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PAPER

Diastereoselective ruthenium porphyrin-catalyzed tandem nitrone formation/1,3-dipolar cycloaddition for isoxazolidines. Synthesis, *in silico* docking study and *in vitro* biological activities†

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Ruthenium porphyrin catalyzes tandem nitrone formation/1,3-dipolar cycloaddition of diazo compounds, nitrosoarenes and alkenes to form isoxazolidines in good to high yields and with excellent regio-, chemo- and diastereo-selectivities. A broad substrate scope of alkenes is applicable to this protocol and various functional groups are compatible with the reaction conditions. *In silico* analysis and *in vitro* biological experiments revealed that some of the new isoxazolidines synthesized in this work could act as leukotriene A4 hydrolase inhibitors.

Introduction

Isoxazolidines are versatile building blocks used in organic synthesis[1–10] and are frequently present in bioactive molecules.[11–15] Through the reductive cleavage of the N–O bond, these five-membered heterocycles can be readily transformed into numerous useful compounds such as 1,3-amino alcohols, amino acids and β-lactams. 1,3-Dipolar cycloaddition of nitrones with alkenes is the most efficient method for the synthesis of isoxazolidines and has been used in the synthesis of nitrogen containing compounds with diversity and complexity. A variety of routes to precursor nitrones have been developed. Among these synthetic methods, the oxidation of amines, acid-catalyzed condensation of N-monosubstituted hydroxylamines with carbonyl compounds, and alkylation of oximes are the most commonly used.[4] In view of the wide utility of isoxazolidines in organic synthesis, there has been a continuing interest to develop a means for the generation of nitrones under neutral and non-oxidative conditions.

Over the past few decades, ruthenium porphyrins have been shown to efficiently catalyze highly selective carbene insertion and transfer reactions.[16–22] It has been reported that reacting ruthenium porphyrin with diazo compounds would furnish ruthenium carbene complexes, some of which have been characterized by X-ray crystallography.[23–27] Recently, we reported that ruthenium porphyrin can decompose diazo compounds in the presence of an imine to form reactive azomethine ylides which can undergo 1,3-dipolar cycloaddition with alkenes.[28,29] Noting the similarity between azomethine ylides and nitrones and the synthetic value of 1,3-dipolar cycloaddition of nitrones, we examined the tandem nitrone formation/1,3-dipolar cycloaddition *via* the ruthenium porphyrin catalyzed reaction of diazo compounds, nitrosoarenes and alkenes, in which cases nitrones are generated *in situ* by the reaction of ruthenium–carbene with nitroso compounds. An example of HOTf-catalyzed 1,3-dipolar cycloaddition of an α-diazo ester, nitrosobenzene and electron-deficient alkenes has recently been reported,[30] but the alkenes were confined to electron-deficient ones and strong acid was employed in the reaction. *The method described herein features a broad scope of alkene substrates, excellent compatibility of various functionalities, high chemo-, regio- and diastereo-selectivity and neutral reaction conditions, all of these features are particularly valuable in organic synthesis. In silico* docking study and *in vitro* biochemical experiments revealed that some of the new isoxazolidines synthesized in this work could act as leukotriene A4 hydrolase inhibitors.

Results and discussion

1. Ruthenium porphyrin-catalyzed tandem nitrone formation/1,3-dipolar cycloaddition of diazo compounds, nitrosoarenes and alkenes

1.1. Intermolecular 1,3-dipolar cycloaddition. At the outset, we examined the cycloaddition reaction of ethyl α-diazo acetate (EDA), nitrosobenzene **2a** and *N*-phenylmaleimide **3a** using [Ru(TTP)(CO)(MeOH)] (H_2TTP = *meso*-tetrakis(4-tolyl)porphyrin)

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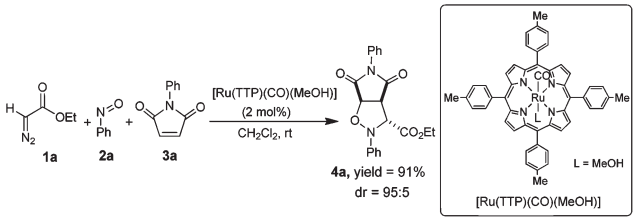
†Electronic supplementary information (ESI) available: Experimental procedures, compound characterization. See DOI: 10.1039/c2ob26518d



Scheme 1

as a catalyst. Slow addition of a CH_2Cl_2 solution of EDA to a mixture of **2a**, **3a** and [Ru(TTP)(CO)(MeOH)] in CH_2Cl_2 *via* a syringe pump afforded the cycloadduct **4a** in 91% yield with a diastereoselectivity of 95 : 5 (Scheme 1). The structure of **4a** is inferred by the X-ray crystal structure of its analogue **5a** (see ESI†). [Fe(TTP)Cl] and [Co(TTP)] were less active than [Ru(TTP)(CO)(MeOH)] resulting in low substrate conversion (71–74%) and product yield (36–47%) under the same conditions (see ESI, Table S1†). When [Ru(*p*-cymene)Cl_2]$_2$ was used as a catalyst, a low product yield of 9% was obtained. The activities of a panel of rhodium and copper catalysts towards this cycloaddition were also examined. $Rh_2(OAc)_4$, $Cu(OTf)_2$ and $[Cu(CH_3CN)_4]PF_6$ gave the product **4a** in 53–60% yields. A trace amount of a cycloadduct (<5%) produced by the reaction of EDA, **2a** and ethyl maleate (which was generated by the dimerization of EDA), and an unidentified mixture were found in the rhodium and copper catalysis. Doyle and co-workers recently found that nitrones can coordinate to Cu(I) salts.[31] Such a coordination would reduce the efficiency of the copper catalyst in the 1,3-dipolar cycloaddition of EDA, **2a** and **3a**. Solvent screening revealed that CH_2Cl_2 was the best solvent. Reducing the loading of [Ru(TTP)(CO)(MeOH)] to 1 mol% did not affect the product yield.

With the optimal conditions, the substrate scope of [Ru(TTP)(CO)(MeOH)]-catalyzed 1,3-dipolar cycloaddition of EDA, **2a** and unsaturated compounds was examined. As depicted in Table 1, a broad array of alkenes including electron-rich, electron-deficient and electron-neutral ones are reactive dipolarophiles to undergo the cycloaddition reaction with high regio- and diastereo-selectivities. For example, the Ru-catalyzed cycloaddition reaction of **1a** and **2a** with ethyl maleate and methyl fumarate gave the cycloadducts **4b** and **4c** in 95% and 90% yield respectively both with a dr value of 95 : 5 (entries 1, 2). The unsymmetric alkene **3d** also underwent the cycloaddition with excellent diastereoselectivity (entry 3). The Boc functionality in **3d**, which is well-documented to be acid sensitive, remained intact in this ruthenium catalysis.

Besides electron-deficient alkenes, this ruthenium catalysis can be extended to styrenes and vinyl acetate giving corresponding products in good to high yields with good to excellent stereoselectivities (entries 4–7). The reaction is highly regioselective to exclusively afford 3,5-isoxazolidines. The stereochemistry of the major isomers was determined by NOE experiments to have a *cis* configuration (see ESI†). In this work, *HOTf was found to fail to catalyze the cycloaddition reaction of **1a**, **2a** and styrene.* No cyclopropanation of styrenes and vinyl acetate was observed under the Ru-catalyzed conditions, revealing that the tandem nitrone formation/1,3-dipolar cycloaddition is much faster than cyclopropanation.

This ruthenium catalysis also worked well when aliphatic alkenes were used (entries 8–12) giving *cis*-3,5-isoxazolidines in 63–94% yields and with high regio- and diastereo-selectivities. When allyl bromide, allyl alcohol or 3-buten-1-ol was used as a dipolarophile, no ylide formation/2,3-sigmatropic rearrangement or O–H insertion was observed (entries 8–10). *When HOTf was used as a catalyst, the cycloaddition reaction of **1a**, **2a** and allyl alcohol led to low product yield (~20%).*

Alkyne is also reactive towards this Ru-catalyzed cycloaddition; dimethyl acetylenedicarboxylate **3n** underwent the cycloaddition to give the cycloadduct **4n** in 81% yield (entry 13).

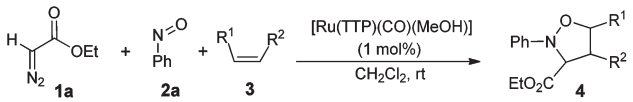
The effect of the substituent(s) of nitrosoarenes on the cycloaddition was investigated. The results are depicted in Table 2. *para*-Electron-withdrawing groups (NO_2, CO_2Et, Cl, Br) gave corresponding cycloadducts in 76–93% yields and dr values of 95 : 5 (entries 1–4). However, the electron-donating group (*p*-OMe) and the *ortho*-substituent (*o*-Me) were found to retard the cycloaddition to give products in low yields (entries 6–7), and the major by-product was the dimer derived from the coupling of EDA. The heteroaromatic nitroso compound is also reactive towards the Ru-catalyzed 1,3-dipolar cycloaddition. For example, 6-methyl-2-nitrosopyridine **2i**, which was reported to be an effective dienophile in the asymmetric nitroso Diels–Alder reaction,[32,33] reacted with EDA and **3a** in the presence of [Ru(TTP)(CO)(MeOH)] to give **5h** in 93% yield as a single isomer (entry 8).

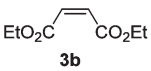
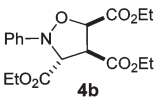
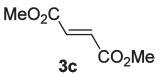
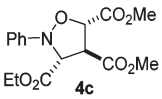
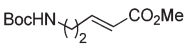
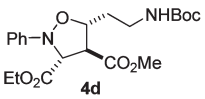
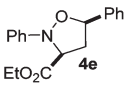
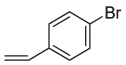
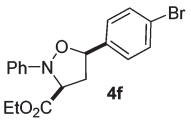
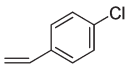
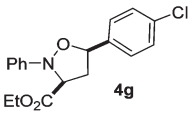
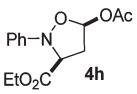
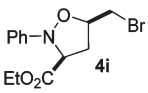
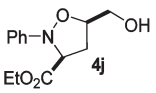
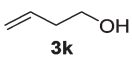
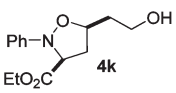
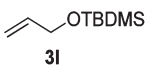
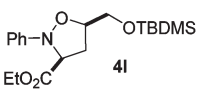
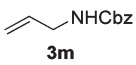
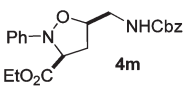
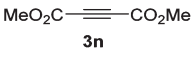
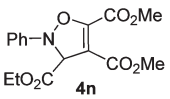
As depicted by entry 9 of Table 2, bulky *t*-butyl diazoacetate can also undergo the cycloaddition to give **5i** in high yield and high diastereoselectivity. Diazoketone is also reactive towards the cycloaddition. The reaction of 1-diazo-6-phenylhexan-2-one with nitrosobenzene and *N*-phenylmaleimide gave cycloadduct **5j** in 82% yield with a dr value of 98 : 2 (entry 10). Intramolecular C–H bond insertion of 1-diazo-6-phenylhexan-2-one, which has been reported to take place in $Rh_2(OAc)_4$ catalysis, has not been observed.[34]

Similarly, substituted nitrosobenzenes and 6-methyl-2-nitrosopyridine also underwent 1,3-dipolar cycloaddition with allyl alcohol and Cbz-protected allyl amine giving corresponding cycloadducts in 62–94% yields and with dr values of 85 : 15 to 99 : 1 (entries 11–15). Cycloadduct **5o** (entry 15), which is a key intermediate used in the synthesis of negamycin, a naturally occurring antibiotic, was obtained in 83% yield and with excellent diastereoselectivity (dr > 99 : 1), the latter is much better than the previously reported dr value of 3 : 2 observed in 1,3-dipolar cycloaddition of *N*-benzyl-*C*-alkoxycarbonylnitrones with Cbz-protected allyl amine.[35,36]

It is noteworthy that the Ru-catalyzed 1,3-dipolar cycloaddition is compatible with a variety of functional groups including ester, hydroxyl, halo, nitro and Cbz as well as acid-sensitive functionalities such as Boc, TBDMS and *t*-butyl ester as depicted in Tables 1 and 2. In addition, the catalysis could be scaled up as demonstrated by the following experiment. A one pot reaction using 2 g of EDA (17.5 mmol), 2.3 g of **2a** (21 mmol) and 3.6 g of **3a** (21 mmol) with 0.1 mol% [Ru(TTP)(CO)(MeOH)] as a catalyst gave **4a** (4.8 g, 13.1 mmol) in 75% yield and 100% substrate conversion, corresponding to a product turnover number of 750. The TLC showed that the catalyst was not exhausted after the reaction was completed.

Table 1 1,3-Dipolar cycloaddition with various dipolarophiles[a]

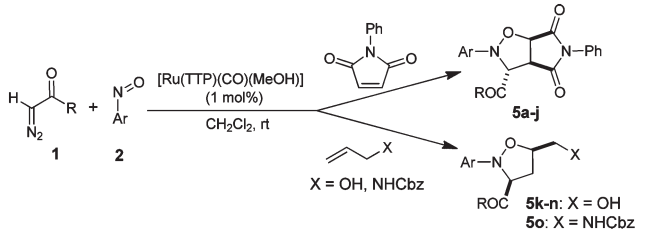


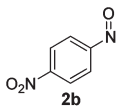
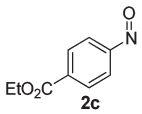
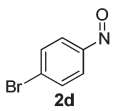
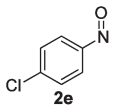
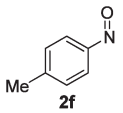
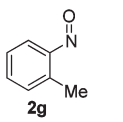
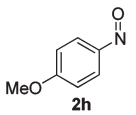
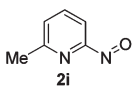
Entry	Dipolarophile	Product	Yield[b] (%)	dr[d]
1	 **3b**	 **4b**	95	95 : 5
2	 **3c**	 **4c**	90	95 : 5
3[c]	 **3d**	 **4d**	55	98 : 2
4	 **3e**	 **4e**	75	86 : 14
5[c]	 **3f**	 **4f**	63	82 : 18
6	 **3g**	 **4g**	77	80 : 20
7	 **3h**	 **4h**	96	98 : 2
8	 **3i**	 **4i**	82	95 : 5
9	 **3j**	 **4j**	94	95 : 5
10	 **3k**	 **4k**	88	95 : 5
11	 **3l**	 **4l**	63	95 : 5
12	 **3m**	 **4m**	91	95 : 5
13	 **3n**	 **4n**	81	

[a] **1a** : **2a** : **3** : [Ru(TTP)(CO)(MeOH)] = 1 : 2 : 2 : 0.01. [b] Isolated yield. [c] 40 °C. [d] Determined by ^{1}H NMR.

1.2. Intramolecular 1,3-dipolar cycloaddition. The tandem nitrone formation/intramolecular 1,3-dipolar cycloaddition for the synthesis of structurally complex isoxazolidines has also been investigated. When allyl α-diazoacetate **6** was treated with **2a** in the presence of [Ru(TTP)(CO)(MeOH)] (1 mol%) at −20 °C, the bicyclic isoxazolidine **7a** was obtained in 57% yield along with 16% of intramolecular cyclopropanation product (Scheme 2, eqn (1)). Compared with **6**, butenyl α-diazoacetate **8** is less reactive as a higher temperature is required for the cycloaddition reaction giving a mixture of fused isoxazolidine **9a** and

Table 2 1,3-Dipolar cycloaddition with various diazo compounds and nitrosoarenes[a]



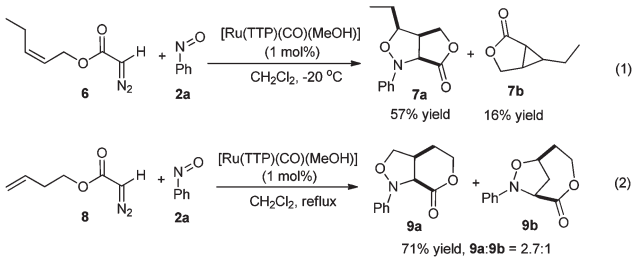
Entry	R	Ar	Product	Yield[b] (%)	dr[c]
1	EtO	 **2b**	**5a**	88	95 : 5
2	EtO	 **2c**	**5b**	76	95 : 5
3	EtO	 **2d**	**5c**	93	95 : 5
4	EtO	 **2e**	**5d**	87	95 : 5
5	EtO	 **2f**	**5e**	79	95 : 5
6	EtO	 **2g**	**5f**	24	95 : 5
7	EtO	 **2h**	**5g**	<5	95 : 5
8	EtO	 **2i**	**5h**	93	>99 : 1
9	^{t}BuO	**2a**	**5i**	73	95 : 5
10	$Ph(CH_2)_4$	**2a**	**5j**	82	98 : 2
11	EtO	**2b**	**5k**	62	95 : 5
12	EtO	**2d**	**5l**	94	95 : 5
13	EtO	**2e**	**5m**	89	95 : 5
14	EtO	**2i**	**5n**	65	85 : 15
15	EtO	**2i**	**5o**	83	>99 : 1

[a] **1** : **2** : **3** : [Ru(TTP)(CO)(MeOH)] = 1 : 2 : 2 : 0.01. [b] Isolated yield. [c] Determined by ^{1}H NMR.

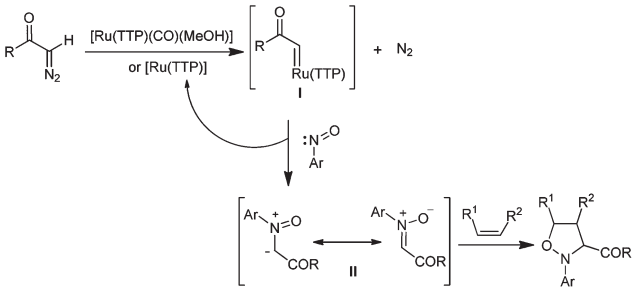
its bridged regioisomer **9b** in a total 71% yield with a ratio of **9a** : **9b** = 2.7 : 1 and without intramolecular cyclopropanation being observed (Scheme 2, eqn (2)).

2. Reaction mechanism and stereoselectivity

2.1. Reaction mechanism. A tentative mechanism is proposed in Scheme 3. Ruthenium(II) porphyrin decomposes a



Scheme 2

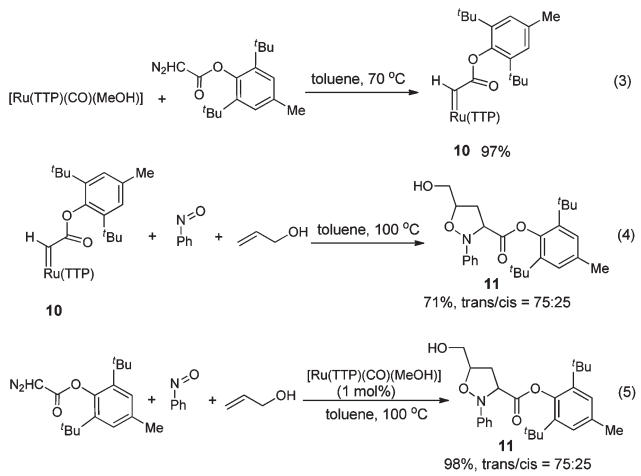


Scheme 3

diazo compound to generate ruthenium–carbene complex **I**.[23–27] Such an intermediate is generally conceived to be reactive and unstable. Recently, the stable derivatives of intermediate **I** have been reported by us[23] and Simonneaux.[24] In Simonneaux's work, a sterically bulky diazo compound 2,6-di-*tert*-butyl-4-methylphenyl diazoacetate reacted with [Ru(TPP)(CO)] to produce a kinetically stable ruthenium porphyrin carbene complex in which the bulky ester group of the carbene ligand protects the carbene group from reaction with incoming substrate. In our approach, the ruthenium complex supported by the sterically encumbered bis-pocket porphyrin ligand 5,10,15,20-tetrakis(2,4,6-triphenylphenyl)porphyrinato(2−) (ttppp) reacted with EDA to generate the corresponding carbene complex which has been isolated and structurally characterized. Both of these two carbene complexes are able to mediate the cyclopropanation with styrene supporting that intermediate **I** is involved in the ruthenium porphyrin catalyzed carbene transfer reaction.

Owing to the lone pair of electrons on the nitrogen atom, nitrosoarenes can act as nucleophiles[37] to attack **I** giving nitrone intermediate **II** and simultaneously regenerating the ruthenium catalyst. This *in situ* generated nitrone undergoes 1,3-dipolar cycloaddition with alkenes to give isoxazolidines. The nitrone intermediate has been detected by ^{1}H NMR and MS in the course of the [Ru(TTP)(CO)(MeOH)] catalyzed reaction of EDA with **2a**; subsequent reaction of **3a** with the nitrone intermediate gave **4a** in 88% yield.

To provide further evidence on the generation of nitrones from the reaction of metallocarbenes with nitrosoarenes, we synthesized the ruthenium porphyrin carbene complex **10** according to the literature[23,24] and used it as a stoichiometric reagent to react with nitrosobenzene and allyl alcohol. Treatment of [Ru(TTP)(CO)(MeOH)] with excess of the bulky diazo compound 2,6-di-*tert*-butyl-4-methylphenyl diazoacetate in toluene at 70 °C afforded **10** in 97% yield (Scheme 4, eqn (3)). When a



Scheme 4

stoichiometric amount of **10** was treated with a solution of nitrosobenzene and allyl alcohol in toluene at 100 °C, the cycloadduct **11** was obtained as an isomeric mixture with a *trans*/*cis* ratio of 75 : 25 and in overall 71% yield (Scheme 4, eqn (4)). This result is comparable to that obtained under catalytic conditions (Scheme 4, eqn (5)). Due to the bulkiness of 2,6-di-*tert*-butyl-4-methylphenyl diazoacetate, a higher temperature is required for the cycloaddition, which may account for the moderate diastereoselectivity. This experiment offers experimental evidence on the intermediacy of metal–carbene in the 1,3-dipolar cycloaddition.

2.2. *E*/*Z* isomerisation of nitrones. *N*-Methyl and *N*-benzyl-*C*-alkoxycarbonylnitrones are known to undergo *E*/*Z* isomerisation with an *E*/*Z* ratio from 0.67 : 1 to 6 : 1 depending on the solvent used. This low isomeric ratio presumably accounts for the moderate diastereoselectivity (dr = 1 : 1–5.9 : 1) found in the 1,3-dipolar cycloaddition of *N*-alkyl-*C*-alkoxycarbonylnitrones with alkenes.[38–41] In contrast, high diastereoselectivity (dr ≈ 95 : 5) has been achieved in our ruthenium porphyrin catalysis wherein an *N*-aryl-*C*-alkoxycarbonylnitrone is involved. Analysis of the reaction mixture of [Ru(TTP)(CO)(MeOH)] (1 mol%), EDA (1 equiv.) and **2a** (1.1 equiv.) by ^{1}H NMR spectroscopy after a reaction time of 3.5 h revealed that the isomeric ratio of the nitrone was high (≈ 93 : 7) (see ESI†). This is advantageous in reducing the number of possible cycloaddition products. Unlike *N*-alkyl-*C*-alkoxycarbonylnitrones, *N*-aryl-*C*-alkoxycarbonylnitrones were found not prone to undergo *E*/*Z* isomerisation in solution. When *N*-phenyl-*C*-ethoxycarbonylnitrones were dissolved in toluene, THF, or acetone, respectively for 2 h, no change in the *E*/*Z* ratio was observed. Heating a solution of *N*-phenyl-*C*-ethoxycarbonylnitrone in toluene at 60 °C for 1 h did not change the *E*/*Z* ratio. This finding is consistent with the result of calculation that the *E*/*Z* isomerisation requires a high energy barrier of more than 55 kcal mol^{-1}. In line with these results, the Ru-catalyzed 1,3-dipolar cycloaddition of EDA, **2a** and **3a** in THF or toluene gave the product with the same diastereoselectivity as that for the reaction conducted in CH_2Cl_2.

2.3 DFT calculation on the diastereoselectivity of the 1,3-dipolar cycloaddition. A DFT calculation was performed at the

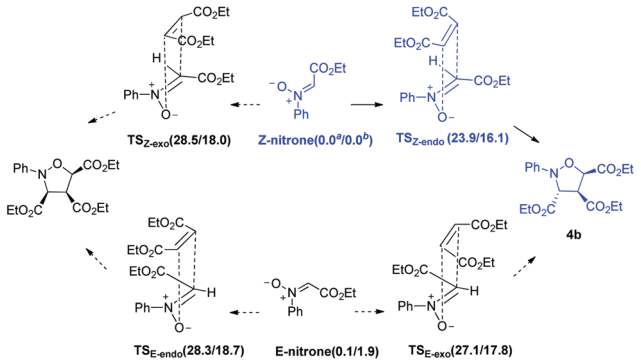


Fig. 1 Calculated free energies (kcal mol^{-1}) of transition states for the cycloaddition of *N*-phenyl-*C*-ethoxycarbonylnitrone with ethyl maleate at the B3LYP/6-31G(d) level; [a]free energies in the gas phase and [b]solvation (CH_2Cl_2) free energies from single point calculations at the UAHF-CPCM-B3LYP/6-31G(d) level using the optimized geometry in the gas phase.

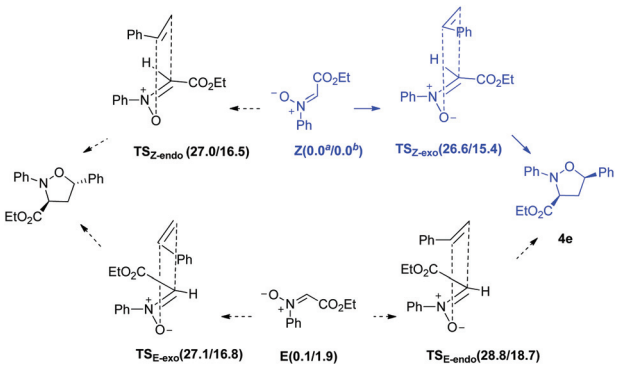


Fig. 2 Calculated free energies (kcal mol^{-1}) of transition states for the cycloaddition of *N*-phenyl-*C*-ethoxycarbonylnitrone with styrene at the B3LYP/6-31G(d) level; [a]free energies in the gas phase and [b]solvation (CH_2Cl_2) free energies from single point calculations at the UAHF-CPCM-B3LYP/6-31G(d) level using the optimized geometry in the gas phase.

B3LYP/6-31G(d) level to provide insight into the stereoselectivity of the cycloaddition (see Fig. 1 and 2). The computational study showed that *Z*-nitrone and *E*-nitrone are energetically neutral in the gas phase. In CH_2Cl_2, *Z*-nitrone is more stable than the *E*-nitrone by 1.9 kcal mol^{-1}. As shown in Fig. 1, when ethyl maleate is used as a dipolarophile, *endo*-cycloaddition with *Z*-nitrone *via* TS$_{Z\text{-}endo}$, leading to 3,4,5-*trans,cis*-isoxazolidine **4b**, is the most favoured pathway. This result is consistent with the stereochemical outcome of our experiments. Styrene prefers to undergo *exo*-cycloaddition with *Z*-nitrone to give 3,5-*cis*-cycloadduct **4e** as the major product (Fig. 2), but the difference (1.1 kcal mol^{-1}) between the free energies of the TS$_{Z\text{-}exo}$ and TS$_{Z\text{-}endo}$ transition states is small compared to that of the reaction when ethyl maleate was used instead of styrene (1.9 kcal mol^{-1}). This would account for the moderate diastereoselectivity obtained in the 1,3-dipolar cycloaddition of styrene with EDA and **2a**.

3. *In silico* docking study and *in vitro* biological activities of the isoxazolidines

Multi-component coupling reactions could be used for the synthesis of structurally diverse compounds for drug discovery. In this work, a library of functionalized isoxazolidines are rapidly synthesized from simple starting materials by a three component coupling reaction. To examine the potential biological applications of isoxazolidines prepared by this one-pot three component coupling reaction, bioinformatics and computational modeling studies were undertaken.

Table 3 Top ranked target sets with compounds chemically similar to isoxazolidine **4e**

Target sets	*E*-value*
DD-carboxypeptidase	3.34×10^{-45}
ADAM9	1.06×10^{-10}
Dopamine transporter	2.87×10^{-10}
Muscarinic acetylcholine receptor M5	2.57×10^{-9}
FK506-binding protein 1A	1.26×10^{-7}
Muscarinic acetylcholine receptor M4	1.63×10^{-5}
Muscarinic acetylcholine receptor M1	2.06×10^{-5}
Sodium-, chloride-dependent GABA transporter	2.22×10^{-5}
Kappa opioid receptor	8.96×10^{-5}
Norepinephrine transporter	1.02×10^{-4}
Sigma opioid receptor	1.08×10^{-3}
Papain	1.81×10^{-3}
Leukotriene A4 hydrolase	2.56×10^{-3}
Adenosine A3	3.44×10^{-3}
Elastase 1	3.91×10^{-3}
Lactam (beta) antibiotic	7.60×10^{-3}
Aldehyde dehydrogenase 1A2	8.49×10^{-3}
Muscarinic acetylcholine receptor M3	9.13×10^{-3}

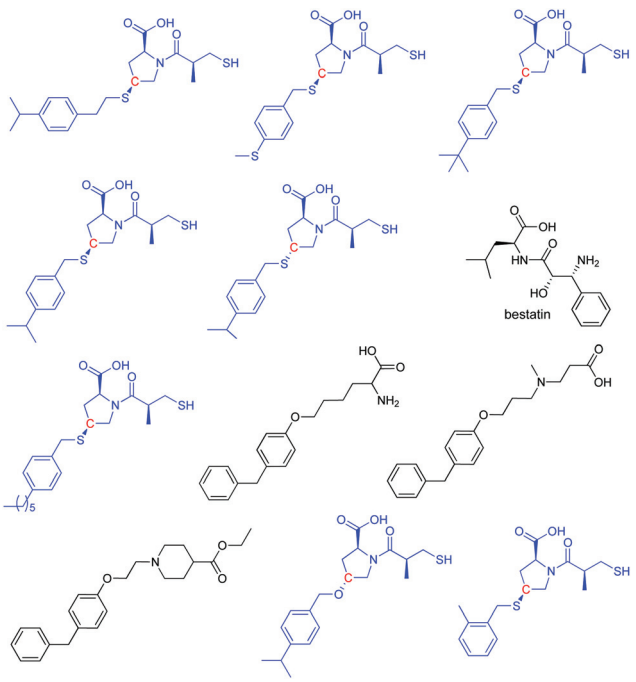


Fig. 3 The top twelve leukotriene A4 hydrolase inhibitors that show chemical similarity to **4e**.

Chemical similarity analysis[42] revealed that **4e** is "chemically similar" to 18 diverse classes of known inhibitors revealing that this compound may have multiple potential targets and could be poly-pharmacology in nature. The details of chemical similarity analysis are given in the Experimental section and ESI.† Out of the 18 potential targets (Table 3), both the *in vitro* screening assay and the X-ray crystal structure of leukotriene A4 hydrolase (LTA4H) are available to us. We therefore chose this target for further study even though it is not the first ranked one. LTA4H is an epoxide hydrolase that catalyzes the final step in the biosynthesis of leukotriene B4, and also exhibits aminopeptidase activity. Inhibitors of LTA4H are potentially useful for treatment of cancer and the inflammatory diseases.[43,44]

The top ranked 12 LTA4H inhibitors that show chemical similarity to **4e** are shown in Fig. 3. We note that most of these LTA4H inhibitors have structural variation at the γ position of the nitrogen atom. In light of these results, we computed the

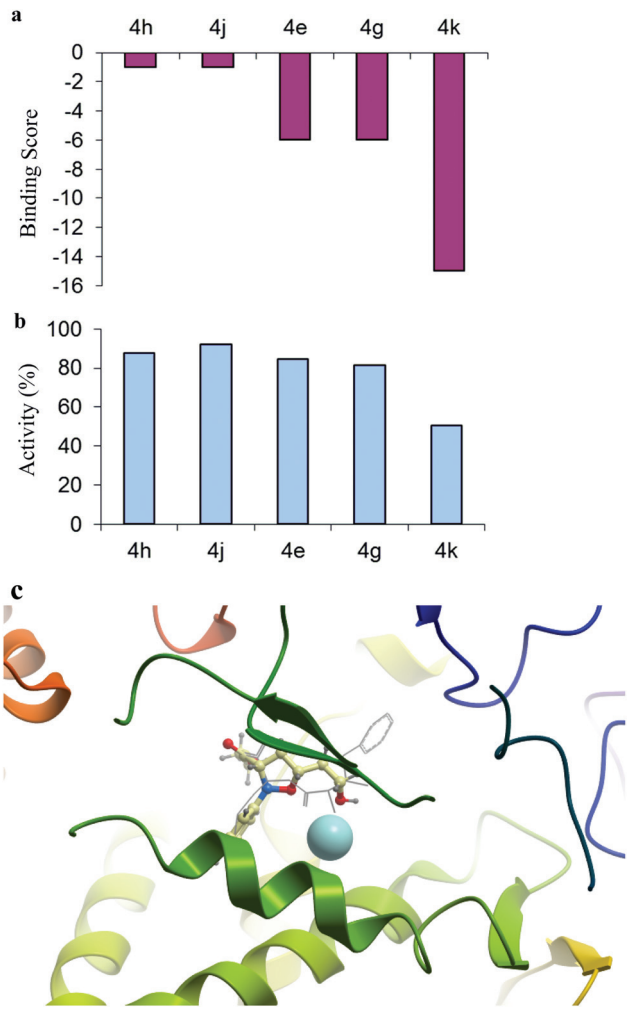


Fig. 4 (a) Binding scores of isoxazolidines to the LTA4H. (b) The aminopeptidase activities of LTA4H treated with 2 mM isoxazolidines compared to that of 0.2% DMSO vehicle control. (c) Schematic model of the binding model of the known inhibitor (bestatin) (grey), and isoxazolidine **4k** (ball-and-stick, yellow = carbons, red = oxygen, blue = nitrogen, Zn^{2+} = blue sphere). The leukotriene A4 hydrolase is represented as a ribbon model.

binding interactions of the five isoxazolidines (**4e**, **4g**, **4j**, **4h** and **4k**) to LTA4H using the program ICM (molsoft LLC).[45] These five isoxazolidines are structurally similar but with different substitutions at the C5 position (γ position of the nitrogen atom), thus allowing the examination of the effect of the C5-substituent of isoxazolidine on the binding score. In addition, these compounds can be readily synthesized in high yields by the [Ru(TTP)(CO)(MeOH)]-catalyzed reaction of EDA, nitrosobenzene and alkenes described in this work. We note that varying the C5-substituent of isoxazolidine affects the binding score (Fig. 4a). The **4j** (–CH_2OH) and **4h** (–OAc) with a hydrophilic C5-substituent has negligible binding energy with LTA4H (average binding energies = −1 kcal mol^{-1}). On the other hand, replacing the C5-substituent with a hydrophobic group as in **4e** (–Ph) and **4g** (–PhCl) renders the isoxazolidines capable of favourably binding (average binding energies = −6 kcal mol^{-1}) to the active site of LTA4H. Notably, **4k** (–CH_2CH_2OH) was found to have the most favourable binding interaction with LTA4H (−15 kcal mol^{-1}). As depicted in Fig. 4c, the binding pocket occupied by **4k** is the same as that of a potent inhibitor;[46] the longer C5-substituent (–CH_2CH_2OH) renders **4k** capable of chelating with the catalytic Zn^{2+} in the active site.

Our docking result has been supported by the following experimental data (Fig. 4b). LTA4H was incubated with the isoxazolidines (2 mM), and the residual aminopeptidase activity of LTA4H compared to the vehicle control was found to be, respectively, 50% for **4k**, ~80% for **4e** and **4g**, and ~90% for **4h** and **4j**. Since various functional groups are compatible with the reaction conditions of the aforementioned [Ru(TTP)(CO)(MeOH)] catalysed three component coupling reaction, we envision that further optimizing the structure of isoxazolidine as a leukotriene A4 hydrolase inhibitor or other potential inhibitors (see Table 3) would be feasible.

Conclusion

We have developed an efficient ruthenium porphyrin-catalyzed tandem nitrone formation/1,3-dipolar cycloaddition reaction of diazo compounds with nitrosoarenes and alkenes. A broad substrate scope of alkenes is applicable to this protocol and various functional groups are compatible with the reaction conditions. Our *in silico* analysis and *in vitro* biochemical experiments illustrate the potential use of the newly developed synthetic method on the optimization of isoxazolidines as better leukotriene A4 hydrolase inhibitors.

Experimental section

Reagents were obtained commercially and used without further purification unless indicated otherwise. All solvents used in the reaction were dried and freshly distilled. [Ru(TTP)(CO)(MeOH)] was prepared according to the reported procedure.[47,48] Flash chromatography was performed using Merck silica gel 60 and a gradient solvent system (EtOAc–*n*-hexane as an eluent). ^1H and ^{13}C NMR spectra were measured on either a Bruker DPX-500, DPX-400 or DPX-300 spectrometer. Chemical shifts (δ ppm) were determined with tetramethylsilane (TMS) as an internal reference. Mass spectra were determined on a Finnigan MAT 95 mass spectrometer.

General procedure for tandem nitrone formation/1,3-dipolar cycloaddition of diazo compounds, nitrosoarenes and alkenes catalyzed by [Ru(TTP)(CO)(MeOH)]

To nitrosoarene (1.0 mmol), alkene (1.0 mmol) and [Ru(TTP)(CO)(MeOH)] (0.005 mmol) in CH_2Cl_2 (5 mL) was added diazo compounds (0.5 mmol) in CH_2Cl_2 (5 mL) over 4 h *via* a syringe pump at room temperature. After addition, the resultant solution was stirred for an additional 0.5–3 h. The solvent was removed and the crude residue was purified by silica gel column chromatography to give the corresponding cycloadduct.

Ethyl 4,6-dioxo-2,5-diphenylhexahydro-2*H*-pyrrolo[3,4-*d*]-isoxazole-3-carboxylate (4a). Oil, yield: 90%; ^1H NMR ($CDCl_3$, 400 MHz), δ 1.30 (t, J = 7.1 Hz, 3H), 4.23–4.33 (m, 2H), 4.41 (d, J = 7.6 Hz, 1H), 5.16 (d, J = 7.6 Hz, 1H), 5.25 (s, 1H), 6.52–6.54 (m, 2H), 7.03 (t, J = 7.3 Hz, 1H), 7.14 (d, J = 7.9 Hz, 2H), 7.25–7.31 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 13.99, 51.28, 62.72, 68.17, 77.37, 114.38, 123.60, 126.02, 128.96, 129.44, 130.66, 147.97, 167.98, 172.33, 173.63; EIMS m/z 366.1 (M^+); HRMS (EI) for $C_{20}H_{18}O_5N_2$, calcd 366.1216, found 366.1203.

Ethyl 5-acetoxy-2-phenylisoxazolidine-3-carboxylate (4h). Oil, yield: 96%; ^1H NMR ($CDCl_3$, 400 MHz), δ 1.34 (t, J = 7.2 Hz, 3H), 2.08 (s, 3H), 2.78–2.81 (m, 2H), 4.17–4.21 (m, 1H), 4.29–4.36 (m, 2H), 6.58–6.60 (m, 1H), 7.04 (t, J = 7.6 Hz, 1H), 7.10 (d, J = 7.6 Hz, 2H), 7.27–7.30 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 14.28, 21.26, 38.61, 62.06, 66.67, 94.56, 116.16, 123.58, 128.90, 150.00, 170.12, 170.32; EIMS m/z 279.1 (M^+); HRMS (EI) for $C_{14}H_{17}O_5N$, calcd 279.1107, found 279.1094.

Ethyl 5-(hydroxymethyl)-2-phenylisoxazolidine-3-carboxylate (4j). Oil, yield: 94%; ^1H NMR ($CDCl_3$, 400 MHz), δ 1.30 (t, J = 7.2 Hz, 3H), 2.46–2.59 (m, 2H), 2.82 (brs, 1H), 3.71–3.74 (m, 1H), 3.91–3.94 (m, 1H), 4.26 (q, J = 7.2 Hz, 2H), 4.33–4.43 (m, 2H), 6.97 (t, J = 7.2 Hz, 1H), 7.06 (d, J = 8.4 Hz, 2H), 7.27 (t, J = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 14.16, 33.63, 61.87, 62.22, 67.86, 78.56, 114.63, 122.50, 129.10, 150.39, 171.70; EIMS m/z 251.1 (M^+); HRMS (EI) for $C_{13}H_{17}O_4N$, calcd 251.1158, found 251.1152.

Ethyl 5-(2-hydroxyethyl)-2-phenylisoxazolidine-3-carboxylate (4k). Oil, yield: 88%; ^1H NMR ($CDCl_3$, 400 MHz), δ 1.31 (t, J = 7.2 Hz, 3H), 1.98–2.03 (m, 2H), 2.35–2.42 (m, 2H), 2.58–2.64 (m, 1H), 3.81 (t, J = 5.6 Hz, 2H), 4.24–4.29 (m, 3H), 4.35 (dd, J = 8.8, 5.6 Hz, 1H), 6.95 (t, J = 7.6 Hz, 1H), 7.04 (d, J = 8.0 Hz, 2H), 7.27 (t, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 14.23, 35.35, 38.48, 60.07, 61.90, 68.12, 76.63, 114.24, 122.14, 129.15, 151.13, 171.76; EIMS m/z 265.1 (M^+); HRMS (EI) for $C_{14}H_{19}O_4N$, calcd 265.1314, found 265.1309.

Ethyl 2-(6-methylpyridin-2-yl)-4,6-dioxo-5-phenylhexahydro-2*H*-pyrrolo[3,4-*d*]isoxazole-3-carboxylate (5h). Oil, yield: 93%; ^1H NMR ($CDCl_3$, 400 MHz), δ 1.36 (t, J = 7.1 Hz, 3H), 2.37 (s, 3H), 4.28–4.40 (m, 2H), 4.47 (d, J = 7.6 Hz, 1H), 5.13 (d, J = 7.6 Hz, 1H), 6.50–6.53 (m, 3H), 6.81 (d, J = 7.2 Hz, 1H), 7.20

(d, J = 8.1 Hz, 1H), 7.29–7.31 (m, 3H), 7.50 (t, J = 7.9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 14.32, 24.16, 50.95, 62.89, 64.19, 78.62, 108.75, 118.92, 125.94, 129.07, 130.87, 139.06, 157.02, 158.85, 169.37, 172.46, 173.20; EIMS m/z 381.1 (M^+); HRMS (EI) for $C_{21}H_{19}O_5N_3$, calcd 381.1325, found 381.1312.

Ethyl 5-((((benzyloxy)carbonyl)amino)methyl)-2-(6-methylpyridin-2-yl)isoxazolidine-3-carboxylate (5o). Oil, yield: 83%; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 1.26 (t, J = 7.1 Hz, 3H), 2.31–2.53 (m, 5H), 3.46–3.60 (m, 2H), 4.21 (q, J = 7.0 Hz, 2H), 4.28–4.39 (m, 2H), 5.16 (dd, J = 12.2, 4.9 Hz, 2H), 5.40 (bs, 1H), 5.50 (dd, J = 9.4, 3.4 Hz, 1H), 6.73 (d, J = 7.3 Hz, 1H), 7.00 (d, J = 8.1 Hz, 1H), 7.30–7.36 (m, 5H), 7.45 (t, J = 7.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 14.24, 24.29, 34.24, 42.40, 61.69, 62.69, 66.92, 106.88, 117.44, 128.13, 128.19, 128.58, 136.52, 138.43, 156.74, 156.79, 160.39, 172.14; EIMS m/z 399.1 (M^+); HRMS (EI) for $C_{21}H_{25}N_3O_5$, calcd 399.1794, found 399.1801.

[Ru(TTP)(CO)(MeOH)]-catalyzed nitrone formation/intramolecular 1,3-dipolar cycloaddition of allyl α-diazoacetate 6 with nitrosobenzene

To nitrosobenzene (0.8 mmol) and [Ru(TTP)(CO)(MeOH)] (0.002 mmol) in CH_2Cl_2 (0.5 mL) was added diazo compound **6** (0.2 mmol) in CH_2Cl_2 (1 mL) over 2 h *via* a syringe pump at −20 °C. After addition, the resultant solution was stirred for an additional 14 h. The solvent was removed and the crude residue was purified by silica gel column chromatography to give cycloadduct **7a** as a solid in 57% yield.

3-Ethyl-1-phenyltetrahydrofuro[3,4-*c*]isoxazol-6(3*H*)-one (7a). ^{1}H NMR ($CDCl_3$, 400 MHz), δ 1.09 (t, J = 7.6 Hz, 3H), 1.61–1.72 (m, 1H), 1.78–1.89 (m, 1H), 3.37–3.44 (m, 1H), 4.06–4.11 (m, 1H), 4.33–4.38 (m, 1H), 4.44–4.48 (m, 1H), 4.61 (d, J = 8.4 Hz, 1H), 7.01 (t, J = 7.2 Hz, 1H), 7.10 (d, J = 7.6 Hz, 2H), 7.28–7.33 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 11.25, 21.29, 45.32, 66.17, 68.67, 81.88, 114.37, 122.77, 129.36, 150.20, 174.52; EIMS m/z 233.1 (M^+); HRMS (EI) for $C_{13}H_{15}O_3N$, calcd 233.1052, found 233.1046.

[Ru(TTP)(CO)(MeOH)]-catalyzed nitrone formation/intramolecular 1,3-dipolar cycloaddition of butenyl α-diazoacetate 8 with nitrosobenzene

To nitrosobenzene (0.4 mmol) and [Ru(TTP)(CO)(MeOH)] (0.002 mmol) in CH_2Cl_2 (2 mL) was added diazo compound **8** (0.2 mmol) in CH_2Cl_2 (3 mL) over 10 h *via* a syringe pump at reflux. After addition, the resultant solution was stirred for an additional 16 h. The solvent was removed and the crude residue was purified by silica gel column chromatography to give two isomeric isoxazolidines **9a** and **9b** in 71% yield.

1-Phenyltetrahydro-1*H*-pyrano[3,4-*c*]isoxazol-7(7aH)-one (9a). Oil, yield: 52%; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 1.91–1.98 (m, 1H), 2.17–2.25 (m, 1H), 3.20–3.27 (m, 1H), 3.97–4.00 (m, 1H), 4.08–4.12 (m, 1H), 4.28 (d, J = 8.4 Hz, 1H), 4.30–4.37 (m, 1H), 4.65–4.70 (m, 1H), 7.02 (t, J = 7.6 Hz, 1H), 7.18–7.21 (m, 2H), 7.29–7.33 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 28.08, 41.77, 67.40, 67.45, 73.31, 114.79, 122.85, 129.28, 151.04, 169.61; EIMS m/z 219.1 (M^+); HRMS (EI) for $C_{12}H_{13}O_3N$, calcd 219.0895, found 219.0890.

8-Phenyl-3,7-dioxa-8-azabicyclo[4.2.1]nonan-2-one (9b). Oil, yield: 19%; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 1.99–2.05 (m, 1H), 2.21–2.28 (m, 1H), 2.49–2.57 (m, 1H), 2.84–2.89 (m, 1H), 4.38–4.45 (m, 3H), 4.53 (dd, J = 9.6, 6.8 Hz, 1H), 6.96 (t, J = 7.2 Hz, 1H), 7.03 (d, J = 7.6 Hz, 2H), 7.24–7.29 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 30.27, 35.58, 61.45, 67.76, 75.96, 114.46, 122.30, 129.23, 150.10, 171.58; EIMS m/z 219.1 (M^+); HRMS (EI) for $C_{12}H_{13}O_3N$, calcd 219.0895, found 219.0904.

For characterization of **4b–4g**, **4i**, **4l–4n**, **5a–5f**, **5i–5n** see ESI.†

[Ru(TTP)(CO)(MeOH)]-catalyzed reaction of EDA with nitrosobenzene

To nitrosobenzene (2.2 mmol) and [Ru(TTP)(CO)(MeOH)] (0.02 mmol) in CH_2Cl_2 (10 mL) was added EDA (2.0 mmol) in CH_2Cl_2 (10 mL) over 3 h *via* a syringe pump at room temperature. After addition, the resultant solution was stirred for an additional 0.5 h. The ^{1}H NMR of the reaction mixture indicated that the intermediate nitrone was formed and the isomeric ratio of nitrone is ≈93 : 7.

Synthesis of [Ru(TTP)(CHCO$_2$-2,6-di^{t}Bu-4-Me-Ph)] (10). To a toluene (12 mL, dry) solution of [Ru(TTP)(CO)(MeOH)] (0.05 mmol) was added a solution of 2,6-di-*tert*-butyl-4-methylphenyldiazoacetate (0.075 mmol) in toluene (2 mL) over 30 min *via* a syringe pump at 70 °C. The reaction was monitored by TLC. After 2 h, the solvent was removed and the crude residue was purified by silica gel column chromatography to give the dark brown product in 97% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C), δ 0.01 (s, 19H), 1.98 (s, 3H), 2.66 (s, 12H), 6.52 (s, 2H), 7.48 (d, J = 8.0 Hz, 8H), 7.91–7.96 (m, 8H), 8.50 (s, 8H), 14.55 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 278.92 (HC═); FABMS m/z 1030.5 (M^+).

Stoichiometric reaction of [Ru(TTP)(CHCO$_2$-2,6-di^{t}Bu-4-Me-Ph)] with nitrosobenzene and allyl alcohol

To a toluene (1 mL) solution of [Ru(TTP)(CHCO$_2$-2,6-di^{t}Bu-4-Me-Ph)] (0.0164 mmol) was added nitrosobenzene (0.0492 mmol) and allyl alcohol (0.0492 mmol). The resultant solution was stirred at 100 °C. The reaction was monitored by TLC. After 5 h, the solvent was removed and the crude residue was purified by silica gel column chromatography to give the cycloadduct 2,6-di-*tert*-butyl-4-methylphenyl 5-(hydroxymethyl)-2-phenylisoxazolidine-3-carboxylate (**11**) as an isomeric mixture with a *trans*/*cis* ratio of 75 : 25 in 71% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C), δ 1.31 (s, 9H), 1.35 (s, 18H), 1.38 (s, 25H), 2.33 (s, 10H), 2.47–2.54 (m, 3H), 2.77–2.83 (m, 2H), 2.90–2.96 (m, 1H), 3.62 (dd, J = 12.0, 6.0 Hz, 1H), 3.77–3.83 (m, 3H), 3.99 (dd, J = 12.4, 2.3 Hz, 2H), 4.45–4.50 (m, 2H), 4.56–4.58 (m, 1H), 4.73 (dd, J = 5.3, 3.5 Hz, 3H), 6.98–7.04 (m, 3H), 7.13 (s, 6H), 7.20 (d, J = 8.5 Hz, 5H), 7.30–7.35 (m, 7H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 21.67, 31.33, 31.36, 31.77, 31.84, 32.42, 32.52, 35.39, 35.44, 35.50, 62.37, 63.88,

68.29, 69.46, 77.40, 79.23, 80.75, 114.45, 114.58, 122.51, 122.68, 127.27, 127.31, 127.33, 129.29, 129.45, 135.04, 135.15, 141.66, 141.73, 142.61, 142.66, 146.21, 146.28, 150.82, 151.57, 170.93, 171.82; EIMS *m/z* 425.3 (M^+); HRMS (EI) for $C_{22}H_{35}O_4N$, calcd 425.2566, found 425.2566.

Computational details

All calculations were carried out with Gaussian 03.[49] Geometries were fully optimized with the density functional method (B3LYP) using the standard 6-31G(d) basis set. Harmonic vibration frequency calculations were performed for all stationary points and transition states to confirm each structure being a minimum (no imaginary frequency) or a transition state with only one imaginary frequency. Intrinsic reaction coordination (IRC) calculations were traced in each case to ensure that the transition state did in fact connect to the proper minima. The solvent effect has been considered by the UAHF-CPCM model in CH_2Cl_2 with single point calculations by B3LYP/6-31G(d) using the optimized geometry in the gas phase. Cartesian coordinates for stationary structures are included in the ESI.†

In silico analysis and *in vitro* biological experiments of the isoxazolidines

The chemical similarity analysis was carried out using the Similarity Ensemble Approach (SEA). SEA searches chemical similarity among compounds (inhibitors in this work) which are organized by their targets.[50] It can be used to rapidly search large compound databases and to build cross-target similarity maps. SEA has been previously used to identify the drugs acting on G-protein coupled receptors and protein farnesyltransferase.[51] Here we used SEA to look for potential targets of isoxazolidines.

Docking was performed using the ICM-Pro 3.7-2b program (Molsoft)[45] to evaluate the binding of isoxazolidines with LTA4H. The coordinates of leukotriene A4 hydrolase were taken from the Protein Data Bank (PDB ID: 1HS6).[46] Hydrogen and missing heavy atoms were added to the receptor structure followed by local minimization using the conjugate gradient algorithm and analytical derivatives in the internal coordinate space. The energetically most favorable tautomeric state of His was chosen. Positions of Asn and Gln were optimized to maximize hydrogen bonding. The correct stereochemistry and formal charges were assigned. Each compound was then assigned the MMFF atom types and charges, and subjected to a global energy optimization using the ICM stochastic optimization algorithm. The molecular system is described in terms of internal coordinate variables, using a modified ECEPP/3 force-field with a distance-dependent dielectric constant for the energy calculations as implemented in ICM. The biased probability Monte Carlo (BPMC) minimization method consists of the following steps: (1) a random conformation change of the free variables according to a predefined continuous probability distribution; (2) local energy minimization of analytical differentiable terms; (3) calculation of the complete energy including non-differentiable terms such as entropy and solvation energy; (4) acceptance or rejection of the total energy based on the metropolis criterion and return to step 1.

Aminopeptidase activity of LTA4H was determined as previously described with modification.[52] Recombinant human LTA4H (Cayman, 0.8 μg) was preincubated with the test compounds (2 mM) or vehicle (0.2% DMSO) for 30 min at room temperature in a volume of 90 μL of assay buffer (50 mM Tris-HCl, pH 8.0, 100 mM KCl). Reaction was started with addition of 10 μL of L-alanine-4-nitro-anilide hydrochloride (Sigma, 5 mM final concentration). The aminopeptidase activity was determined by monitoring absorbance at 405 nm for 2 h.

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