addition of an anti-invasive agent resulted in a clear histological separation between the confronting partners; the invasion grade is denoted as I/II. Panel C displays the outcome for a product with selective toxicity for MCF-7/6 cells with respect to the PHF. In this case, no judgment of the anti-invasive potency can be made; this pattern is coined grade 0.

Table 1
In vitro anti-invasive activity data against MCF7/6 cells, obtained via the CHI-assay (0 = toxic for MCF7/6; I and II = no invasion, the compound is coined active; III and IV: invasion, the compound is inactive)

	Structure	Invasion grade at different concentrations (μmol L^{-1})				Anti-invasive activity class[a]
		100	10	1	0.1	
4b		II[b]/II[b]	II/II	III/III/IV	IV	1
5a		II[b]/III[b]	I[c]	II/II	III/III	0
13c		III/III	II/III	III/IV		⩾2
4c			IV/IV	III/IV		⩾2
26a		II[b]/II[b]	III/III	III/III	III/III	2

[a] Log c_{min}.
[b] Toxic.
[c] Slightly toxic for PHF, few MCF7/6 cells left.

the rest of the set. The observed cytotoxicity of compounds **4b**, **5a** and **26a** at 100 μmol L^{-1} can be considered normal at this elevated dose.

At 10 μmol L^{-1}, on the other hand, isoxazole **5a** exhibits an interesting behavior: few MCF7-6 cells were left upon histological examination, while only a slight toxicity vis-à-vis the PHF was observed. Compound **5a** thus exerts—to a certain extent—selective cytotoxicity versus the malignant cells.

Although only one of the assessed compounds proved reasonably active, the acquired data is surely valuable in the broader perspective of our quest for anti-invasive molecules. Indeed, these results provides us with important information on the conformation-activity relationship of the highly potent chalcones on which this study was based.

Firstly, both isoxazoles completely lack potency (class ⩾1). These molecules should, however, be mimics of the s-*cis* conformation of their parent chalcones, compounds which proved active up to 10 nmol L^{-1} (class-2). This geometrical similarity was confirmed by flexible superposition of s-*cis* conformer **1a** of 3-(4-fluorophenyl)-1-(3′,4′,5′-trimethoxyphenyl)propenone(Fig. 4, above, green) and its isoxazole analogue **4b** (yellow). Both molecules are planar and a good overlap of methoxy and fluorine substituents, aromatic rings and the α,β-unsaturated enone or its imine equivalent can be appreciated, resulting in an RMS of 0.7466. Given the absence of activity for isoxazole **4b**, the active geometry of chalcone **1** may therefore clearly deviate from that of s-*cis* conformer **1a**.

In a similar way, we have analyzed the geometrical similarity between the most active of the tested compounds, 4,5-diarylisoxazole **5a**, and the s-*trans* conformer of chalcone **1**. A good match between these two geometries is apparent from their three-dimensional representations in Figure 4, and from the RMS of 0.7038.

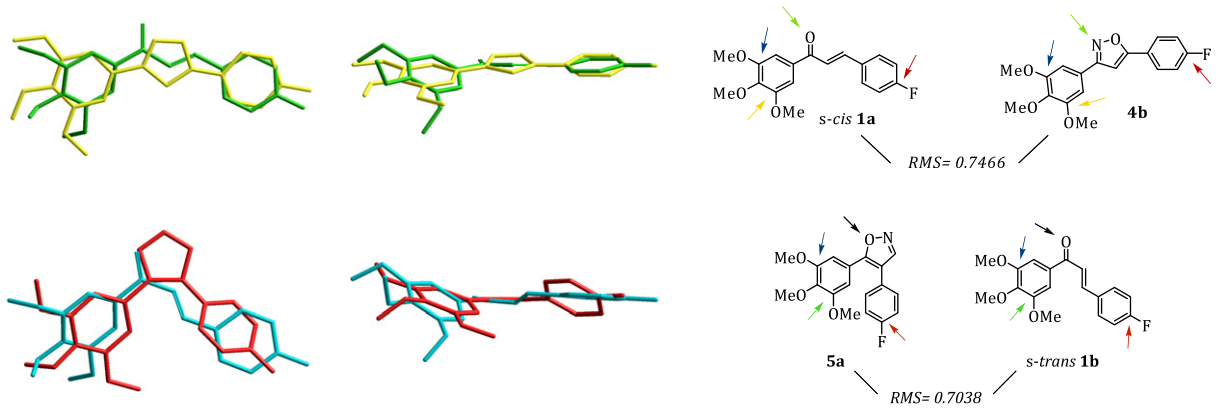


Figure 4. Geometrical comparisons of various compounds using flexible superposition. Above: Isoxazole **4b** (yellow) and s-*cis* conformer **1a** of 3-(4-fluorophenyl)-1-(3′,4′,5′-trimethoxyphenyl)propenone (green). Below: Isoxazole **5a** (red) and s-*trans* conformer **1b** of 3-(4-fluorophenyl)-1-(3′,4′,5′-trimethoxyphenyl)propenone (cyan). Root mean square (RMS) values were calculated for the atom–atom distances between the 3D-structures of atoms indicated by colored arrows.

This adds value to the assumption that the active conformer of the potent chalcones is more s-*trans* than s-*cis* like. Such a conformation furthermore better resembles that of (*Z*)-stilbenes, for which we earlier observed a high anti-invasive potency. Indeed, isoxazole **5a** was the only molecule in the test set designed as a (*Z*)-stilbene mimic.

The above corollaries concerning the active conformation of potent anti-invasive chalcones seem plausible, but surely warrant further investigation in order to obtain a more complete view of the SAR-landscape of chalcones. Hence, we are currently synthesizing further conformationally restricted analogues of the most potent 1,3-diarylpropenones in our library, for which the results will be communicated in due course.

3. Conclusion

Although further exploration of their SAR-landscape is necessary, the present study provides initial indications that the active conformation of anti-invasive chalcones resembles the s-*trans* geometry more closely than the s-*cis* counterpart. This observation furthermore correlates with the interesting anti-invasive activity profile of (*Z*)-stilbenes.

4. Experimental part

4.1. Chemistry

All reagents were purchased from commercial sources (Aldrich, Acros) and used without further purification. Solvents were purchased from commercial sources (Aldrich) and employed as is. Microwave reactions were carried out in a CEM Benchmate apparatus using a standard temperature program (maximum power: 300 W). Crude reaction mixtures were analyzed on LC/MS/UV. Thin layer chromatography was carried out on silica gel 60F254 plates (Merck).

High resolution ^{1}H (300 MHz), ^{13}C (75 MHz) and ^{19}F (282 MHz) magnetic resonance (NMR) spectra were recorded on a Jeol Eclipse+300 FT NMR spectrometer in deuterated solvents. Chemical shifts are reported using TMS and/or $CFCl_3$ as an internal reference, unless otherwise indicated. Peak assignments were obtained with the aid of DEPT, HSQC, HMBC and COSY spectra. Attenuated total reflection (ATR) IR spectra were recorded on a Perkin Elmer Spectrum BX spectrometer, equipped with a ZnSe-crystal, at room temperature. Low-resolution mass spectra were recorded on an Agilent 1100 series VL mass spectrometer (ES, 70 eV). Melting points were measured with a Büchi B-540 apparatus and are uncorrected.

4.1.1. Synthesis of 1-trimethylsilylmethyl-1*H*-benzotriazole (15)

In a flame-dried round-bottomed flask, 49.6 mmol (5.95 g) of benzotriazole is dissolved in 50 mL of a freshly prepared 1 M solution of NaOEt in EtOH (1.15 g of Na). Then, the solvent is removed under reduced pressure and the residue is dried overnight at 50 °C in a vacuum oven. Subsequently, the flask is cooled to room temperature, and 50 mL of DMF is added, followed by 1 equiv (6.95 mL) of chlorimethyltrimethylsilane. The mixture is stirred for one hour at 50 °C, and then for 23 h at room temperature.

Afterwards, 40 mL of water is added, and the resulting mixture is extracted with Et_2O (7 × 50 mL). The combined organic layers are subsequently dried over $MgSO_4$. Solvent removal affords a red oil, which is taken up in hexanes. Overnight storage at −20 °C affords crystals of **15**, which are isolated in a yield of 40% after washing and thorough drying.

4.1.2. Synthesis of 2-benzotriazol-1-yl-1-arylethanones (16)

To a flame-dried round-bottomed flask, kept under an N_2-atmosphere, 4.87 mmol (1 g) of 1-trimethylsilylmethyl-1*H*-benzotriazole **15** and 25 mL of dry THF are added, followed by 0.1 equiv (0.06 g) of DMAP and 1 equiv of a suitable benzoyl chloride. The resulting mixture is stirred at reflux and under an N_2-atmosphere for 24 h. The volatiles are subsequently removed under reduced pressure, upon which crystallization of the resulting crude in EtOH affords the desired 2-benzotriazol-1-yl-1-arylethanone **16** in high purity.

4.1.3. 2-Benzotriazol-1-yl-1-(3,4,5-trimethoxyphenyl) ethanone (16b)

Yield: 65%, as white crystals; mp: 151.5–153.0 °C; IR (ATR, cm^{-1}) ν_{max}: 1002, 1123, 1160, 1229, 1316, 1415, 1456, 1586, 1694; ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): δ 3.93 (6H, s, 2x OMe), 3.94 (3H, s, 1x OMe), 6.07 (2H, s, CH_2), 7.32 (2H, s, $H^{2'}$, $H^{6'}$), 7.33–7.55 (3H, m, 3x CH), 8.11 (1H, d, J_{vic} = 8.3 Hz, CH); ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 55.97 (CH_2), 58.31 (2x OMe), 62.95 (OMe), 107.82 ($C^{2'}$, $C^{6'}$), 111.53 (CH), 122.02 (CH), 126.04 (CH), 129.82 (CH), 130.94 (C_q), 135.66 (C_q), 145 (C_q), 147.99 (C_q) 155.26 ($C^{3'}$, $C^{5'}$), 191.34 (C=O); ESI-MS (+) *m*/*z*: 318.1 ($[M+H]^+$, 100).

4.1.4. Synthesis of 2-benzotriazol-1-yl-1-(4-fluorophenyl)-3-(3,4,5-trimethoxyphenyl) propenone (12a)

0.85 mmol (0.22 g) of 2-benzotriazol-1-yl-1-(4-fluorophenyl)ethanone **16a** is loaded into a 10 mL microwave vial, upon which of 7 mL abs EtOH, 1 equiv (75 mg) of piperidine and 0.9 mmol (0.176 g) of 3,4,5-trimethoxybenzaldehyde are added. The headspace is flushed with nitrogen, the vial is subsequently sealed with

a Teflon® 'snap-on' cap, and the mixture is heated at 70 °C under microwave irradiation for 3 h. The resulting precipitate is the desired propenone **12a**, which can be isolated by filtration, washing and thorough drying.

4.1.5. 2-Benzotriazol-1-yl-1-(4-fluorophenyl)-3-(3,4,5-trimethoxyphenyl) propenone (12a)

Yield: 51%, as white crystals; mp: 179.1–180.7 °C; IR (ATR, cm^{-1}) ν_{max}: 998, 1051, 1070, 1130, 1148, 1241, 1261, 1333, 1505; ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 3.42 (6H, s, 2x OMe), 3.79 (3H, s, OMe), 5.98 (2H, s, $H^{2''}$, $H^{6''}$), 7.12 (2H, dd, J_{vic} = 8.6 Hz, J_{HF} = 8.6 Hz, $H^{3'}$, $H^{5'}$), 7.25 (1H, td, J_{vic} = 7.2 Hz, J_{allyl} = 1.1 Hz, H^5 or H^6), 7.39 (1H, td, J_{vic} = 7.2 Hz, J_{allyl} = 1.1 Hz, H^5 or H^6), 7.46 (1H, dd, J_{vic} = 7.2 Hz, J_{allyl} = 1.1 Hz, H^4 or H^7), 7.76 (1H, s, H^{β}), 7.84 (2H, dd, J_{vic} = 8.8 Hz, J_{HF} = 5.5 Hz, $H^{2''}$, $H^{6''}$), 8.11 (1H, dd, J_{vic} = 7.2 Hz, J_{allyl} = 1.1 Hz, H^4 or H^7); ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 55.77 (2x OMe), 60.98 (OMe), 77.16 (t, $CDCl_3$), 107.71 ($C^{2''}$, $C^{6''}$), 110.20 (C^5 or C^6), 115.97 (d, J_{CF} = 21.9 Hz, $C^{3'}$, $C^{5'}$.), 120.14 (C^4 or C^7), 124.67 (C^5 or C^6), 126.05 (C^q), 128.72 (C^4 or C^7), 130.16 (C^q), 131.79 (d, J_{CF} = 9.2 Hz, $C^{2'}$, $C^{6'}$) 133.09 (d, J_{CF} = 2.3 Hz, $C^{1'}$), 133.32 (C^q), 141.09 (C^4), 142.21 (C^{β}), 145.92 (C^q), 153.12 (C^3, C^5), 165.51 (d, J_{CF} = 255.0 Hz, $C^{4'}$), 189.66 (C=O); ESI-MS (+) m/z: 434.1 ($[M+H]^+$, 100), 435.3 ($[M+H+1]^+$, 28).

4.1.6. General synthetic procedure for chalcones (1–3)

15 mmol of an acetophenone, 0.1 equiv of $LiOH.H_2O$ and 20 mL of absolute EtOH are brought into a round bottomed flask, and the resulting suspension is stirred for 15 min. Subsequently, 1 equiv of the appropriate benzaldehyde is added and the mixture is stirred until conversion has reached its maximum, as monitored by TLC and/or LC/MS. The reaction is quenched with a 10 mL of a 1% aqueous solution of HCl, followed by 10 mL of H_2O.

When a precipitate is obtained, it is isolated by filtration and thoroughly washed with water until the filtrate turns clear. Recrystallization of the precipitate in absolute EtOH furnishes crystals of chalcone in high purity. If, upon quenching, the product separates as a thick oily layer at the bottom of the bulb, it is extracted with Et_2O (2 × 20 mL). The combined organic layers are subsequently washed with brine (2 × 20 mL) and dried over $MgSO_4$. Solvent evaporation under reduced pressure furnishes the crude chalcone **3** as a solid, which can be purified by recrystallization in absolute EtOH.

4.1.7. Synthesis of 3,5-diaryl-4,5-dihydroisoxazoles (13a–c)

In a round-bottomed flask containing 20 mL of absolute ethanol and kept under a nitrogen atmosphere, 0.1 mmol of a chalcone, 6 equiv (41.7 mg) of $NH_2OH.HCl$ and 7 equiv (39.3 mg) of KOH are added respectively. The resulting mixture is stirred at reflux and under a nitrogen atmosphere for 24 h. Upon cooling of the contents to ambient temperature, an equal volume of H_2O is added and the resulting suspension is extracted with ethyl acetate (2 × 20 mL). The combined organic layers are subsequently dried over $MgSO_4$, and the solvent is removed under reduced pressure. The resulting solid can be recrystallized in absolute ethanol, furnishing the pure isoxazoline **13**.

4.1.8. 3-(4-Fluorophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole (13a)

Yield: 30%, as white crystals; mp: 125.4–126.9 °C; IR (ATR, cm^{-1}) ν_{max}: 832, 896, 1007, 1123, 1218, 1236, 1324, 1426, 1509, 1592; ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ: 3.33 (1H, dd, J_{gem} = 16.5 Hz, J_{vic} = 8.3 Hz, CH_aH_b), 3.75 (1H, dd, J_{gem} = 16.5 Hz, J_{vic} = 10.0 Hz, CH_aH_b), 3.84 (3H, s, OCH_3), 3.87 (6H, s, 2x OCH_3), 5.69 (1H, dd, $J_{vic,1}$ = 10.0 Hz, $J_{vic,2}$ = 8.3 Hz, H^{β}), 6.61 (2H, s, H^2, H^6), 7.11 (2H, dd, J_{vic} = 8.5 Hz, J_{HF} = 8.5 Hz, $H^{3'}$, $H^{5'}$), 7.69 (2H, dd, J_{vic} = 8.8 Hz, J_{HF} = 5.5 Hz, $H^{2'}$, $H^{6'}$); ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 43.51 (C^{α}), 56.33 (2x OCH_3), 60.98 (OCH_3), 77,16 (t, $CDCl_3$), 82.90 (C^{β}), 102.81 (C^2, C^6), 116.07 (d, J_{CF} = 21.9 Hz, $C^{3'}$, $C^{5'}$), 125.80 (d, J_{CF} = 3.5 Hz, $C^{1'}$), 128.82 (d, J_{CF} = 8.1 Hz, $C^{2'}$, $C^{6'}$), 136.49 (C^4), 137.97 (C^1), 153.71 (C^3, C^5), 155.44 (C=N), 163.96 (d, J_{CF} = 251.5 Hz, $C^{4'}$); ESI-MS (+) m/z: 332.1 ($[M+H]^+$, 100), 333.1 ($[M+H+1]^+$, 22), 457.2 (73), 458.2 (23).

4.1.9. 5-(4-Fluorophenyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole (13b)

Yield: 34%, as white crystals; mp: 115.8–117.4 °C; IR (ATR, cm^{-1}) ν_{max} = 826, 838, 914, 1005, 1132, 1215, 1374; ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 3.20 (1H, dd, J_{gem} = 16.5 Hz, J_{vic} = 8.3 Hz, CH_aH_b), 3.78 (1H, dd, J_{gem} = 16.5 Hz, J_{vic} = 10.9 Hz, CH_aH_b), 3.88 (3H, s, OCH_3), 3.89 (6H, s, 2x OCH_3), 5.73 (1H, dd, $J_{vic.1}$ = 10.9 Hz, $J_{vic.2}$ = 8.3 Hz, H^{β}), 6.92 (2H, s, $H^{2'}$, $H^{6'}$), 7.07 (2H, dd, J_{vic} = 8.8 Hz, J_{HF} = 8.8 Hz, H^3, H^5), 7.37 (2H, dd, J_{vic}= 8.8 Hz, J_{HF} = 5.5 Hz, H^2, H^6); ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 43.31 (C^{α}), 56.26 (2x OCH_3), 60.93 (OCH_3), 77.16 (t, $CDCl_3$) 82.08 (C^{β}), 104.14 ($C^{2'}$, $C^{6'}$), 115.67 (d, J_{CF} = 21.9 Hz, C^3, C^5), 124.75 ($C^{1'}$), 127.69 (d, J_{CF} = 8.1 Hz, C^2, C^6), 136.71 (d, J_{CF} = 2.3 Hz, C^1), 139.99 ($C^{4'}$), 153.39 ($C^{3'}$, $C^{5'}$), 156.00 (C=N), 162.59 (d, J_{CF} = 246.9 Hz, C^4); ESI-MS (+) m/z: 332.1 ($[M+H]^+$, 100), 333.0 ($[M+H+1]^+$, 22).

4.1.10. 5-(4-Chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazole (13c)

Spectral data are in full accordance with those reported earlier by Mizuno.[22]

Yield: 94%, as white crystals; mp: 128.3–129.4 °C; IR (ATR, cm^{-1}) ν_{max} = 808, 834, 862, 892, 1021, 1091, 1177, 1254, 1491, 1606, 2853, 2922.03, 2955; ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ 3.27 (1H, dd, J_{gem} = 16.7 Hz, J_{vic} = 7.8 Hz, CH_aH_b), 3.76 (1H, dd, J_{gem} = 16.7 Hz, J_{vic} = 11.0 Hz, CH_aH_b), 3.84 (3H, s, OCH_3), 5.68 (1H, dd, J_1 = 11.0 Hz, J_2 = 7.8 Hz, H^{β}), 6.93 (2H, d, J_{vic} = 9.2 Hz, $H^{3'}$, $H^{5'}$), 7.34 (4H, br. m, H^2, H^6, H^3, H^5), 7.62 (2H, d, J_{vic} = 9.2 Hz, $H^{2'}$, $H^{6'}$); ^{13}C NMR *($CDCl_3$, 75 MHz, ppm)*: δ 42.46 (C^{α}), 54.34 (OCH_3), 80.48 (C^{β}), 113.17 ($C^{3'}$, $C^{5'}$), 120.74 ($C^{1'}$), 126.24 (C^2, C^6), 127.27 ($C^{2'}$, $C^{6'}$), 127.87 (C^3, C^5), 132.91, 138.63 (C^1, C^4), 154.62 (C=N), 160.16 ($C^{4'}$); ESI-HRMS (+) m/z: Anal. Calcd for $C_{16}H_{14}ClNO_2$ $(M+H)^+$: 288.0786. Found: 288.0790.

4.1.10.1. Synthesis of 5-(4-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole (20). In a round-bottomed flask kept under a nitrogen atmosphere, 0.01 mol (3.15 g) of chalcone **2** is dissolved in 20 mL of absolute EtOH. Subsequently, 6 equiv (3 g) of $NH_2NH_2.H_2O$ and 7 equiv (3.91 g) of KOH are added. The resulting mixture is stirred at reflux for 24 h. Upon cooling, water (20 mL) is added and the pyrazole is extracted with EtOAc (2 × 15 mL). The combined organic layers are dried over $MgSO_4$; solvent removal by rotary evaporation furnishes the desired pyrazoline **20** as a solid residue.

4.1.11. Synthesis of isoxazoles (4a–c)

A microwave vial is loaded with 0.9 mmol of an isoxazoline **13**, 6 mL of benzene, 10 equiv (0.89 g) of activated MnO_2 and 2.4 equiv (0.26 g) of $MgSO_4$. Electrolytically precipitated, active MnO_2 (88%) from Acros was used in this reaction. Next, the vial is sealed and heated at 100 °C under microwave irradiation during 1.5 h. Subsequently, the reaction mixture is filtered over Celite®, and the residue is washed with CH_2Cl_2. Solvent removal from the filtrate furnishes the pure isoxazole **4** as a solid residue.

4.1.12. 5-(4-Fluorophenyl)-3-(3,4,5-trimethoxyphenyl) isoxazole (4b)

Yield: 65%, as white crystals; ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ = 3.91 (3H, s, OCH_3), 3.95 (6H, s, 2x OCH_3), 6.75 (1H, s, H^{α}), 7.08 (2H, s, $H^{2''}$, $H^{6''}$), 7.19 (2H, dd, J_{vic} = 8.5 Hz, J_{HF} = 8.5 Hz, $H^{3'}$, $H^{5'}$),

7.84 (2H, ddd, J_{vic} = 8.5 Hz, J_{HF} = 5.1 Hz, J_{allyl} = 1.9 Hz, $H^{2'}$, $H^{6'}$); ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ = 56.20 (2x OCH_3), 61.07 (OCH_3), 77.16 (t, $CDCl_3$), 97.31 (C^{α}), 103.96 ($C^{2''}$, $C^{6''}$), 116.18 (d, J_{CF} = 21.9 Hz, $C^{3'}$, $C^{5'}$), 123.70 (d, J_{CF} = 3.5 Hz, $C^{1'}$), 124.35 ($C^{1''}$), 127.84 (d, J_{CF} = 8.1 Hz, $C^{2'}$, $C^{6'}$), 139.61 ($C^{4''}$), 153.59 ($C^{3''}$, $C^{5''}$), 162.89 (C=N), 163.73 (d, J_{CF} = 251.5 Hz, $C^{4'}$), 169.41 (C^{β}); ESI-HRMS (+) *m*/*z*: Anal. Calcd for $C_{18}H_{16}FNO_4$ $(M+H)^+$: 330.1136. Found: 330.1144.

4.1.13. 5-(4-Chlorophenyl)-3-(4-methoxyphenyl)isoxazole (4c)

Spectral data were in full accordance with those reported earlier by Upadhyay.[23]

ESI-HRMS (+) *m*/*z*: Anal. Calcd for $C_{16}H_{12}ClNO_2$ $(M+H)^+$: 286.0629. Found: 286.0634.

4.1.14. Synthesis of dimethyl [hydroxyl(3,4,5-trimethoxyphenyl)-methyl]phosphonate (22a) and dimethyl [(4-fluorophenyl)-hydroxymethyl]phosphonate (22b)

25 mmol of an appropriate benzaldehyde is dissolved in 25 mL of Et_2O, upon which 2 equiv (4.6 mL) of dimethyl phosphite and 14 mol % (0.6 mL) of *n*-Bu_2NH are added. The mixture is stirred at ambient temperature for 3 h, and the resulting precipitate is isolated by filtration. The residue is subsequently washed with Et_2O, yielding the pure α-hydroxyphosphonate **22**.

4.1.15. Synthesis of 2-(4-fluorophenyl)-1-(3,4,5-trimethoxyphenyl)ethanone (26a) and 1-(4-fluorophenyl)-2-(3,4,5-trimethoxyphenyl)ethanone (26b)

In a round-bottomed flask kept under a nitrogen atmosphere, 10 mmol of phosphonate **22**, 2.2 equiv (2 mL) of 3,4-dihydro-2*H*-pyran and 2.5 mol % (50 mg) of *para*-$TsOH{\cdot}H_2O$ are dissolved in 50 mL of benzene. The resulting mixture is heated to reflux temperature for 2 h. Upon cooling to room temperature, 1 equiv of an appropriate aldehyde, 4 equiv (0.6 g) of NaH and 1.7 equiv (1 mL) of dry EtOH are added. The contents of the flask is stirred during 18 h under a nitrogen atmosphere, after which water is added (30 mL). The organic phase is separated, washed with water (30 mL) and dried over $MgSO_4$. Upon solvent removal via rotary evaporation, the residue is taken up in a mixture of 30 mL of MeOH and 0.95 equiv aq HCl (3 ml of a 10% solution) and stirred at room temperature for 1 h. Next, the flask is stored at −20 °C to allow the ethanone to precipitate as a solid. Pure **26** can be obtained from this crude mixture by chromatographic separation, employing a solvent mixture of EtOAc and petroleum ether in a 2:1 ratio.

4.1.16. Synthesis of 4-(4-fluorophenyl)-5-(3,4,5-trimethoxyphenyl)isoxazole (5a) and 5-(4-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)isoxazole (5b)

A solution 1.61 mmol of ethanone **26** in 10 mL of DMFDMA, kept under a nitrogen atmosphere, is stirred at reflux for 14 h. Next, the solvent is removed under reduced pressure, and the residue is taken up in a mixture of MeOH and H_2O in a 2:1 ratio. Subsequently, 1.96 equiv (0.22 g) of $NH_2OH.HCl$ and 1 equiv (0.17 g) of Na_2CO_3 are added. The pH of the reaction mixture is adjusted to 4–5 by addition of AcOH, whereupon the contents of the flask, still under a nitrogen atmosphere, is heated to reflux for 2 h. Afterward, NH_4OH is added until the pH of the mixture equals 8, and the isoxazole is extracted with CH_2Cl_2. Upon drying over $MgSO_4$, rotary evaporation of the solvent furnishes the crude isoxazole **5**, which can be recrystallized from MeOH.

4.1.17. 4-(4-Fluorophenyl)-5-(3,4,5-trimethoxyphenyl) isoxazole (5a)

Yield: 28%, as white crystals; ^1H NMR ($CDCl_3$, 300 MHz, ppm): δ = 3.72 (6H, s, 2x OCH_3), 3.88 (3H, s, OCH_3), 6.84 (2H, s, $C^{2''}$, $C^{6''}$), 7.13 (2H, dd, J_{vic} = 8.5 Hz, J_{HF} = 8.5 Hz, $H^{3'}$, $H^{5'}$), 7.40 (2H, dd, J_{vic} = 8.5 Hz, J_{HF} = 5.2 Hz, $H^{2'}$, $H^{6'}$), 8.32 (1H, s, H^3); ^{19}F NMR *($CDCl_3$, 282 MHz, ppm)*: δ (−)112.90–(−)112.75 (m); ESI-HRMS (+) *m*/*z*: Anal. Calcd for $C_{18}H_{16}FNO_4$ $(M+H)^+$: 330.1136. Found: 330.1145.

4.2. Molecular modeling

Two-dimensional drawings of compounds were made using ChemDraw Ultra 7.0.1.[24] These structures were subsequently loaded into HyperChem 8.0.3[25] and their refined equilibrium molecular geometry was obtained by (i) 2D–3D conversion using parameters included in HyperChem, (ii) pre-optimization using the molecular mechanics force field (MM+) method and (iii) final optimization at the semi-empirical AM1-level of theory using a Polak-Ribière conjugated gradient and an RMS gradient of 0.01 kcal $Å^{-1}$ mol^{-1} as the termination condition for optimized structures. These lowest energy conformers were used to generate molecular alignments via the 'RMS fit and overlay' algorithm, embedded in HyperChem 8.0.3. The potential energy scan, for which the result is shown in Scheme 1, was obtained in HyperChem 8.0.3 at the AM1 level of theory.

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