

Identifying Simultaneous Matrix Metalloproteinases/Soluble Epoxide Hydrolase Inhibitors

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Research Article

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Abstract

Matrix metalloproteinase (MMP) and soluble epoxide hydrolase (sEH) have completely unrelated biological functions, however, their dysregulation produce similar effects on biological systems. Based on the similarity in the reported structural requirements for their inhibition, the current study aimed to identify a simultaneous inhibitor for MMP and sEH. Six compounds were identified as potential simultaneous MMP/sEH inhibitors and tested for their capacity to inhibit MMP and sEH. Inhibition of MMP and sEH activity using their endogenous and exogenous substrates was measured by liquid chromatography/mass spectrometry, spectrophotometry and zymography. Two compounds, CTK8G1143 and ONO-4817, were identified to inhibit both MMP and sEH activity. CTK8G1143 and ONO-4817 inhibited the recombinant human sEH activity by an average of 67.4% and 55.2%, respectively. The IC_{50} for CTK8G1143 and ONO-4817 to inhibit recombinant human sEH were 5.2 and 3.5 μ M, respectively, whereas, their maximal inhibition values were 71.4% and 42.8%, respectively. Also, MMP and sEH activity of human cardiomyocytes were simultaneously inhibited by CTK8G1143 and ONO-4817. Regarding other compounds, they showed either MMP or sEH inhibitory activity but not both. In conclusion, these two simultaneous inhibitors of MMP and sEH could provide a promising intervention for the prevention and control of several diseases, especially cardiovascular diseases.

Introduction

Matrix metalloproteinases (MMP) comprise a family of more than 20 zinc-dependent endopeptidases best known for their ability to mediate degradation of extracellular matrix (Jabłońska-Trypuć, Matejczyk, & Rosochacki, 2016; Laronha & Caldeira, 2020; X. Wang & Khalil, 2018).. Extracellular matrix consists of a collection of fibrous proteins, collagen and enzymes imbedded in a hydrated polysaccharide gel, and provides the required structural and biochemical support to cells to form tissues and organs (Kular, Basu, & Sharma, 2014; Laronha & Caldeira, 2020). Therefore, degradation of extracellular matrix is the key step for tissue and organ remodeling either at normal physiological level, such as cell migration, proliferation and growth, or at pathological level, such as hypertrophy, carcinogenesis and metastasis (Azevedo, Prado, Antonio, Issa, & Gerlach, 2014; Raeeszadeh-Sarmazdeh, Do, & Hritz, 2020; X. Wang & Khalil, 2018). On the other hand, soluble epoxide hydrolase (sEH) is the enzyme responsible for the inactivation of a group of important lipid mediators, namely epoxyeicosatrienoic acids (EETs) (Jorge H. Capdevila & Wang, 2013; Xu et al., 2016). The formation of EETs is mediated by cytochrome P450 enzymes, typically CYP2Cs and CYP2Js, from arachidonic acid (J. H. Capdevila, Falck, & Harris, 2000). The reported biological activities of EETs include anti-inflammatory, analgesic, vasodilatory, anti-platelet, anti-hypertrophic, fibrinolytic and vascular smooth muscle anti-migratory effects (Katakura et al., 2015; Christophe Morisseau & Bruce D. Hammock, 2013; Yang et al., 2014).

Apparently, the independent inhibition of either MMP or sEH has several beneficial effects, therefore, they have been the focus of extensive biological research (Castro, Kandasamy, Youssef, & Schulz, 2011; C. Morisseau & B. D. Hammock, 2013). Despite the biological roles of MMP and sEH are completely unrelated, there is a remarkable agreement in the *in vivo* effects of their inhibitors, such as vasodilatory,

anti-hypertrophic, anti-inflammatory and fibrinolytic effects (Imig, 2012; Vargova, Pytliak, & Mechirova, 2012). Therefore, simultaneous inhibition of both MMP and sEH has great potential to yield synergistic and profound effect to control and prevent several diseases, especially cardiovascular diseases. Moreover, the simultaneous inhibition can attenuate adverse effects; there are rising concerns about the clinical use of sEH inhibitors due to their promoting effect on cancer progression (D. Wang & Dubois, 2012). This major adverse effect can be controlled by the simultaneous inhibition of MMP which is known to attenuate cancer progression and metastasis (Fields, 2019; Hu, Van den Steen, Sang, & Opdenakker, 2007; Vargova et al., 2012). Interestingly, structure-activity relationship studies show several structural characteristics shared between number of MMP and sEH inhibitors (Levy et al., 1998; Morisseau et al., 1999; Morisseau et al., 2002; Pirard, 2007; Rezaee Zavareh et al., 2014; Shen & Hammock, 2012; Tripathi et al., 2018). Accordingly, simultaneous inhibition of MMP and sEH by a single compound is feasible, therefore, in the current study, our objective was to identify a simultaneous inhibitor of MMP and sEH. We examined the chemical structure of several MMP and sEH inhibitors, consequently, 6 compounds (Table S1) were selected and tested for their capacity to simultaneously inhibit both MMP and sEH.

Materials And Methods

Materials

14,15-epoxyeicosatrienoic acid (14,15-EET), 14,15-dihydroxyeicosatrienoic acid (14,15-DHET), internal standards (14,15-EET-d11 and 14,15-DHET-d11), 4-nitrophenyl-2S,3S-epoxy-3 phenylpropyl carbonate (S-NEPC) and 12-[[tricyclo[3.3.1.13,7]dec-1-ylamino)carbonyl]amino]-dodecanoic acid (AUDA) were purchased from Cayman Chemical (Ann Arbor, MI). High performance liquid chromatography (HPLC) grade acetonitrile, water and ethyl acetate were purchased from EM Scientific (Gibbstawn, NJ). Acrylamide, N'N'-bis-methylene-acrylamide, β -mercaptoethanol, ammonium persulfate, glycine, and N,N,N',N'-tetramethyleneethylenediamine (TEMED) were purchased from Bio-Rad Laboratories (Hercules, CA). 1-(1-methanesulfonyl-piperidin-4-yl)- 3-(4-trifluoromethoxy-phenyl)-urea (TUPS) was synthesized by Paul Jones (University of California, Davis) as described in Tsai *et al* (Tsai et al., 2010). Trans-4-[4-(3-Adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (t-AUCB) was synthesized by Sung Hwang (University of California, Davis) as described in Hwang *et al* (Hwang, Tsai, Liu, Morisseau, & Hammock, 2007). N-[(4,5-dihydro-5-thioxo-1,3,4-thiadiazol-2-yl)amino]carbonyl]-L-phenylalanine methyl ester (CTK8G1143) and N-[(1S,3S)-1-[(Ethoxymethoxy)methyl]-4-(hydroxyamino)-3-methyl-4-oxobutyl]-4-phenoxybenzamide (ONO-4817) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). 4-aminophenyl mercuric acetate and doxycycline was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). The catalytic domain of human recombinant MMP-2, human recombinant pro-MMP-2, human recombinant sEH and OmniMMP[®] fluorogenic peptide substrate were purchased from Oxford Biomedical Research (Oxford, MI). Other chemicals were purchased from Fisher Scientific Co. (Toronto, ON, Canada), or Sigma-Aldrich Chemical Co. (St. Louis, MO).

Determination of sEH activity by spectrophotometric assay

The effect of MMP inhibitors on sEH activity was determined by using spectrophotometric substrate, S-NEPC, as previously described by Dietze *et al* (Dietze, Kuwano, & Hammock, 1994). The principle of assay is that the epoxide group in S-NEPC is hydrolysed by sEH, releasing 4-nitrophenol, which can be quantified spectrophotometrically. Briefly, 96-well plates were pretreated by 25 mg/mL BSA, 2% Triton X-100 and 2% Tween 20 for 2 hr. Thereafter, 10 nM of sEH was added to 0.1 mM ethylenediaminetetraacetate and 2.5 mg/mL of BSA in 76 mM phosphate buffer (pH=6.4) in the absence or presence of 0.5 μ M of sEH inhibitor, t-AUCB, or 100 μ M of MMP inhibitors, CTK8G1143, ONO-4817 or doxycycline. After the initiation of the reaction by the addition of S-NEPC (30 μ M final concentration), test and blank (without sEH) samples were monitored at 405 nm every 10 sec for 5 min at 25°C using BioTek Synergy H1 Hybrid Reader (BioTek Instruments, Inc., VT, USA). The initial rate of product formation in each well was determined by linear regression of absorbance-time data using GraphPad Prism (version 5.0, GraphPad software, San Diego, CA).

The effect of MMP inhibitors on sEH activity of immortalized human cardiomyocytes cell line, RL14, (American Type Culture Collection, Manassas, VA) was determined. Cells were maintained in Dulbecco's modified Eagle's medium F-12 medium supplemented with 12.5% dialysed Fetal Bovine serum, and grown in 75 cm² tissue culture flasks at 37°C in a 5% CO₂ humidified incubator. Cells were grown at a density of 5 X 10⁵ cells per well in a 24-well tissue culture plate. On ~80% confluence (2 days), the media was replaced by 300 μ L phosphate buffered saline in the absence or presence of 0.1 μ M of sEH inhibitor, t-AUCB, or 10 μ M of MMP inhibitors, CTK8G1143 or ONO-4817. Thereafter, S-NEPC (30 μ M final concentration) was added to the cells. After 10 min incubation at 37°C, the formation of 4-nitrophenol in test and blank (without cells) samples were measured at 405 nm in 96-well plate using BioTek Synergy H1 Hybrid Reader (BioTek Instruments, Inc., VT, USA).

Determination of sEH activity by liquid chromatography-mass spectrometry (LC-MS)

The most important endogenous substrate of sEH is 14,15-EET, which is hydrolyzed by sEH to 14,15-DHET. Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) (Waters Micromass ZQ 4000 spectrometer) was used to quantify the formation of 14,15-DHET as described previously by Morisseau and Hammock (Morisseau & Hammock, 2007). Briefly, 5 nM of sEH was added to 0.1 mg/mL BSA in 100 mM phosphate buffer (pH=7.4) in the absence or presence of 0.5 μ M of sEH inhibitor, t-AUCB, or 100 μ M of MMP inhibitors, CTK8G1143, ONO-4817 or doxycycline. The reaction was initiated by the addition of 14,15-EET (50 μ M final concentration) and terminated after 7 min at 30°C by the addition of 600 ml ice-cold acetonitrile followed by the internal standards. 14,15-EET and its corresponding 14,15-DHET in test and blank (without sEH) samples were extracted by 1ml ethyl acetate twice and dried using speed vacuum (Savant, Farmingdale, NY). Extracted 14,15-EET and 14,15-DHET were analyzed using LC-MS (Waters Micromass ZQ 4000 spectrometer) method as described previously (El-Sherbeni, Aboutabl, Zordoky, Anwar-Mohamed, & El-Kadi, 2013). To determine the kinetics of sEH inhibition, concentration range of 0.05-500 nM for TUPS or t-AUCB or 0.01-100 μ M for CTK8G1143 or 0.004-100 μ M for ONO-4817 were tested using the same procedure as described above. Inhibitor

concentrations required for 50% inhibition (IC_{50}) and maximal inhibition (I_{max}) were determined by Enzyme Kinetics module from GraphPad Prism (version 5.0, GraphPad software, San Diego, CA).

Determination of MMP-2 activity by fluorescence assay

The effect of sEH inhibitors on MMP-2 activity was determined by assessing MMP-dependent hydrolysis of Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH₂ AcOH (OmniMMP) fluorogenic substrate as previously described by Castro *et al* (Castro et al., 2013). 96-well black polystyrene half-area plates were pretreated by 25 mg/mL BSA, 2% Triton X-100 and 2% Tween 20 for 2 hr. Thereafter, 0.5 nM of MMP-2 catalytic domain was added to 10 mM CaCl₂, 0.05% Brij 35 and 10 mM ZnSO₄ in 50 mM Tris-HCl (pH=7.0) in the absence or presence of 100 μ M of MMP inhibitor, ONO-4817, or 50 μ M of sEH inhibitors, AUDA, TUPS or t-AUCB. The reaction was initiated by the addition of OmniMMP substrate (30 μ M final concentration). The fluorescence associated with the breakdown of OmniMMP in test and blank (without MMP-2) samples were measured at 30 s intervals (excitation and emission wavelengths of 328 nm and 393 nm) for 1 hr at 37°C using a BioTek Synergy H1 Hybrid Reader (BioTek Instruments, Inc., VT, USA). The initial rate of product formation in each well was determined by linear regression of fluorescence-time data using GraphPad Prism (version 5.0, GraphPad software, San Diego, CA).

Determination of MMP activity by zymography

The effect of sEH inhibitors on the gelatinolytic activity of MMP was also determined using substrate-gel electrophoresis (zymography) as previously described by Hawkes *et al* (Hawkes, Li, & Taniguchi, 2010). Briefly, pro-MMP-2 was activated by 1 mM 4-aminophenyl mercuric acetate in 150 mM NaCl, 5 mM CaCl₂, 50mM Tris HCl (pH=7.5). Then, pro-MMP-2 and MMP-2 were electrophoresed on an 8% polyacrylamide gel containing 1 mg/ml gelatin for 90 min (125 V, ambient temperature). Thereafter, the gels were cut into individual strips and then washed in 2.5% Triton X-100 and 5 mM CaCl₂ in 50 mM Tris-HCl buffer (pH=8.0). These strips were incubated separately overnight at 37°C in development buffer (50 mM Tris-HCl, 10 mM CaCl₂, 1 μ M ZnSO₄, pH 7.6) in the absence or presence of 100 μ M of MMP inhibitor, ONO-4817, or 50 μ M of sEH inhibitors, AUDA, TUPS or t-AUCB. Gels were stained with 1 mg/mL Coomassie blue, followed by destaining and, finally, gelatinolytic activity appeared as a clear band on a blue background. Moreover, gelatinolytic activity of MMP-2 and MMP-9 in the conditioned medium of cultured RL14 was determined by zymography, where 8 μ L aliquots of conditioned RL14 medium were electrophoresed as described above. After washing, gel strips were incubated separately overnight at 37°C in development buffer, as described above, in the absence or presence of 10 μ M CTK8G1143 or ONO-4817.

Results

The effect of MMP inhibitors on sEH activity

The chemical structure of several MMP inhibitors were reviewed, and consequently, 2 MMP inhibitors, CTK8G1143 and ONO-4817, were selected based on the presence of sEH inhibitors pharmacophores as

previously reported (Morisseau et al., 1999, Morisseau et al., 2002, Ulu et al., 2012). In addition, we included doxycycline, which exerts its MMP inhibitory activity through the same mechanism of CTK8G1143 and ONO-4817, chelating the catalytic metal (Zn^{2+}) ions, albeit having totally different chemical structure (Table S1) (Castro et al., 2011). Using S-NEPC as a spectrophotometric exogenous substrate, CTK8G1143 and ONO-4817 were found to exhibit inhibitory activity against recombinant human sEH enzyme (Fig. 1A). CTK8G1143 and ONO-4817 inhibited sEH activity by 59.8% and 71.2%, respectively, whereas the potent sEH inhibitor, t-AUCB, inhibited sEH activity by 85.5% compared with dimethyl sulfoxide (DMSO) control (Fig. 1A). Using 14,15-EET, which is the endogenous substrate of sEH, CTK8G1143 and ONO-4817 inhibited sEH activity by 74.9% and 39.1%, respectively, and t-AUCB inhibited sEH activity by 84.2% compared with DMSO control (Fig. 1A). In both cases, doxycycline did not significantly affect the activity of sEH compared with DMSO control (Fig. 1A). **The effect of sEH inhibitors on MMP-2 activity**

In contrast to MMP inhibitors, sEH inhibitors display a more homogenous group with respect to chemical structure, from which we selected 3 potent sEH inhibitors, AUDA, TUPS and t-AUCB. The selected sEH inhibitors show some general structural characteristics of MMP inhibitors, most importantly Zn-binding moieties, such as carboxyl and sulfonyl groups (Table S1) (Fisher & Mobashery, 2006, Hu et al., 2007, Scozzafava & Supuran, 2000). Either by using fluorogenic substrate or zymography, AUDA, TUPS or t-AUCB did not cause any significant alteration in MMP-2 activity compared with DMSO control (Fig. 1B). On the other hand, ONO-4817 inhibited MMP-2 activity by 98.3% and 99.4% using fluorescence assay and zymography, respectively (Fig. 1B).

The determination of sEH inhibition kinetics of MMP inhibitors

In order to determine whether the inhibitory effect of MMP inhibitors on sEH can occur at biologicallyrelevant concentrations of MMP inhibitors, we determined sEH inhibition kinetics of CTK8G1143 and ONO-4817 by LC-MS. The maximal inhibition (I_{max}) of sEH activity by CTK8G1143 and ONO-4817 were 71.4% and 42.8%, respectively, compared with DMSO control (Fig. 2 & Table 1). The IC_{50} values were 5.2 μM and 3.5 μM for CTK8G1143 and ONO-4817, respectively (Fig. 2 & Table 1). The IC_{50} values for the potent sEH inhibitors, TUPS and t-AUCB, were 6.5 nM and 12.3 nM and I_{max} values were 60.7% and 84.5%, respectively (Fig. 2 & Tab.1). Despite MMP inhibitors were remarkably less potent than sEH inhibitors with respect to sEH inhibition, the concentration range of sEH inhibition by MMP inhibitors is within the reported plasma concentration range (Ro et al., 2007, Shiraga et al., 2002).

The effect of MMP inhibitors on sEH and MMP activities in RL14 cells

MMP enzymes are mostly secreted proteins, in contrast to sEH, which is located in cell cytoplasm. Therefore, we examined whether MMP inhibitors can freely penetrate cell membrane in order to simultaneously inhibit MMP outside the cell and sEH inside the cell at a biologicallyrelevant concentration. Exposing RL14 cells to 10 μM of CTK8G1143 or ONO-4817 significantly inhibited the intracellular sEH activity by 63.3% and 34.5%, respectively, whereas, t-AUCB inhibited sEH activity by

86.4% compared with DMSO control (Fig. 3A). Also, 10 μ M of CTK8G1143 and ONO-4817 inhibited the extracellular MMP-2 activity by 52.2% and 61.3% and MMP-9 activity by 57.7% and 71.6%, respectively, compared with DMSO control (Fig. 3B).

Discussion

Following the discovery of the important biological roles of MMP and sEH, great efforts have been made to design potent inhibitors for them. MMP comprise several enzymes, which depend on zinc for their activity. Zinc as a divalent ion is bound to a common catalytic domain shared between all MMP. Therefore, the most important feature for MMP inhibitor is the presence of zinc-binding group, besides the proper positioning in the catalytic site by non-covalent interactions. Aside of these features, MMP inhibitors are remarkably diverse and are usually subdivided into 8 classes, succinyl hydroxamates, sulphonamide hydroxamates, phosphinamide hydroxamates, carboxylates, thiol inhibitors, aminomethyl benzimidazole analogue, peptides and tetracyclines. In contrast, sEH inhibitors are structurally similar, Morisseau *et al.* first reported that carbamide compounds with appropriate substituents can exhibit potent sEH inhibitory activity (Morisseau et al., 1999). Till now, the carbamide pharmacophore presents in all current sEH inhibitors that can be divided into urea, benzamide and benzoxazole compounds (Chen, Zhang, Li, & Han, 2012, C. Morisseau & B. D. Hammock, 2013, Shen & Hammock, 2012).

Due to the diversity of MMP inhibitors, we scanned MMP inhibitors for urea, benzamide and benzoxazole moiety. We did not find any MMP inhibitor with benzoxazole moiety, on the other hand, urea moiety was found in CTK8G1143, and benzamide moiety was found in ONO-4817. In addition to MMP-2 and MMP-3 inhibitory activity of CTK8G1143, it possesses several structural characteristics critical for a successful urea-based sEH inhibitor (Table S1). The sEH inhibition structural characteristics of CTK8G1143 include: 1) urea group, 2) each of the two urea nitrogen atoms is singly substituted by a bulky group (6-12 carbons), 3) the urea N-substituents are of unequal size, and 4) a secondary pharmacophore, an ester or ether group, present at a distance from urea carbonyl group, which reported to improve the effectiveness of sEH inhibitors (Huang et al., 2010, Kim et al., 2005, Morisseau et al., 1999, Morisseau et al., 2002, Shen & Hammock, 2012). In addition, ONO-4817, which is a broad-spectrum MMP inhibitor, shows several structural characteristics of a benzamide-based sEH inhibitor (Table S1). This includes benzamide moiety with an electronegative function group in the para-position and N-attached to a very bulky group (12-15 carbons) ended with methylsulfonyl or carboxyl group (Eldrup et al., 2009, C. Morisseau & B. D. Hammock, 2013, Rezaee Zavareh et al., 2014). We were also interested in measuring the MMP inhibitory activity of 3 urea-based sEH inhibitors, AUDA, TUPS and t-AUCB, due to their structural similarity with CTK8G114 (Table S1). In the current study, we examined these 5 compounds and determined CTK8G114 and ONO-4817 as simultaneous inhibitors of MMP and sEH. We can conclude that sEH inhibitory activity of CTK8G114 and ONO-4817 cannot be attributed to zinc-binding activity of these MMP inhibitors based on doxycycline lacking of sEH inhibitory activity. With respect to AUDA, TUPS and t-AUCB, they did not inhibit MMP-2 activity, despite their potent inhibitory effect on sEH. This can be explained by their many-fold less potent zinc-binding groups, carboxyl and methylsulfonyl. Typically, MMP inhibitors have

hydroxamate as zinc-binding group, which has been reported to be 20-fold to 50-fold more potent than thiol group and 100-2000 folds more potent than carboxyl group (Hu et al., 2007).

Over 20 years ago, there was great enthusiasm for MMP inhibitors as anticancer agents by impairing tumor angiogenesis and metastasis. However, disappointing results from multiple cancer clinical trials have reduced the interest in MMP inhibitors (Vandenbroucke & Libert, 2014, Vargova et al., 2012). In fact, elevated MMP activity has been reported not only in malignant diseases but also in several inflammatory and degenerative cardiovascular diseases (Cabral-Pacheco et al., 2020). The maladaptive remodeling mediated by MMP is an important aspect in cardiac hypertrophy, heart failure, hypertension, and atherosclerosis, therefore, MMP inhibition could be beneficial for these diseases. Similarly, hypertension and several inflammatory diseases were once regarded the prime indications for the use of sEH inhibitors. However, sEH inhibitors failed in clinical trials to show promising results and the only sEH inhibitor in clinical trial now is GSK2256294 that has entered Phase I clinical trial for a chronic obstructive pulmonary disease in male obese smokers (Podolin et al., 2013). Consequently, simultaneous MMP/sEH inhibition could be a promising solution to potentiate the benefits of the individual MMP or sEH inhibition. In conclusion, we identified 2 compounds that exhibit simultaneous inhibitory activity against MMP and sEH at biologically relevant feasible concentration range. Further investigation is required to evaluate their use for the prevention and control of several diseases, especially cardiovascular diseases.

Declarations

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Contributions

Ahmed A. El-Sherbeni: Performing experiments, data analysis, writing the manuscript

Rabia Bhatti: Performing some experiments

Fadumo A. Isse: data analysis, writing the manuscript

Ayman O.S. El-Kadi: data analysis, writing the manuscript

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Data availability

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval

No animals were used for these studies.

Conflict of Interest

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Tables

Table 1. Inhibitor concentration required for 50% inhibition (IC_{50}) and maximal inhibition (I_{max}) mean values and SE.

	$IC_{50} \pm SE$	$I_{max} \pm SE$	R^2
TUPS	0.01 ± 0.00	60.73 ± 5.78	0.95
tAUCB	0.01 ± 0.00	84.53 ± 5.64	0.93
CTK8G1143	5.20 ± 0.19	71.39 ± 2.85	0.89
ONO-4817	3.48 ± 0.12	42.82 ± 7.28	0.88

IC_{50} is in μM , and I_{max} is % of DMSO control.

Figures

FIGURE 1

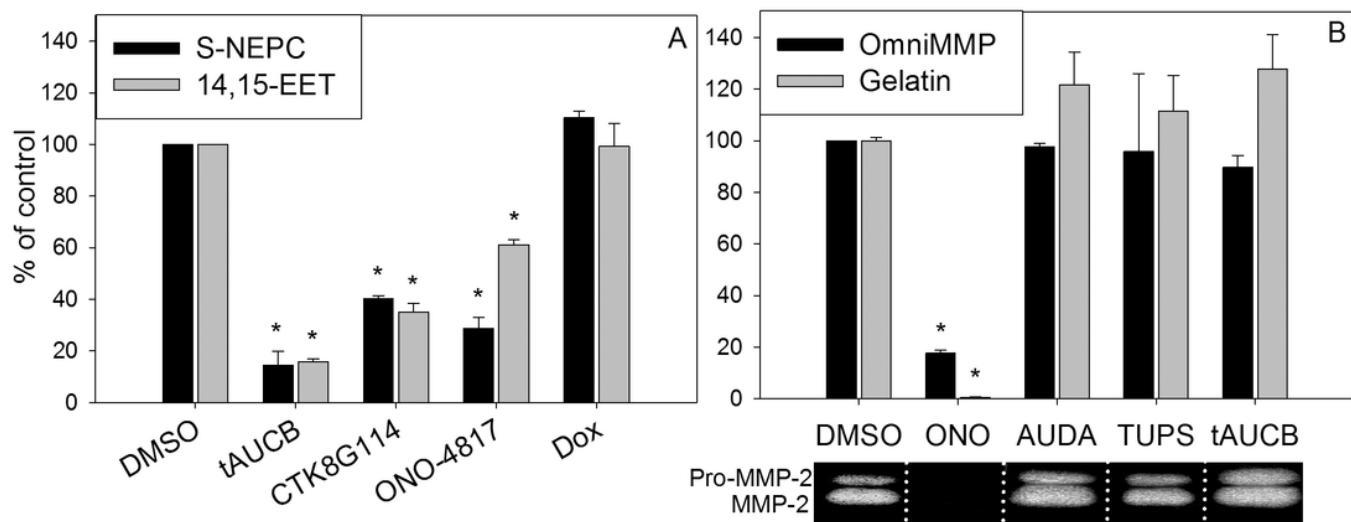


Figure 1

Effect of CTK8G114, ONO-4817, doxycycline, AUDA, TUPS and tAUCB on MMP-2 and sEH activities. (A) the activity of human recombinant sEH was measured by using exogenous substrate, S-NEPC, and by using sEH endogenous substrate, 14,15-EET, as described under Materials and Methods in the absence or presence of 0.5 μ M of sEH inhibitor, t-AUCB, or 100 μ M of MMP inhibitors, CTK8G1143, ONO-4817 or doxycycline. (B) the activity of human recombinant MMP-2 catalytic domain or the activated human recombinant Pro-MMP-2 was measured by using exogenous substrate, OmniMMP and by using MMP endogenous substrate, gelatin, as described under Materials and Methods in the absence or presence of 100 μ M of MMP inhibitor, ONO-4817, or 50 μ M sEH inhibitors, AUDA, TUPS or t-AUCB. Results are presented as mean % of DMSO control and SE, and are based on 4 individual experiments. * $p<0.05$.

FIGURE 2

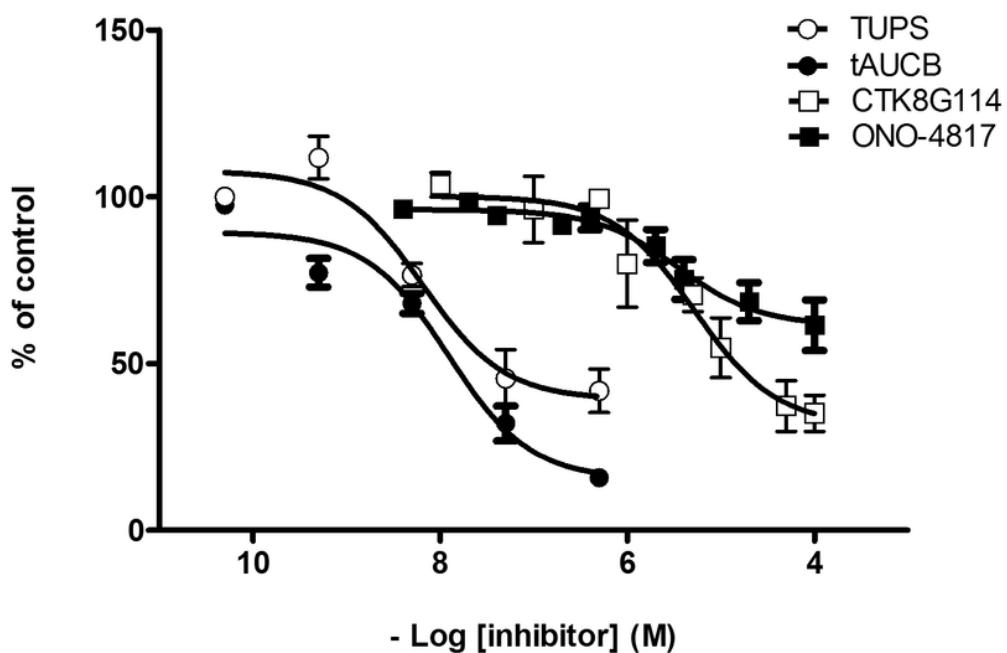


Figure 2

sEH inhibition kinetics of CTK8G114, ONO-4817, TUPS and tAUCB. Concentration range of 0.05-500 nM for TUPS or t-AUCB or 0.01-100 μ M for CTK8G1143 or 0.004-100 μ M for ONO-4817 were incubated with human recombinant sEH. Activity was measured by the formation of 14,15-DHET from 14,15-EET, as described under Materials and Methods. Results are presented as mean % of DMSO control and SE, and are based on 4 individual experiments.

FIGURE 3

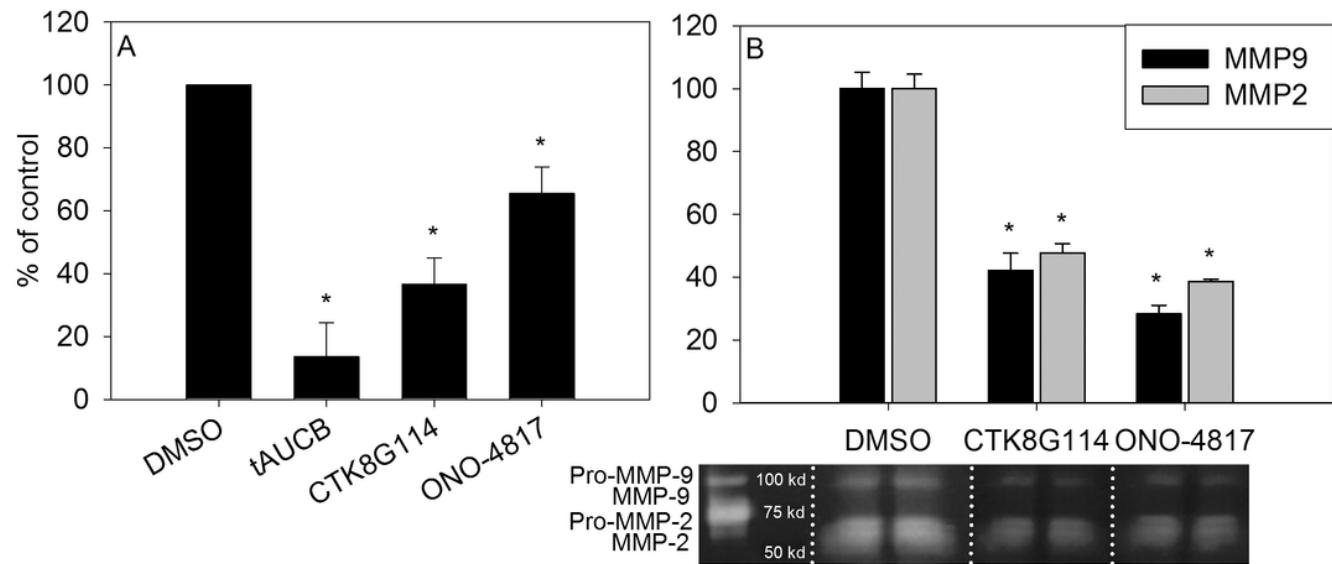


Figure 3

Effect of CTK8G114, ONO-4817 and tAUCB on MMP and sEH activities in human cardiomyocytes, RL14. These compounds were incubated with RL14 cells or conditioned medium of cultured RL14. sEH activity in the absence or presence of 0.1 μ M of sEH inhibitor, t-AUCB, or 10 μ M of MMP inhibitors, CTK8G114 or ONO-4817 (A), as well as MMP-2 and MMP-9 activities in the absence or presence of 10 μ M CTK8G114 or ONO-4817 (B) were measured as described under Materials and Methods. Results are presented as mean % of DMSO control and SE, and are based on 4 individual experiments. * $p<0.05$.

Supplementary Files

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