Bioorganic & Medicinal Chemistry 20 (2012) 1100–1112

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and evaluation of polar head group containing 2-keto-oxazole inhibitors of FAAH

Marion Rusch [a], Stefan Zahov [b], Ingrid R. Vetter [c], Matthias Lehr [b], Christian Hedberg [a,*]

[a] Department of Chemical Biology, Max-Planck Institute of Molecular Physiology, Otto-Hahn Strasse 11, D-44227 Dortmund, Germany
[b] Institute for Pharmacy and Medicinal Chemistry, Westfalian Wilhelms-University, Münster, Hittorfstr. 58-62, 48149 Münster, Germany
[c] Department of Physical Biochemistry, Max-Planck Institute of Molecular Physiology, Otto-Hahn Strasse 11, D-44227 Dortmund, Germany

ARTICLE INFO

Article history:
Received 1 September 2011
Revised 8 November 2011
Accepted 11 November 2011
Available online 28 November 2011

Keywords:
Fatty acid amide hydrolase
FAAH inhibitor
Endocannabinoid
2-Keto oxazole
Pain

ABSTRACT

2-α-Keto oxazoles containing polar head groups in their C5-side chains were designed as fatty acid amide hydrolase (FAAH) inhibitors. Variation in the spacer length resulted in submicromolar α-keto-oxazole FAAH inhibitor (IC_{50} = 436 nM) presenting electrostatic stabilizing interactions between its polar head group contained in the C5-side chain and the hydrophilic pocket of the enzyme.

1. Introduction

Since the discovery and the isolation of fatty acid amide hydrolase (FAAH) from rat liver,[1] FAAH has been shown to be an important membrane-bound serine hydrolase involved in the endocannabinoid metabolism. FAAH rapidly cleaves the analgesic and anti-inflammatory endocannabinoid anandamide into arachidonic acid and ethanolamine, and is considered as an attractive therapeutic target for the treatment of pain and inflammation, as well as a target for various psychiatric indications related to endocannabinoid receptors.[2–5]

Over the past decade, extensive drug discovery efforts have led to the development of various FAAH inhibitors.[6–9] First-generation FAAH covalent inhibitors consisted of structures derived from anandamide, oleoylethanolamide (OEA) and palmitoyl-ethanolamide (PEA). However, these compounds have been extensively used as tools for FAAH-functional studies, exemplified by the use of ethoxyoleyl fluorophosphonate (EOFP) for the identification of Ser241 as the nucleophilic residue of FAAH catalytic machinery, or the use of methylarachidonoyl fluorophosphonate (MAFP) for FAAH co-crystallization.[10,11] Not surprisingly, they were poor drug candidates due to selectivity issues.[6] In 2003, Piomelli and co-workers reported the arylcarbamate URB-597 (cyclohexylcarbamic acid 3′-carbamoyl-biphenyl-3-yl ester) to inhibit FAAH (IC_{50} value of 4.6 nM in brain membrane and 0.5 nM in intact neurons) by rreversible carbamylation of the nucleophilic serine (Ser241) leading to anxiolytic and analgesic activities.[12] A second class of FAAH inhibitors reported in 2005 by Cravatt and Boger, was based on the α-2-keto oxazole OL-135 (1-oxo-1-[5-(2-pyridyl)oxazol-2-yl]-7-phenylheptane), which turned out to be a potent (K_i = 4.7 nM upon FAAH) and selective FAAH inhibitor versus other mammalian serine hydrolases.[13] With the discovery of the ability of OL-135 to promote analgesia in vivo,[14] OL-135 has become the starting point for several structure–activity relationships studies (SARs), exploring successively the C2-acyl side chain and the C5-position of the central oxazole ring.[15,16] Recently, a crystal structure of inhibitor OL-135 in complex with h/rFAAH was reported, confirming the mode of inhibition of FAAH via a reversible hemiketal formation with the nucleophilic Ser241 of the unusual Ser241-Ser217-Lys142 catalytic triad.[17]

2. Results and discussion

2.1. Compound design

The recent discovery by Mileni et al.[17] regarding the FAAH inhibition mode via a water-mediated hydrogen bond between 2-keto oxazoles C5-substituted with H-bond acceptor groups (such as pyridine (OL-135), $-CO_2Me$ and $-CON(Me)_2$) and the Thr236 residue (located into the hydrophilic FAAH binding side), encouraged us to further investigate the potency of such stabilizing interactions. To address this issue, a series of 2-keto oxazoles, carrying various polar heteroatoms ($—NMe_3^+$, $—NMe_2$, —S—, $—SO_2—$) along their linear C5-side chain allowing stabilizing interactions (hydrogen bond

* Corresponding author. Tel.: +49 231 133 2429; fax: +49 231 1332498.
E-mail address: christian.hedberg@mpi-dortmund.mpg.de (C. Hedberg).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.11.027

and/or polar interactions), were synthesized and screened for FAAH inhibition. The effect of variations in spacer length (n = 2–6) was also investigated in order to optimize the distance between the electrophilic 2-keto-oxazole and the polar head groups with respect to binding affinities (Fig. 1).

2.2. Chemistry

The synthesis of the compound library started from recently reported ethyl esters **1**–**5**.[18] Compounds **1**–**5** were treated with $LiCH_2NC$ at low temperature, according to Schöllkopfs protocol,[19–21] leading to oxazoles **6–10** isolated in good yields (84–94%). Subsequent C2-metallation of compounds **6**–**10** by the treatment with iPrMgCl,[22] followed by quenching with Weinreb amide *N*-methoxy-*N*-methyldodecanamide (R = n-$C_{11}H_{23}$, **11**) or *N*-methoxy-*N*-methyloctanamide (R = n-C_7H_{15}, **12**) concluded the synthesis of sub-library A (**13**–**22**). Subsequent *S*-oxidation of **13–22** with Oxone® yielded the corresponding sulfones denoted sub-library B (**23**–**32**).[23] Sub-libraries A (**13**–**22**) and B (**23**–**32**) were quaternized at the dimethylamino functionality by the treatment with an excess of methyl iodide in acetonitrile, yielding respectively sub-libraries C (**33**–**38**) and D (**39**–**48**) (Scheme 1).[24]

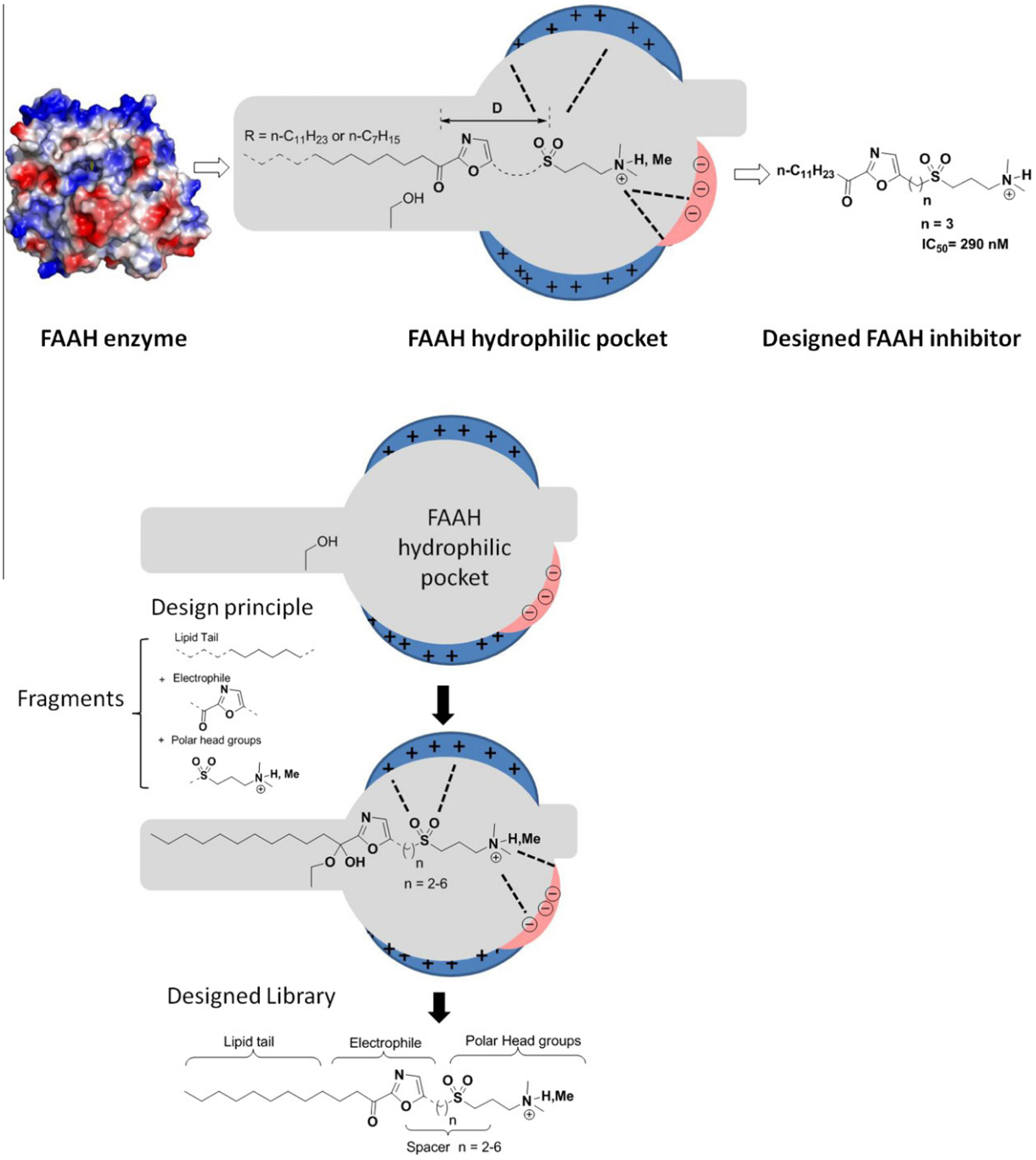
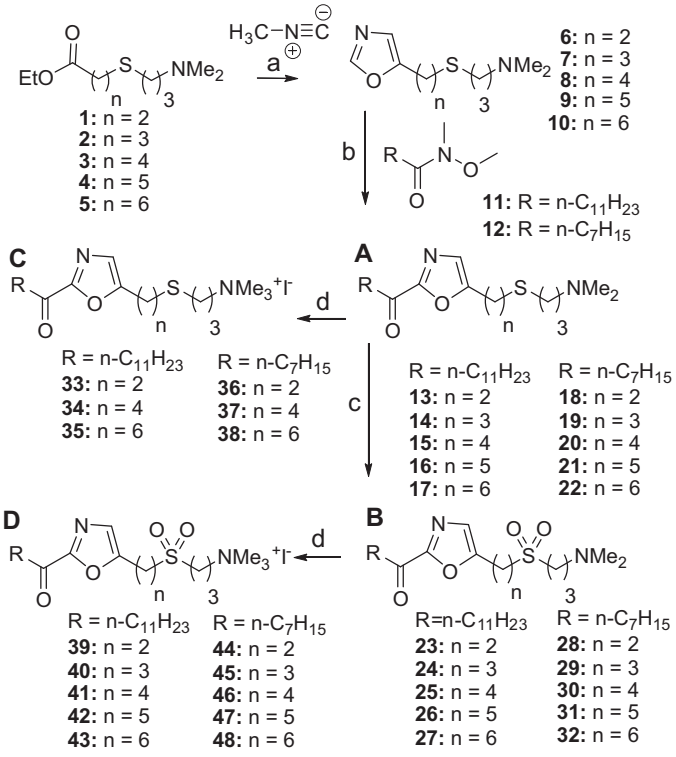


Figure 1. Design of the α-keto oxazole FAAH inhibitor candidates based on the electrostatic potential of the enzyme in its hydrophilic pocket. The position of polar head groups in the enzyme was investigated by variation of the spacer length (n varying from 2 to 6 methylene units) to profit from electrostatic stabilizing interactions.



Scheme 1. Synthesis of α-keto-oxazole sub-libraries A–D. Reagents and conditions: (a) $CH_3N{\equiv}C$ (1.34 equiv), *n*-Buli (1.5 equiv), dry THF, −78 °C (2 h) then ester **1**–**5**, dry THF, −78 °C (3 h), rt (3 h); (b) *i*-PrMgCl (1.4 equiv), dry THF, −15 °C to −20 °C (2 h), Weinreb amide **11**–**12** (1.0 equiv), −20 °C to −25 °C (30 min), rt (25 h); (c) Oxone (3.0 equiv), MeOH/H_2O (3/2), rt (60 h); (d) MeI (1.2 equiv), CH_3CN, rt (5 h).

2.3. Compound evaluation

Sub-libraries A–D were evaluated for FAAH inhibition applying a modified version of an assay published recently.[25] Briefly, microsomes from rat brain were employed as enzyme source. *N*-(2-Hydroxyethyl)-4-pyren-1-ylbutanamide, holding a fluorophore in the acyl part of the molecule, was used as substrate in combination with Triton X-100 as detergent. Inhibitory potencies of the tested compounds were assessed by comparing the amount of 4-pyren-1-ylbutanoic acid released from the substrate in their absence and presence, after an incubation time of 60 min by reversed-phase HPLC with fluorescence detection applying 6-pyren-1-ylhexanoic acid as internal standard.[25]

We modified the enzyme isolation procedure (rat brain) from a recently published procedure, which led to a two- to threefold decrease of the IC_{50} values of the references in comparison with the previous protocol.[25,26] The three known FAAH inhibitors cyclohexylcarbamic acid 3′-carbamoylbiphenyl-3-ylester (URB 597), 1-oxazolo[4,5-*b*]pyridin-2-yl-6-phenylhexan-1-one (PHOP)[27] and (6-(2-methyl-4,5-diphenyl-1*H*-imidazol-1-yl)hexyl)carbamic acid phenyl ester[28] were used as references for the evaluation of new inhibitor candidates, with URB-597, PHOP and the hexylcarbamic acid phenyl ester showing an IC_{50} value of 67 nM, 2.9 nM and 340 nM, respectively, under the assay conditions applied.

Screening for FAAH inhibition of our α-keto-oxazole libraries A–D revealed an enzyme preference for inhibitors substituted with long C2-alkyl chains (R = *n*-$C_{11}H_{23}$), as replacement by shorter chains (R = *n*-C_7H_{15}) resulted in a decreased inhibitory activity (Fig. 2A, Table 1). Although α-keto oxazolopyridines with C2-alkyl chains varying from R = *n*-C_7H_{15} to R = *n*-$C_{11}H_{23}$ were shown by Boger et al. to have the greatest potency,[27] our observation is consistent with the significant reduction in activity reported for

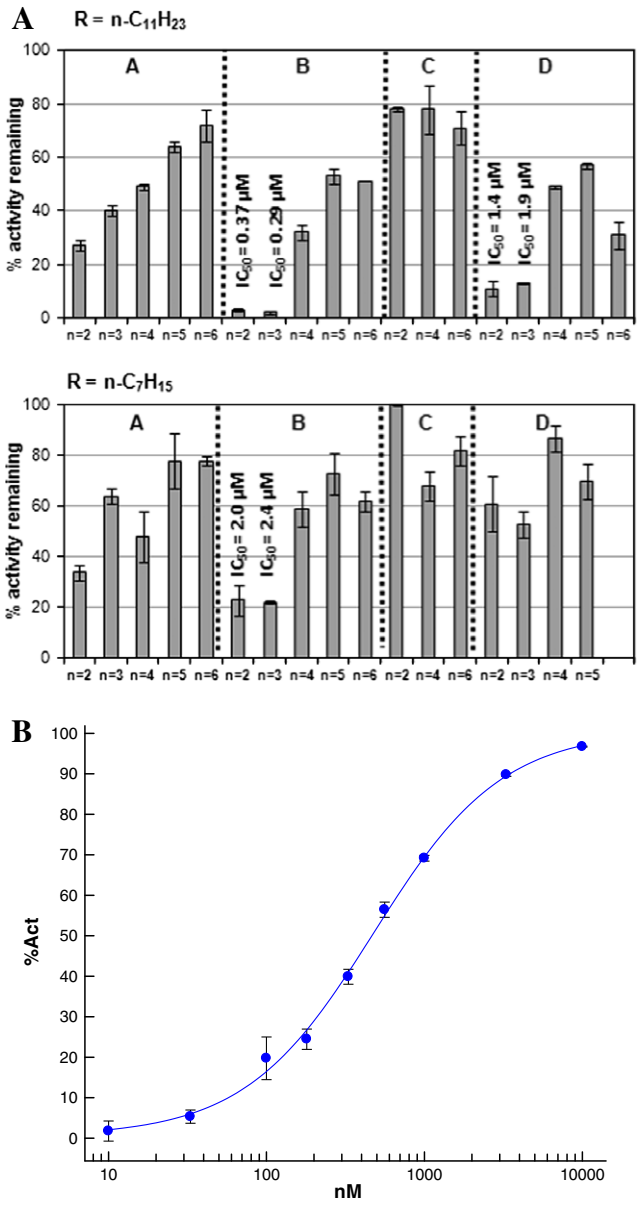
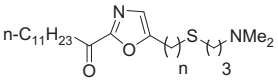
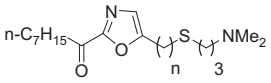
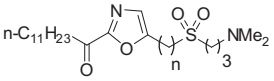
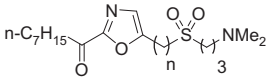
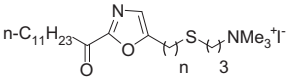
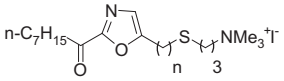
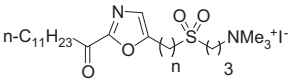
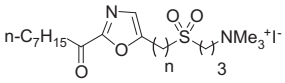


Figure 2. Evaluation of α-keto oxazole libraries A–D for FAAH inhibition. (A) Graphical representation of the residual FAAH enzyme activity for the two series of α-keto oxazole inhibitors (respectively R = *n*-$C_{11}H_{23}$ or R = *n*-C_7H_{15}) at 10 μM concentration, IC_{50} values of the more potent oxazoles were evaluated based on three concentration data points (10 μM, 3.3 μM and1 μM). Values obtained based on two independents measurements. (B) IC_{50} curve of the more potent oxazole **24** presenting an IC_{50} value of 436 nM when including additional data points.

1,1,1-trifluorononan-2-one (R = C_7H_{15}, K_i = 1.2 μM) compared to the four-carbon longer homolog (R = $C_{11}H_{23}$, K_i = 0.14 μM).[29] Jonsson et al. also addressed a similar observation later, with a decreased inhibition observed for ethanolamides of aliphatic straight chain fatty acids holding chain lengths below C_{12}.[30] An increased number of polar heteroatoms (SO_2, vs S) in the oxazole-C5 side-chain resulted in a significant increase in inhibitory potency, with sulfones generally more potent than their non-oxidized homologs (Fig. 2A, Table 1). Those results are consistent with the hydrophilic character of the protein suggested by X-ray study of h/rFAAH in complex with OL-135, where a wide hydrophilic pocket defined by various polar residues such as Ser217, Lys142 and Thr236 (allowing stabilizing polar interactions) was shown to accommodate the oxazole-C5 side-chain (Fig. 3A). Although

Table 1
Evaluation of the α-keto oxazole libraries A,B,C and D for FAAH inhibition

	FAAH activity remaining (10 μM, HLPC based-assay)[a]		FAAH activity remaining (10 μM, HLPC based-assay)[a]
A			
13: *n* = 2	27 ± 2%	**18**: *n* = 2	34 ± 3%
14: *n* = 3	40 ± 2%	**19**: *n* = 3	64 ± 3%
15: *n* = 4	49 ± 1%	**20**: *n* = 4	48 ± 10%
16: *n* = 5	64 ± 2%	**21**: *n* = 5	78 ± 11%
17: *n* = 6	72 ± 6%	**22**: *n* = 6	78 ± 2%
B			
23: *n* = 2	3 ± 1%	**28**: *n* = 2	23 ± 6%
24: *n* = 3	2 ± 1%	**29**: *n* = 3	22 ± 1%
25: *n* = 4	32 ± 3%	**30**: *n* = 4	59 ± 7%
26: *n* = 5	53 ± 3%	**31**: *n* = 5	73 ± 8%
27: *n* = 6	51 ± 0%	**32**: *n* = 6	62 ± 4%
C			
33: *n* = 2	78 ± 1%	**36**: *n* = 2	No activity
34: *n* = 4	78 ± 9%	**37**: *n* = 4	68 ± 6%
35: *n* = 5	71 ± 6%	**38**: *n* = 5	82 ± 6%
D			
39: *n* = 2	11 ± 3%	**44**: *n* = 2	61 ± 11%
40: *n* = 3	13 ± 0%	**45**: *n* = 3	53 ± 5%
41: *n* = 4	49 ± 1%	**46**: *n* = 4	83 ± 5%
42: *n* = 5	57 ± 1%	**47**: *n* = 5	70 ± 7%
43: *n* = 6	31 ± 5%	**48**: *n* = 6	–

Residual FAAH enzyme activity given in percentage for the two series of α-keto oxazole inhibitors (R = *n*-$C_{11}H_{23}$ or R = *n*-C_7H_{15} respectively) at 10 μM concentration. Values obtained based on two independents measurements.

[a] All FAAH residual activity values were determined using the HPLC-based assay described previously with *N*-(2-hydroxyethyl)-4-pyren-1-ylbutanamide as substrate, and rat brain as enzyme source. Released 4-pyren-1-ylbutanoic acid was quantified after 60 min incubation time by reversed-phase HPLC with fluorescence applying 6-pyren-1-ylhexanoic acid as internal standard. Data were determined based on two independent measurements.

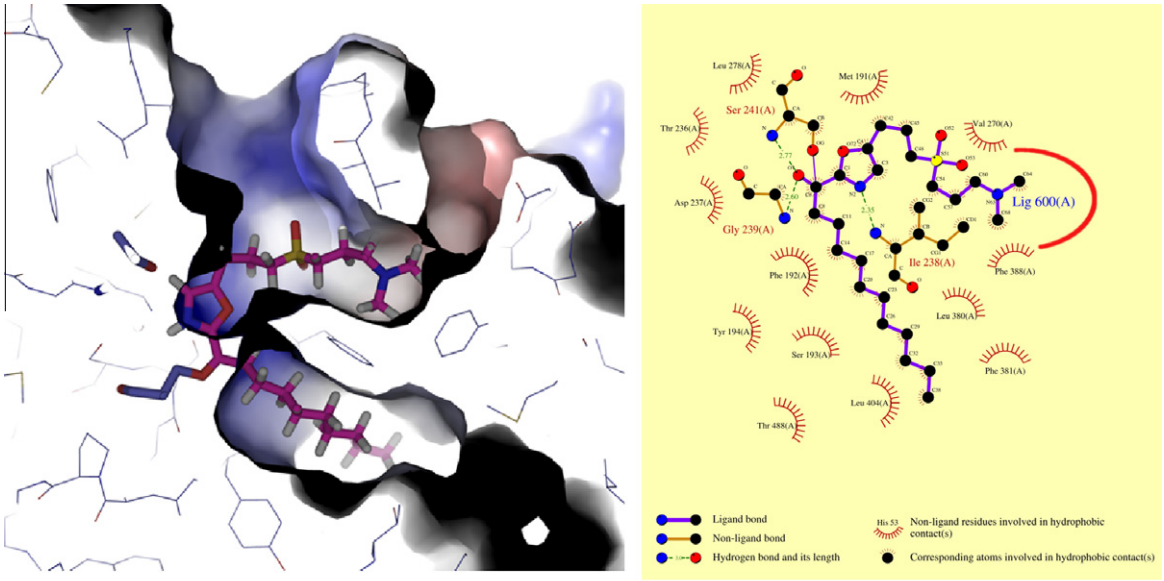


Figure 3. Covalent docking of α-keto oxazole inhibitor **24** in FAAH active site with the oxazole carbonyl group connected to the nucleophilic serine residue (Ser 241). (A) The electrostatic potential surface of the enzyme in the proximity of the C5-side chain of the inhibitor, possibly accounts for the stabilizing interactions respectively with SO_2 and NMe_2 groups. Positively charged regions are shown in blue and negative regions in red. (B) Corresponding ligand plot image. The red half-ellipsoid represents the negatively charged region of the enzyme, present at the proximity of the inhibitor's dimethylamino group. The destabilizing effect observed with inhibitors quaternized at the dimethylamino group may be explained by steric repulsions. Figure 3A was created using the Pymol software.

narrower and deeper, the hydrophobic 'acyl chain-binding' channel was found to be quite flexible and to accept various substrates,[31] provided that the side chain was long enough to confer sufficient affinity, as addressed above. The introduction of a terminal quaternary amino group was also investigated to compensate the negatively charged region located in the enzyme hydrophilic part. However, such modification gave a decreased inhibitory activity, possibly due to steric repulsions (Fig. 3B).

Finally, the optimal location of the polar heteroatoms (–S–, –SO_2–, –NMe_2) in the FAAH hydrophilic pocket was further investigated by varying their position within the C5-side chain to benefit from potential hydrogen bond stabilizing interactions with the protein. Comparison of the inhibition potency among (*n*-$C_{11}H_{23}$)-compounds from the series A–D suggested an optimal spacer length (*n*) at 2- or 3-methylene units. An IC_{50} value of 290 nM was obtained for the most potent inhibitor **24** (*n* = 3, SO_2, NMe_2, *n*-$C_{11}H_{23}$) at standard assay condition when determined from three concentration data points. When measuring more data points to get an IC_{50} curve, a slightly higher IC_{50} value of 436 nM was obtained for compound **24** (Fig. 2B). In addition, the α-keto oxazole **24** was shown not to inhibit several additional serine hydrolases investigated, such as cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$) at a concentration of 1 μM,[32] acyl protein thioesterase 1 (APT1) and acyl protein thioesterase 2 (APT2) at a concentration of 50 μM.[18]

3. Conclusion

A novel series of FAAH-inhibitors is described. In silico exploration of the binding pocket of FAAH led to design features allowing for more polar contacts from inhibitor to hydrophilic parts of FAAH, compared to previously reported compounds. Importantly, the resulting compound design can be used for further improvement of FAAH inhibitors in terms of interactions to hydrophilic parts of the enzyme. Additional work is required to optimize the liphophilic part of the molecule, and possibly further lower molecular weight, Log P and total polar surface area.

4. Experimental section

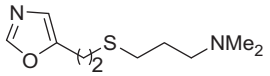
4.1. Chemistry

4.1.1. General

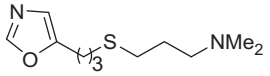
All chemicals were obtained from Aldrich, Acros or Novabiochem, and were used without further purification except where noted. Solvents were used as received or passed over a drying column (DCM, THF, DMF). All reactions were carried out under argon atmosphere using oven-dried glassware. Flash chromatography was performed using silica gel for chromatography 0.035–0.070 mm, 60 Å purchased from Acros Organic. ^{1}H spectra were recorded on a Varian Mercury—400 Oxford NMR spectrometer. Chemical shifts are reported in δ values relative to tetramethylsilane and coupling constants (*J*) are reported in Hertz. Melting points were recorded on a Büchi Melting point B-540. HPLC measurements were performed using an Agilent 1100 Series instrument. GCMS spectra were recorded on a Hewlett Packard, HP6890 series GC system, equipped with a Hewlett Packard, HP5973 series mass selective detector. Ethyl esters **1–6** were synthesized accordingly to the literature.[18]

4.1.2. Synthesis of C5-substituted oxazoles (6–10)

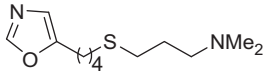
C5-substituted oxazoles were synthesized following a modified literature protocol.[19–21] To a solution of methyl isocyanide (5.8 mmol, 1.34 equiv) in anhydrous THF (13.5 ml), *n*BuLi (2.5 M in hexane, 6.5 mmol, 1.5 equiv) was added dropwise at −78 °C to the reaction mixture which was then stirred at −78 °C for 2 h. A solution of ethyl ester **1–5** (4.3 mmol, 1.0 equiv) in dry THF (3 ml) was added dropwise (over 30 min) to the reaction mixture which was then stirred for 3 h at −78 °C followed by 3 h at rt. The mixture was quenched with brine (100 ml) and extracted with Et_2O (3 × 100 ml). The combined organic extracts were subsequently dried over anhydrous $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography (gradient 1–4% MeOH/DCM using NEt_3 pre-treated column) leading to the corresponding C5-substituted oxazole **(6–10)**.



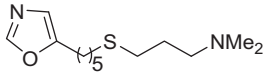
4.1.2.1. *N,N*-Dimethyl-3-(2-(oxazol-5-yl)ethylthio)propan-1-amine (6). Compound **6** was synthesized following the general procedure for the C5-substituted oxazole formation on 4.3 mmol of ethyl ester **1**. Yield = 84% (yellow oil). R_f = 0.42 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (s, 1H, *H*–C═N), 6.77 (t, 1H, *J* = 0.8 Hz, *H*–C═C), 2.81–2.97 (m, 2H, –CH_2–C═C), 2.76–2.71 (m, 2H, –CH_2S), 2.49 (t, 2H, *J* = 7.4 Hz, –CH_2S), 2.26 (t, 2H, *J* = 7.2 Hz, –CH_2NMe_2), 2.14 (s, 6H, $N(CH_3)_2$), 1.67 (dd, 2H, *J* = 7.4, *J* = 7.2 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 150.9, 150.1, 122.6, 58.3, 45.3, 29.9, 29.4, 27.5, 26.1. HRMS (ESI) calcd for $C_{10}H_{18}N_2OS$ $[M+H]^+$ 215.1213, found 215.1211. GCMS found 214 for $[M^{+\cdot}]$.



4.1.2.2. *N,N*-Dimethyl-3-(3-(oxazol-5-yl)propylthiol)propan-1-amine (7). Compound **7** was synthesized following the general procedure for the C5-substituted oxazole formation on 5.8 mmol of ethyl ester **2** (no purification needed). Yield = 95% (yellow oil). R_f = 0.43 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (s, 1H, *H*–C═N), 6.74 (t, 1H, *J* = 0.8 Hz, *H*–C═C), 2.75 (dt, 2H, *J* = 7.4, *J* = 0.8 Hz, –CH_2–C═C), 2.55 (t, 2H, *J* = 7.2 Hz, –CH_2S), 2.54 (t, 2H, *J* = 7.2 Hz, –CH_2S), 2.30 (t, 2H, *J* = 7.2 Hz, –CH_2NMe_2), 2.17 (s, 6H, $N(CH_3)_2$), 1.96–1.84 (m, 2H), 1.79–1.65 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 151.7, 149.8, 121.8, 58.1, 45.0 (2C), 30.8, 29.5, 27.3, 27.0, 23.9. HRMS (ESI) calcd for $C_{11}H_{20}N_2OS$ $[M+H]^+$ 229.1369, found 229.1368. GCMS found 228 for $[M^{+\cdot}]$.

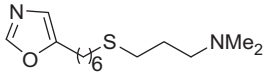


4.1.2.3. *N,N*-Dimethyl-3-(4-(oxazol-5-yl)butylthio)propan-1-amine (8). Compound **8** was synthesized following the general procedure for the C5-substituted oxazole formation on 14 mmol of ethyl ester **3** (no purification needed). Yield = 94% (orange oil). R_f = 0.47 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (s, 1H, *H*–C═N), 6.75 (t, 1H, *J* = 0.8 Hz, *H*–C═C), 2.66 (dt, 2H, *J* = 7.6, *J* = 0.8 Hz, –CH_2–C═C), 2.52 (t, 2H, *J* = 7.2 Hz, –CH_2S), 2.51 (t, 2H, *J* = 7.4 Hz, –CH_2S), 2.32 (t, 2H, *J* = 7.2 Hz, –CH_2NMe_2), 2.20 (s, 6H, $N(CH_3)_2$), 1.74–1.64 (m, 4H), 1.62–1.54 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 152.5, 149.9, 121.9, 58.5, 45.3 (2C), 31.6, 29.9, 28.8, 27.6, 26.6, 24.8. HRMS (ESI) calcd for $C_{12}H_{22}N_2OS$ $[M+H]^+$ 243.1526, found 243.1526. GCMS found 242 for $[M^{+\cdot}]$.



4.1.2.4. *N,N*-Dimethyl-3-(5-(oxazol-5-yl)pentylthio)propan-1-amine (9). Compound **9** was synthesized following the general procedure for the C5-substituted oxazole formation on

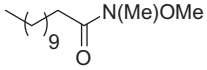
3.9 mmol of ethyl ester **4** (no purification needed). Yield = 90% (orange oil). R_f = 0.50 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (s, 1H, *H*–C═N), 6.73 (t, 1H, *J* = 0.8 Hz, *H*–C═C), 2.64 (dt, 2H, *J* = 7.6, *J* = 0.8 Hz, –C*H*$_2$–C═C), 2.51 (t, 2H, *J* = 7.6 Hz, –C*H*$_2$S), 2.49 (t, 2H, *J* = 7.6 Hz, –C*H*$_2$S), 2.32 (t, 2H, *J* = 7.4 Hz, –C*H*$_2$NMe$_2$), 2.19 (s, 6H, N(C*H*$_3$)$_2$), 1.74–1.51 (m, 6H), 1.45–1.34 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 152.7, 149.8, 121.7, 58.5, 45.3 (2C), 31.9, 29.9, 29.1, 28.1, 27.6, 27.0, 25.1. HRMS (ESI) calcd for $C_{13}H_{24}N_2OS$ [M+H]$^+$ 257.1682, found 257.1682. GCMS found 256 for [M$^{+\cdot}$].



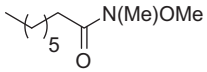
4.1.2.5. *N,N*-Dimethyl-3-(6-(oxazol-5-yl)hexylthio)propan-1-amine (10). Compound **10** was synthesized following the general procedure for the C5-substituted oxazole formation on 9.8 mmol of ethyl ester **5** (no purification needed). Yield = 92% (orange oil). R_f = 0.50 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (s, 1H, *H*–C═N), 6.69 (t, 1H, *J* = 0.8 Hz, *H*–C═C), 2.59 (dt, 2H, *J* = 7.6, *J* = 0.8 Hz, –C*H*$_2$–C═C), 2.47 (t, 2H, *J* = 7.4 Hz, –C*H*$_2$S), 2.45 (t, 2H, *J* = 7.4 Hz, –C*H*$_2$S), 2.29 (t, 2H, *J* = 7.2 Hz, –C*H*$_2$NMe$_2$), 2.16 (s, 6H, N(C*H*$_3$)$_2$), 1.73–1.47 (m, 6H), 1.40–1.25 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 152.9, 149.8, 121.7, 58.6, 45.4 (2C), 32.0, 29.9, 29.4, 28.5, 28.4, 27.7, 27.3, 25.2. HRMS (ESI) calcd for $C_{14}H_{26}N_2OS$ [M+H]$^+$ 271.1839, found 271.1840. GCMS found 270 for [M$^{+\cdot}$].

4.1.3. Synthesis of Weinreb amides (11–12)

Weinreb amides **11–12** were synthesized following a modified literature protocol.[33,34] To a solution of *N,O*-dimethylhydroxylamine hydrochloride (3.9 g, 39 mmol, 1.1 equiv) and pyridine (82 mmol, 2.2 equiv) in dry DCM (55 ml), was added slowly the corresponding acid chloride (36.5 mmol, 1.0 equiv) at 0 °C over 15 min. The mixture was allowed to warm to rt and was stirred for 5 h at rt. The mixture was then diluted with EtOAc (200 ml), washed with 1 N HCl (100 ml × 2), saturated aqueous $NaHCO_3$ (50 ml × 2) and brine (50 ml). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure leading to the corresponding Weinreb amides **11–12** which were used directly in the next step.



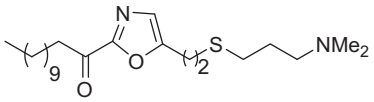
4.1.3.1. *N*-Methoxy-*N*-methyldodecanamide (11). Compound **11** was synthesized following the general procedure for the Weinreb amide synthesis using 115 mmol of acyl chloride (dodecanoyl chloride). Yield = 98% (colorless oil). R_f = 0.71 (EtOAc/cyclohexane 1/1). IR: 1668 cm^{-1} (amine C═O stretch), 2922–2853 cm^{-1} (amine N–H stretch). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.64 (s, 3H, OC*H*$_3$), 3.16 (s, 3H, –NC*H*$_3$), 2.37 (t, 2H, *J* = 7.4 Hz, –C*H*$_2$CO), 1.62–1.56 (m, 2H), 1.34–1.13 (m, 16H), 0.83 (t, 3H, *J* = 6.6 Hz, C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.7, 61.0, 31.8, 29.5 (4C), 29.4, 29.3 (2C), 29.2, 24.5, 22.6, 13.9. HRMS (ESI) calcd For $C_{14}H_{29}NO_2$ [M+H]$^+$ 244.2271, found 244.2270. GCMS found 243 for [M$^{+\cdot}$].



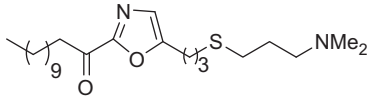
4.1.3.2. *N*-Methoxy-*N*-methyloctanamide (12). Compound **12** was synthesized following the general procedure for the Weinreb amide synthesis using 164 mmol of acyl chloride (octanoyl chloride). Yield = 94% (colorless oil). R_f = 0.67 (EtOAc/cyclohexane 1/1). IR: 1665 cm^{-1} (amine C═O stretch), 2955–2855 cm^{-1} (amine N–H stretch). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.64 (s, 3H, OC*H*$_3$), 3.14 (s, 3H, –NC*H*$_3$), 2.37 (t, 2H, *J* = 7.6 Hz, –C*H*$_2$CO), 1.62–1.55 (m, 2H), 1.34–1.19 (m, 8H), 0.84 (t, 3H, *J* = 7.4 Hz, C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.7, 61.1, 32.1, 31.8, 31.6, 29.3, 29.0, 24.5, 22.5, 13.9. HRMS (ESI) calcd for $C_{10}H_{21}NO_2$ [M+H]$^+$ 188.1645, found 188.1641. GCMS found 187 for [M$^{+\cdot}$].

4.1.4. Synthesis of C2-functionalized oxazoles (13–22)[22,34]

To a 100 ml round bottomed flask equipped with a stirring bar, argon inlet, was added C5-substituted oxazole **6–10** (8.7 mmol, 1 equiv) as a solution in freshly distilled THF (20 ml). *I*-PrMgCl (2 M in THF, 11.3 mmol, 1.4 equiv) was added over 10 min to the mixture previously cooled to −15 °C which was then stirred for 2 h between −15 °C and −20 °C. A solution of Weinreb amides **11–12** (8.65 mmol, 1.0 equiv) as a solution in distilled THF (15 ml) was added dropwise to the reaction mixture at such a rate that the temperature was kept between −20 °C and −25 °C (addition over 25 min). The reaction was stirred at −20 °C for 10 min and at rt for 25 h. The mixture was quenched with water (25 ml) and extracted with EtOAc (4x150 ml) and brine (100 ml). Saturated NH_4Cl solution was added to the aqueous layer which was extracted with EtOAc. The combined organics layers were dried over anhydrous $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography (eluent: gradient 1–2% MeOH/DCM) leading to the C2-functionalized oxazoles **13–22**.

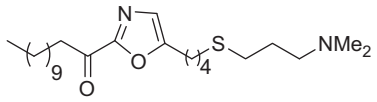


4.1.4.1. 1-(5-(2-(3-(Dimethylamino)propylthio)ethyl)oxazol-2-yl)dodecan-1-one (13). Compound **13** was synthesized following the general procedure for the oxazole C2-functionalization using 9.8 mmol of Weinreb amide **11**. Yield = 24% (orange oil). R_f = 0.74 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1699 cm^{-1} (ketone C═O stretch). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01 (t, 1H, *J* = 0.8 Hz, *H*–C═C), 2.99 (t, 2H, *J* = 7.0 Hz, –C*H*$_2$CO), 2.98 (t, 2H, *J* = 6.8 Hz, –C*H*$_2$S), 2.83 (dt, 2H, *J* = 7.4, *J* = 0.8 Hz, –C*H*$_2$–C═C), 2.56 (t, 2H, *J* = 7.2 Hz, –C*H*$_2$S), 2.34 (t, 2H, *J* = 7.2 Hz, –C*H*$_2$NMe$_2$), 2.22 (s, 6H, N(C*H*$_3$)$_2$), 1.78–1.66 (m, 4H), 1.39–1.14 (m, 16H), 0.86 (t, 3H, *J* = 6.8 Hz, –C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.5, 157.4, 154.6, 125.5, 58.3, 45.3 (2C), 38.8, 31.9, 30.0, 29.7, 29.5 (2C), 29.4, 29.3, 29.3, 29.1, 27.4, 26.4, 24.0, 22.6, 14.1. HRMS (ESI) calcd for $C_{22}H_{40}N_2O_2S$ [M+H]$^+$ 397.2883, found 397.2873. LC–MS (ESI) found 397.23 for [M+H]$^+$.

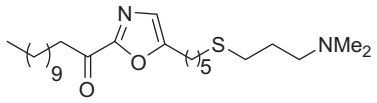


4.1.4.2. 1-(5-(3-(3-(Dimethylamino)propylthio)propyl)oxazol-2-yl)dodecan-1-one (14). Compound **14** was synthesized following the general procedure for the oxazole C2-functionalization using 8.6 mmol of Weinreb amide **11**. Yield = 37% (yellow oil). R_f = 0.64 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1698 cm^{-1} (ketone C═O stretch). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.96 (t, 1H, *J* = 0.8 Hz, *H*–C═C), 3.00 (t, 2H, *J* = 7.6 Hz, –C*H*$_2$CO), 2.85 (dt, 2H, *J* = 7.2, *J* = 0.8 Hz, –C*H*$_2$–C═C), 2.55 (t, 2H, *J* = 7.2 Hz, –C*H*$_2$S), 2.54 (t, 2H, *J* = 7.2 Hz, –C*H*$_2$S), 2.39 (t, 2H, *J* = 7.2 Hz, –C*H*$_2$NMe$_2$), 2.25 (s, 6H, N(C*H*$_3$)$_2$), 2.01–1.92 (m, 2H), 1.82–1.75 (m, 2H), 1.74–1.67 (m, 2H), 1.43–1.16 (m, 16H), 0.85 (t, 3H, *J* = 6.8 Hz, –C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.5, 157.4, 155.8, 124.9, 58.3, 45.1 (2C), 38.8, 31.8, 31.2, 29.8, 29.5 (2C), 29.4, 29.3, 29.3, 29.1, 27.3, 27.1,

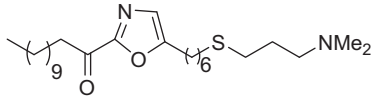
24.6, 24.0, 22.6, 14.1. HRMS (EI) calcd for $C_{23}H_{42}N_2O_2S$ [$M^{+\cdot}$] 410.2962, found 410.2951. GCMS found 410 for [$M^{+\cdot}$].



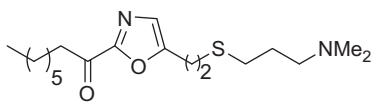
4.1.4.3. 1-(5-(4-(3-(Dimethylamino)propylthio)butyl)oxazol-2-yl)dodecan-1-one (15). Compound **15** was synthesized following the general procedure for the oxazole C2-functionalization using 8.4 mmol of Weinreb amide **11**. Yield = 29% (yellow oil). R_f = 0.71 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1699 cm^{-1} (ketone C=O stretch). ^1H NMR (400 MHz, $CDCl_3$): δ 6.95 (t, 1H, J = 0.8 Hz, H–C=C), 3.01 (t, 2H, J = 7.6 Hz, –CH_2CO), 2.75 (dt, 2H, J = 7.6, J = 0.8 Hz, –CH_2–C=C), 2.55 (t, 2H, J = 7.2 Hz, –CH_2S), 2.54 (t, 2H, J = 7.4 Hz, –CH_2S), 2.43 (t, 2H, J = 7.0 Hz, –CH_2NMe$_2$), 2.28 (s, 6H, N(CH_3)$_2$), 1.84–1.59 (m, 8H), 1.39–1.18 (m, 16H), 0.87 (t, 3H, J = 6.8 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.5, 157.3, 156.4, 124.7, 58.4, 45.1 (2C), 38.8, 31.8, 31.6, 29.9, 29.5 (2C), 29.4, 29.3, 29.3, 29.1, 28.9, 27.3, 26.5, 25.4, 24.0, 22.6, 14.1. HRMS (ESI) calcd for $C_{24}H_{44}N_2O_2S$ [M+H]$^+$ 425.3196, found 425.3191. LC–MS (ESI) found 425.23 for [M+H]$^+$.



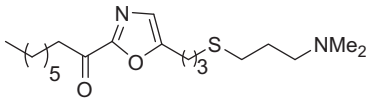
4.1.4.4. 1-(5-(5-(3-(Dimethylamino)propylthio)pentyl)oxazol-2-yl)dodecan-1-one (16). Compound **16** was synthesized following the general procedure for the oxazole C2-functionalization using 7.6 mmol of Weinreb amide **11**. Yield = 34% (yellow oil). R_f = 0.71 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1699 cm^{-1} (ketone C=O stretch). ^1H NMR (400 MHz, $CDCl_3$): δ 6.93 (t, 1H, J = 0.8 Hz, H–C=C), 3.00 (t, 2H, J = 7.4 Hz, –CH_2CO), 2.72 (dt, 2H, J = 7.4, J = 0.8 Hz, –CH_2–C=C), 2.52 (t, 2H, J = 7.4 Hz, –CH_2S), 2.50 (t, 2H, J = 7.4 Hz, –CH_2S), 2.39 (t, 2H, J = 7.4 Hz, –CH_2NMe$_2$), 2.25 (s, 6H, N(CH_3)$_2$), 1.80–1.65 (m, 6H), 1.64–1.55 (m, 2H), 1.49–1.39 (m, 2H), 1.37–1.19 (m, 16H), 0.86 (t, 3H, J = 6.8 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.5, 157.2, 156.6, 124.6, 58.3, 45.0 (2C), 38.8, 31.9, 31.8, 29.9, 29.5 (2C), 29.4, 29.3, 29.3, 29.1, 29.1, 28.2, 27.2, 26.9, 25.6, 24.0, 22.6, 14.1. HRMS (ESI) calcd for $C_{25}H_{46}N_2O_2S$ [M+H]$^+$ 439.3353, found 439.3346. LC–MS (ESI) found 439.26 for [M+H]$^+$.



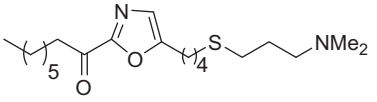
4.1.4.5. 1-(5-(6-(3-(Dimethylamino)propylthio)hexyl)oxazol-2-yl)dodecan-1-one (17). Compound **17** was synthesized following the general procedure for the oxazole C2-functionalization using 5.7 mmol of Weinreb amide **11**. Yield = 36% (yellow oil). R_f = 0.75 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1698 cm^{-1} (ketone C=O stretch). ^1H NMR (400 MHz, $CDCl_3$): δ 6.91 (t, 1H, J = 0.8 Hz, H–C=C), 2.99 (t, 2H, J = 7.4 Hz, –CH_2CO), 2.70 (dt, 2H, J = 7.6, J = 0.8 Hz, –CH_2–C=C), 2.52 (t, 2H, J = 7.2 Hz, –CH_2S), 2.48 (t, 2H, J = 7.2 Hz, –CH_2S), 2.45 (t, 2H, J = 7.6 Hz, –CH_2NMe$_2$), 2.29 (s, 6H, N(CH_3)$_2$), 1.82–1.62 (m, 6H), 1.60–1.50 (m, 2H), 1.43–1.17 (m, 20H), 0.85 (t, 3H, J = 6.6 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.5, 157.2, 156.8, 124.6, 58.2, 44.8 (2C), 38.7, 32.0, 31.8, 29.8, 29.5(2C), 29.4, 29.3, 29.3, 29.2, 29.1, 28.6, 28.3, 27.2, 26.9, 25.6, 24.0, 22.6, 14.0. HRMS (ESI) calcd for $C_{26}H_{48}N_2O_2S$ [M+H]$^+$ 453.3509, found 453.3502. LC–MS (ESI) found 453.32 for [M+H]$^+$.



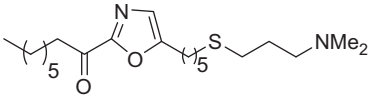
4.1.4.6. 1-(5-(2-(3-(Dimethylamino)propylthio)ethyl)oxazol-2-yl)octan-1-one (18). Compound **18** was synthesized following the general procedure for the oxazole C2-functionalization using 9.1 mmol of Weinreb amide **12**. Yield = 25% (orange oil). R_f = 0.71 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1698 cm^{-1} (ketone C=O stretch). ^1H NMR (400 MHz, $CDCl_3$): δ 6.96 (t, 1H, J = 0.8 Hz, H–C=C), 2.96 (t, 2H, J = 7.2 Hz, –CH_2CO), 2.94 (t, 2H, J = 7.4 Hz, –CH_2S), 2.77 (dt, 2H, J = 7.4, J = 0.8 Hz, –CH_2–C=C), 2.49 (t, 2H, J = 7.4 Hz, –CH_2S), 2.27 (t, 2H, J = 7.2 Hz, –CH_2NMe$_2$), 2.14 (s, 6H, N(CH_3)$_2$), 1.70–1.61 (m, 4H), 1.37–1.16 (m, 8H), 0.79 (t, 3H, J = 7.0 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.4, 157.3, 154.6, 125.4, 58.2, 45.2 (2C), 38.8, 31.5, 30.0, 29.6, 29.0, 28.9, 27.3, 26.4, 23.9, 22.5, 13.9. HRMS (ESI) calcd for $C_{18}H_{32}N_2O_2S$ [M+H]$^+$ 341.2257, found 341.2258. LC–MS (ESI) found 341.15 for [M+H]$^+$.



4.1.4.7. 1-(5-(3-(3-(Dimethylamino)propylthio)propyl)oxazol-2-yl)octan-1-one (19). Compound **19** was synthesized following the general procedure for the oxazole C2-functionalization using 8.5 mmol of Weinreb amide **12**. Yield = 34% (yellow oil). R_f = 0.65 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1698 cm^{-1} (ketone C=O stretch). ^1H NMR (400 MHz, $CDCl_3$): δ 6.95 (t, 1H, J = 0.8 Hz, H–C=C), 2.99 (t, 2H, J = 7.4 Hz, –CH_2CO), 2.84 (dt, 2H, J = 7.4, J = 0.8 Hz, –CH_2–C=C), 2.54 (t, 2H, J = 6.8 Hz, –CH_2S), 2.52 (t, 2H, J = 7.2 Hz, –CH_2S), 2.36 (t, 2H, J = 7.2 Hz, –CH_2NMe$_2$), 2.22 (s, 6H, N(CH_3)$_2$), 1.93–1.85 (m, 2H), 1.72–1.58 (m, 4H), 1.34–1.12 (m, 8H), 0.85 (t, 3H, J = 7.0 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.5, 157.3, 155.8, 124.9, 58.3, 45.2 (2C), 38.7, 31.6, 31.2, 29.8, 29.1, 28.9, 27.3, 27.0, 24.6, 24.0, 22.5, 14.0. HRMS (EI) calcd for $C_{19}H_{34}N_2O_2S$ [$M^{+\cdot}$] 354.2336, found 354.2330. LC–MS (ESI) found 355.16 for [M+H]$^+$.

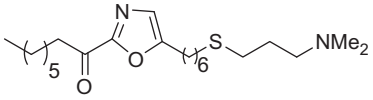


4.1.4.8. 1-(5-(4-(3-(Dimethylamino)propylthio)butyl)oxazol-2-yl)octan-1-one (20). Compound **20** was synthesized following the general procedure for the oxazole C2-functionalization using 8.7 mmol of Weinreb amide **12**. Yield = 32% (yellow oil). R_f = 0.74 (EtOAc/AcOH/MeOH/H_2 3/3/3/2). IR: 1698 cm−1 (ketone C=O stretch). ^1H NMR (400 MHz, $CDCl_3$): δ 6.96 (t, 1H, J = 0.8 Hz, H–C=C), 3.02 (t, 2H, J = 7.6 Hz, –CH_2CO), 2.76 (dt, 2H, J = 7.4, J = 0.8 Hz, –CH_2–C=C), 2.56 (t, 2H, J = 7.2 Hz, –CH_2S), 2.55 (t, 2H, J = 7.2 Hz, –CH_2S), 2.36 (t, 2H, J = 7.2 Hz, –CH_2NMe$_2$), 2.22 (s, 6H, N(CH_3)$_2$), 1.90–1.77 (m, 4H), 1.76–1.61 (m, 4H), 1.42–1.21 (m, 8H), 0.87 (t, 3H, J = 7.0 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.5, 157.3, 156.4, 124.7, 58.5, 45.3 (2C), 38.7, 31.6, 31.6, 30.0, 29.1, 28.9, 28.8, 27.5, 26.4, 25.3, 24.0, 22.5, 14.0. HRMS (ESI) calcd for $C_{20}H_{36}N_2O_2S$ [M+H]$^+$ 369.2570, found 369.2571. LC–MS (ESI) found 369.19 for [M+H]$^+$.



4.1.4.9. 1-(5-(5-(3-(Dimethylamino)propylthio)pentyl)oxazol-2-yl)octan-1-one (21). Compound **21** was synthesized following the general procedure for the oxazole C2-functionalization using 8.5 mmol of Weinreb amide **12**. Yield = 28% (yellow oil). R_f = 0.77 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1698 cm−1 (ketone C=O stretch). ^1H NMR (400 MHz, $CDCl_3$): δ 6.92 (t, 1H,

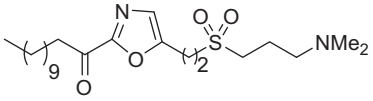
J = 0.8 Hz, H–C=C), 2.99 (t, 2H, J = 7.6 Hz, –CH_2CO), 2.71 (dt, 2H, J = 7.4, J = 0.8 Hz, –CH_2–C=C), 2.51 (t, 2H, J = 7.4 Hz, –CH_2S), 2.49 (t, 2H, J = 7.4 Hz, –CH_2S), 2.35 (t, 2H, J = 7.2 Hz, –CH_2NMe$_2$), 2.21 (s, 6H, N(CH_3)$_2$), 1.80–1.66 (m, 6H), 1.64–1.55 (m, 2H), 1.48–1.39 (m, 2H), 1.37–1.19 (m, 8H), 0.85 (t, 3H, J = 7.0 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.5, 157.2, 156.6, 124.6, 58.5, 45.2 (2C), 38.7, 31.9, 31.6, 29.9, 29.0 (2C), 28.9, 28.2, 27.4, 26.9, 25.6, 24.0, 22.5, 14.0. HRMS (ESI) calcd for $C_{21}H_{38}N_2O_2S$ [M+H]$^+$ 383.2727, found 383.2733. LC–MS (ESI) found 383.22 for [M+H]$^+$.



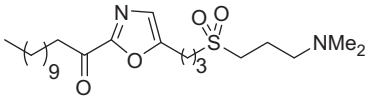
4.1.4.10. 1-(5-(6-(3-(Dimethylamino)propylthio)hexyl)oxazol-2-yl)octan-1-one (22). Compound **22** was synthesized following the general procedure for the oxazole C2-functionalization using 6.6 mmol of Weinreb amide **12**. Yield = 32% (yellow oil). R_f = 0.73 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1699 cm−1 (ketone C=O stretch). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.92 (t, 1H, J = 0.8 Hz, H–C=C), 2.99 (t, 2H, J = 8.0 Hz, –CH_2CO), 2.76 (dt, 2H, J = 7.6, J = 0.8 Hz, –CH_2–C=C), 2.51 (t, 2H, J = 7.2 Hz, –CH_2S), 2.50 (t, 2H, J = 7.0 Hz, –CH_2S), 2.41 (t, 2H, J = 7.4 Hz, –CH_2NMe$_2$), 2.26 (s, 6H, N(CH_3)$_2$), 1.81–1.62 (m, 6H), 1.61–1.50 (m, 2H), 1.45–1.17 (m, 12H), 0.85 (t, 3H, J = 7.0 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.5, 157.2, 156.8, 124.6, 58.4, 45.1(2C), 38.8, 32.0, 31.6, 29.9, 29.3, 29.1, 28.9, 28.6, 28.3, 27.2 (2C), 25.6, 24.0, 22.5, 14.0. HRMS (ESI) calcd for $C_{22}H_{40}N_2O_2S$ [M+H]$^+$ 397.2883, found 397.2873. LC–MS (ESI) found 397.24 for [M+H]$^+$.

4.1.5. Synthesis of S-oxidized oxazoles (23–32)[23]

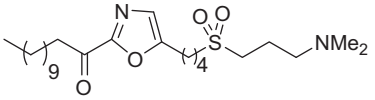
To a 25 ml round bottomed flask equipped with a stirring bar, was added *C2*-substituted oxazoles **13–22** (0.46 mmol, 1.0 equiv) in MeOH (14 ml). The mixture was cooled to 0 °C and a suspension of oxone® (1.40 mmol, 3.0 equiv) in water (9 ml) was added over 5 min to the mixture which was subsequently stirred at rt for 60 h. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ (30 ml) and extracted with EtOAc (100 ml). The aqueous layer was basified to pH 12.0 using saturated $NaHCO_3$ solution and extracted with EtOAc (4 × 50 ml). The combined organic extracts were dried over anhydrous $MgSO_4$, concentrated under reduced pressure and purified by reverse phase chromatography (reverse C18 cartridge (5 g), gradient MeOH/H_2O up to 100% MeOH) leading to compounds **23–32**.



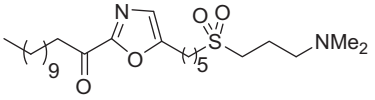
4.1.5.1. 1-(5-(2-(3-(Dimethylamino)propylsulfonyl)ethyl)oxazol-2-yl)dodecan-1-one (23). Compound **23** was synthesized following the general procedure for oxidation into sulfone using 0.42 mmol of sulfide derivative **13**, reaction time 60 h. Purification by reverse phase chromatography using RPC_{18} column (eluent 0–100% MeOH). Yield = 76% (white semi-solid). R_f = 0.54 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1694 cm^{-1} (ketone C=O stretch), 1304 cm^{-1} (SO_2 asym. stretch), 1112 cm^{-1} (SO_2 sym. stretch). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.10 (t, 1H, J = 0.8 Hz, H–C=C), 3.42–3.27 (m, 4H, –CH_2SO_2), 3.18–3.11 (m, 2H, –CH_2CO), 3.04–2.98 (m, 2H, –CH_2–C=C), 2.70–2.61 (m, 2H, –CH_2NMe$_2$), 2.39 (s, 6H, N(CH_3)$_2$), 2.16–2.07 (m, 2H), 1.76–1.66 (m, 2H), 1.41–1.15 (m, 16H), 0.87 (t, 3H, J = 6.8 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.5, 157.7, 151.8, 126.1, 56.9, 50.8, 50.3, 44.5 (2C), 38.9, 31.9, 29.6, 29.4, 29.3 (2C), 29.1, 23.9, 22.7, 19.1, 18.7, 14.1. HRMS (ESI) calcd for $C_{22}H_{40}N_2O_4S$ [M+H]$^+$ 429.2782, found 429.2776. LC–MS (ESI) found 429.22 for [M+H]$^+$.



4.1.5.2. 1-(5-(3-(3-(Dimethylamino)propylsulfonyl)propyl)oxazol-2-yl)dodecan-1-one (24). Compound **24** was synthesized following the general procedure for oxidation into sulfone using 0.43 mmol of sulfide derivative **14**, reaction time 60 h. Purification by reverse phase chromatography using RPC_{18} column (eluent 0–100% MeOH). Yield = 75% (white semi-solid). R_f = 0.53 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1699 cm^{-1} (ketone C=O stretch), 1318 cm^{-1} (SO_2 asym. stretch), 1129 cm^{-1} (SO_2 sym. stretch). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (t, 1H, J = 0.8 Hz, H–C=C), 3.20–3.14 (m, 2H, –CH_2SO_2), 3.11–3.05 (m, 2H, –CH_2SO_2), 3.02 (t, 2H, J = 7.6 Hz, –CH_2CO), 2.96 (dt, 2H, J = 7.4, J = 0.8 Hz, –CH_2–C=C), 2.83–2.74 (m, 2H, –CH_2NMe$_2$), 2.49 (s, 6H, N(CH_3)$_2$), 2.30–2.20 (m, 2H), 2.16–2.07 (m, 2H), 1.76–1.66 (m, 2H), 1.41–1.15 (m, 16H), 0.87 (t, 3H, J = 7.0 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.5, 157.6, 154.3, 125.5, 57.0, 51.8, 50.6, 44.6 (2C), 38.9, 31.9, 29.6(2C), 29.5, 29.3, 29.3, 29.2, 24.4, 24.0, 22.7, 20.1, 19.1, 14.1. HRMS (ESI) calcd for $C_{23}H_{42}N_2O_4S$ [M+H]$^+$ 443.2938, found 443.2936. GCMS found 442 for [M$^{+\cdot}$].

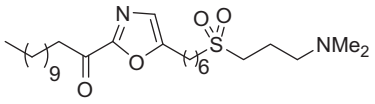


4.1.5.3. 1-(5-(4-(3-(Dimethylamino)propylsulfonyl)butyl)oxazol-2-yl)dodecan-1-one (25). Compound **25** was synthesized following the general procedure for oxidation into sulfone using 0.38 mmol of sulfide derivative **15**, reaction time 60 h. Purification by reverse phase chromatography using RPC_{18} column (eluent 0–100% MeOH). Yield = 73% (white semi-solid). R_f = 0.53 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1701 cm^{-1} (ketone C=O stretch), 1274 cm^{-1} (SO_2 asym. stretch), 1111 cm^{-1} (SO_2 sym. stretch). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.99 (t, 1H, J = 0.8 Hz, H–C=C), 3.22–3.16 (m, 2H, –CH_2SO_2), 3.11–3.05 (m, 2H, –CH_2SO_2), 3.01 (t, 2H, J = 7.4 Hz, –CH_2CO), 2.97–2.90 (m, 2H, –CH_2–C=C), 2.83–2.76 (m, 2H, –CH_2NMe$_2$), 2.61 (s, 6H, N(CH_3)$_2$), 2.30–2.19 (m, 2H), 1.97–1.81 (m, 4H), 1.75–1.66 (m, 2H), 1.41–1.18 (m, 16H), 0.87 (t, 3H, J = 6.8 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.6, 157.4, 155.5, 125.1, 56.7, 52.5, 50.0, 43.9 (2C), 38.8, 31.9, 29.6 (2C), 29.4, 29.3, 29.3, 29.1, 26.2, 25.2, 24.0, 22.6, 21.3, 18.4, 14.1. HRMS (ESI) calcd for $C_{24}H_{44}N_2O_4S$ [M+H]$^+$ 457.3095, found 457.3087. LC–MS (ESI) found 457.26 for [M+H]$^+$.

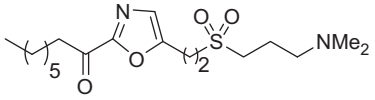


4.1.5.4. 1-(5-(5-(3-(Dimethylamino)propylsulfonyl)pentyl)oxazol-2-yl)dodecan-1-one (26). Compound **26** was synthesized following the general procedure for oxidation into sulfone using 0.38 mmol of sulfide derivative **16**, reaction time 60 h. Purification by reverse phase chromatography using RPC_{18} column (eluent 0–100% MeOH). Yield = 74% (white semi-solid). R_f = 0.58 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1701 cm^{-1} (ketone C=O stretch), 1320 cm^{-1} (SO_2 asym. stretch), 1127 cm^{-1} (SO_2 sym. stretch). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.95 (t, 1H, J = 0.8 Hz, H–C=C), 3.10–3.05 (m, 2H, –CH_2SO_2), 3.01 (t, 2H, J = 7.4 Hz, –CH_2CO), 2.99–2.96 (m, 2H, –CH_2SO_2), 2.76 (dt, 2H, J = 7.4, J = 0.8 Hz, –CH_2–C=C), 2.56 (t, 2H, J = 6.8 Hz, –CH_2NMe$_2$), 2.33 (s, 6H, N(CH_3)$_2$), 2.12–2.02 (m, 2H), 1.94–1.83 (m, 2H), 1.80–1.68 (m, 4H), 1.58–1.48 (m, 2H), 1.42–1.17 (m, 16H), 0.87 (t, 3H, J = 6.8 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.6, 157.4, 156.1, 124.8, 57.2, 52.8, 50.4,

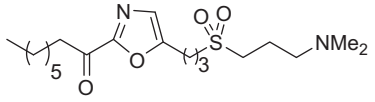
44.7 (2C), 38.8, 31.9, 29.6 (2C), 29.4, 29.3, 29.3, 29.2, 27.9, 26.9, 25.4, 24.0, 22.7, 21.6, 19.3, 14.1. HRMS (ESI) calcd for $C_{25}H_{46}N_2O_4S$ $[M+H]^+$ 471.3251, found 471.3243. LC–MS (ESI) found 471.27 for $[M+H]^+$.



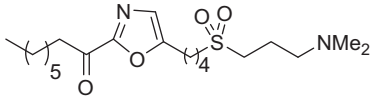
4.1.5.5. 1-(5-(6-(3-(Dimethylamino)propylsulfonyl)hexyl)oxazol-2-yl)dodecan-1-one (27). Compound **27** was synthesized following the general procedure for oxidation into sulfone using 0.35 mmol of sulfide derivative **17**, reaction time 60 h. Purification by reverse phase chromatography using RPC_{18} column (eluent 0–100% MeOH). Yield = 69% (white semi-solid). R_f = 0.62 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1703 cm^{-1} (ketone C=O stretch), 1276 cm^{-1} (SO_2 asym. stretch), 1109 cm^{-1} (SO_2 sym. stretch). ^1H NMR (400 MHz, $CDCl_3$): δ 6.94 (t, 1H, J = 0.8 Hz, H–C=C), 3.08–3.05 (m, 2H, –CH_2SO_2), 3.01 (t, 2H, J = 7.4 Hz, –CH_2CO), 2.99–2.96 (m, 2H, –CH_2SO_2), 2.73 (dt, 2H, J = 7.4, J = 0.8 Hz, –CH_2–C=C), 2.61–2.52 (m, 2H, –CH_2NMe_2), 2.33 (s, 6H, $N(CH_3)_2$), 2.12–2.02 (m, 2H), 1.90–1.79 (m, 2H), 1.77–1.67 (m, 4H), 1.54–1.19 (m, 20H), 0.87 (t, 3H, J = 7.0 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.6, 157.3, 156.5, 124.7, 57.3, 53.0, 50.3, 44.8 (2C), 38.8, 31.9, 29.6 (2C), 29.5, 29.3, 29.3, 29.2, 28.5, 28.1, 27.0, 25.6, 24.1, 22.7, 21.8, 19.3, 14.1. HRMS (ESI) calcd for $C_{26}H_{48}N_2O_4S$ $[M+H]^+$ 485.3408, found 485.3399. LC–MS (ESI) found 485.30 for $[M+H]^+$.



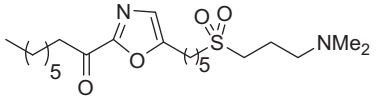
4.1.5.6. 1-(5-(2-(3-(Dimethylamino)propylsulfonyl)ethyl)oxazol-2-yl)octan-1-one (28). Compound **28** was synthesized following the general procedure for oxidation into sulfone using 0.48 mmol of sulfide derivative **18**, reaction time 60 h. Purification by reverse phase chromatography using RPC_{18} column (eluent 0–100% MeOH). Yield = 79% (white semi-solid). R_f = 0.51 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1694 cm^{-1} (ketone C=O stretch), 1287 cm^{-1} (SO_2 asym. stretch), 1111 cm^{-1} (SO_2 sym. stretch). ^1H NMR (400 MHz, $CDCl_3$): δ 7.10 (t, 1H, J = 0.8 Hz, H–C=C), 3.40–3.29 (m, 4H, –CH_2SO_2), 3.16–3.10 (m, 2H, –CH_2CO), 3.04–2.99 (m, 2H, –CH_2–C=C), 2.56 (t, 2H, J = 6.8 Hz, –CH_2NMe_2), 2.33 (s, 6H, $N(CH_3)_2$), 2.13–2.03 (m, 2H), 1.76–1.66 (m, 2H), 1.40–1.22 (m, 8H), 0.87 (t, 3H, J = 6.8 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.5, 157.7, 151.8, 126.1, 57.0, 51.0, 50.3, 44.7 (2C), 38.9, 31.6, 29.0, 28.9, 23.9, 22.5, 19.3, 18.7, 14.0. HRMS (ESI) calcd for $C_{18}H_{32}N_2O_4S$ $[M+H]^+$ 373.2156, found 373.2157. LC–MS (ESI) found 373.16 for $[M+H]^+$.



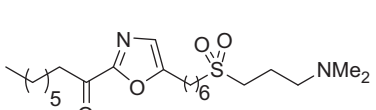
4.1.5.7. 1-(5-(3-(3-(Dimethylamino)propylsulfonyl)propyl)oxazol-2-yl) octan-1-one (29). Compound **29** was synthesized following the general procedure for oxidation into sulfone using 0.46 mmol of sulfide derivative **19**, reaction time 60 h. Purification by reverse phase chromatography using RPC_{18} column (eluent 0–100% MeOH). Yield = 69% (white semi-solid). R_f = 0.50 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1701 cm^{-1} (ketone C=O stretch), 1318 cm^{-1} (SO_2 asym. stretch), 1130 cm^{-1} (SO_2 sym. stretch). ^1H NMR (400 MHz, $CDCl_3$): δ 7.04 (t, 1H, J = 0.8 Hz, H–C=C), 3.19–3.12 (m, 2H, –CH_2SO_2), 3.11–3.05 (m, 2H, –CH_2SO_2), 3.01 (t, 2H, J = 7.4 Hz, –CH_2CO), 2.97 (dt, 2H, J = 7.4, J = 0.8 Hz, –CH_2–C=C), 2.74 (t, 2H, J = 7.0 Hz, –CH_2NMe_2), 2.46 (s, 6H, $N(CH_3)_2$), 2.33–2.22 (m, 2H), 2.20–2.10 (m, 2H), 1.77–1.67 (m, 2H), 1.40–1.16 (m, 8H), 0.87 (t, 3H, J = 6.8 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.5, 157.6, 154.3, 125.5, 56.9, 51.8, 50.4, 44.4 (2C), 38.8, 31.6, 29.1, 29.0, 24.3, 23.9, 22.5, 20.1, 18.9, 14.0. HRMS (EI) calcd for $C_{19}H_{34}N_2O_4S$ $[M^{+\cdot}]$ 386.2239, found 386.2227. GCMS found 386 for $[M^{+\cdot}]$.



4.1.5.8. 1-(5-(4-(3-(Dimethylamino)propylsulfonyl)butyl)oxazol-2-yl)octan-1-one (30). Compound **30** was synthesized following the general procedure for oxidation into sulfone using 0.45 mmol of sulfide derivative **20**, reaction time 60 h. Purification by reverse phase chromatography using RPC_{18} column (eluent 0–100% MeOH). Yield = 76% (white semi-solid). R_f = 0.51 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1692 cm−1 (ketone C=O stretch), 1277 cm^{-1} (SO_2 asym. stretch), 1110 cm^{-1} (SO_2 sym. stretch). ^1H NMR (400 MHz, $CDCl_3$): δ 6.98 (t, 1H, J = 0.8 Hz, H–C=C), 3.15–3.09 (m, 2H, –CH_2SO_2), 3.06–3.02 (m, 2H, –CH_2SO_2), 3.01 (t, 2H, J = 7.4 Hz, –CH_2CO), 2.83–2.77 (m, 2H, –CH_2–C=C), 2.69 (t, 2H, J = 7.0 Hz, –CH_2NMe_2), 2.43 (s, 6H, $N(CH_3)_2$), 2.17–2.09 (m, 2H), 1.96–1.81 (m, 4H), 1.76–1.67 (m, 2H), 1.41–1.19 (m, 8H), 0.87 (t, 3H, J = 6.8 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.6, 157.4, 155.4, 125.1, 57.0, 52.5, 50.3, 44.5 (2C), 38.8, 31.6, 29.1, 28.9, 26.3, 25.2, 24.0, 22.6, 21.4, 19.0, 14.0. HRMS (ESI) calcd for $C_{20}H_{36}N_2O_4S$ $[M+H]^+$ 401.2469, found 401.2460. LC–MS (ESI) found 401.21 for $[M+H]^+$.



4.1.5.9. 1-(5-(5-(3-(Dimethylamino)propylsulfonyl)pentyl)oxazol-2-yl)octan-1-one (31). Compound **31** was synthesized following the general procedure for oxidation into sulfone using 0.42 mmol of sulfide derivative **21**, reaction time 60 h. Purification by reverse phase chromatography using RPC_{18} column (eluent 0–100% MeOH). Yield = 74% (yellow semi-solid). R_f = 0.54 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1696 cm−1 (ketone C=O stretch), 1278 cm^{-1} (SO_2 asym. stretch), 1118 cm^{-1} (SO_2 sym. stretch). ^1H NMR (400 MHz, $CDCl_3$): δ 6.96 (t, 1H, J = 0.8 Hz, H–C=C), 3.24–3.18 (m, 2H, –CH_2SO_2), 3.10–2.97 (m, 6H, –CH_2SO_2, –CH_2CO, –CH_2–C=C), 2.75 (t, 2H, J = 7.4 Hz, –CH_2NMe_2), 2.71 (s, 6H, $N(CH_3)_2$), 2.35–2.23 (m, 2H), 1.93–1.81 (m, 2H), 1.80–1.66 (m, 4H), 1.57–1.47 (m, 2H), 1.39–1.19 (m, 8H), 0.87 (t, 3H, J = 7.0 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.6, 157.3, 156.2, 124.8, 56.5, 52.9, 49.8, 43.7 (2C), 38.8, 31.6, 29.1, 29.0, 27.8, 26.9, 25.3, 24.0, 22.5, 21.4, 18.0, 14.0. HRMS (ESI) calcd for $C_{21}H_{38}N_2O_4S$ $[M+H]^+$ 415.2625, found 415.2619. LC–MS (ESI) found 415.21 for $[M+H]^+$.

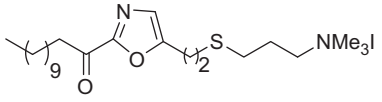


4.1.5.10. 1-(5-(6-(3-(Dimethylamino)propylsulfonyl)hexyl)oxazol-2-yl)octan-1-one (32). Compound **32** was synthesized following the general procedure for oxidation into sulfone using 0.38 mmol of sulfide derivative **22**, reaction time 60 h. Purification by reverse phase chromatography using RPC_{18} column (eluent

0–100% MeOH). Yield = 84% (white semi-solid). R_f = 0.56 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 1694 cm−1 (ketone C=O stretch), 1278 cm^{-1} (SO_2 asym. stretch), 1110 cm^{-1} (SO_2 sym. stretch). ^1H NMR (400 MHz, $CDCl_3$): δ 6.95 (t, 1H, J = 0.8 Hz, H–C=C), 3.34–3.25 (m, 4H, –CH_2SO_2), 3.09–2.97 (m, 4H, –CH_2CO, –CH_2–C=C), 2.84 (s, 6H, N(CH_3)$_2$), 2.73 (t, 2H, J = 7.6 Hz, –CH_2NMe_2), 2.50–2.41 (m, 2H), 1.90–1.81 (m, 2H), 1.77–1.66 (m, 4H), 1.55–1.22 (m, 12H), 0.87 (t, 3H, J = 6.8 Hz, –CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.6, 157.3, 156.5, 124.7, 56.3, 53.5, 49.3, 43.2 (2C), 38.8, 31.6, 29.1, 28.9, 28.4, 27.9, 26.9, 25.5, 24.0, 22.6, 21.7, 17.4, 14.0. HRMS (ESI) calcd for $C_{22}H_{40}N_2O_4S$ $[M+H]^+$ 429.2782, found 429.2776. LC–MS (ESI) found 429.24 for $[M+H]^+$.

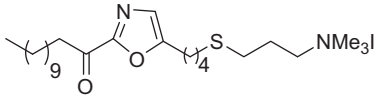
4.1.6. Synthesis of quaternized oxazoles (33–48)[24]

To a solution of oxazoles **13–32** (0.074 mmol, 1.0 equiv) in CH_3CN (0.8 ml) was added dropwise at 0 °C methyl iodide (0.085 mmol, 1.2 equiv) as a solution in CH_3CN (0.3 ml). The reaction mixture was stirred at rt for 5 h and the quaternized ammonium salts **33–48** were precipitated with Et_2O, filtered and washed with Et_2O.



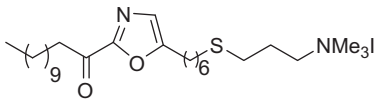
4.1.6.1. *N,N,N*-Trimethyl-3-(2-(2-dodecanoyloxazol-5-yl)ethylthio)propan-1-ammonium iodide (33).

Compound **33** was synthesized following the general procedure for the quaternization using 0.071 mmol of oxazole **13**. Yield = 71% (yellow semi-solid). R_f = 0.38 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3450 cm^{-1} (quaternary amine), 1696 cm^{-1} (ketone C=O stretch). ^1H NMR (400 MHz, DMSO): δ 7.29 (s, 1H, H–C=C), 3.34–3.30 (m, 2H, –$CH_2NMe_3^+$), 3.08–3.06 (m, 2H, –CH_2–C=C), 3.05 (s, 9H, N(CH_3)$_3^+$), 2.97 (t, 2H, J = 7.4 Hz, –CH_2CO), 2.87 (t, 2H, J = 7.2 Hz, –CH_2S), 2.57 (t, 2H, J = 7.0 Hz, –CH_2S), 2.02–1.90 (m, 2H), 1.65–1.53 (m, 2H), 1.34–1.16 (m, 16H), 0.85 (t, 3H, J = 7.0 Hz, –CH_3). ^{13}C NMR (125 MHz, DMSO): δ 187.7, 156.7, 154.8, 125.6, 64.3, 52.2 (3C), 38.0, 31.2, 28.9 (2C), 28.8, 28.7, 28.6, 28.4, 28.3, 27.5, 25.4, 23.3, 22.3, 22.0, 13.9. HRMS (ESI) calcd for $C_{23}H_{43}N_2O_2S$ $[M]^+$ 411.3040, found 411.3036. LCMS (ESI) found 411.27 for $[M]^+$.



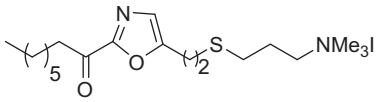
4.1.6.2. *N,N,N*-Trimethyl-3-(4-(2-dodecanoyloxazol-5-yl)butylthio)propan-1-ammonium iodide (34).

Compound **34** was synthesized following the general procedure for the quaternization using 0.060 mmol of oxazole **15**. Yield = 70% (white semi-solid). R_f = 0.53 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3470 cm^{-1} (quaternary amine), 1696 cm^{-1} (ketone C=O stretch). ^1H NMR (400 MHz, DMSO): δ 7.22 (s, 1H, H–C=C), 3.37–3.28 (m, 2H, –$CH_2NMe_3^+$), 3.05 (s, 9H, N(CH_3)$_3^+$), 2.96 (t, 2H, J = 7.2 Hz, –CH_2CO), 2.78 (t, 2H, J = 7.4 Hz, –CH_2–C=C), 2.57 (t, 2H, J = 7.2 Hz, –CH_2S), 2.52 (t, 2H, J = 7.2 Hz, –CH_2S), 2.01–1.88 (m, 2H), 1.76–1.64 (m, 2H), 1.64–1.52 (m, 4H), 1.34–1.14 (m, 16H), 0.85 (t, 3H, J = 6.6 Hz, –CH_3). ^{13}C NMR (125 MHz, DMSO): δ 187.7, 156.7, 156.3, 124.8, 64.3, 52.2 (3C), 37.9, 31.2, 30.3, 28.9 (2C), 28.8, 28.7, 28.6, 28.4, 28.1, 27.5, 25.9, 24.3, 23.3, 22.3, 22.0, 13.9. HRMS (ESI) calcd for $C_{25}H_{47}N_2O_2S$ $[M]^+$ 439.3353, found 439.3351. LCMS (ESI) found 439.28 for $[M]^+$.



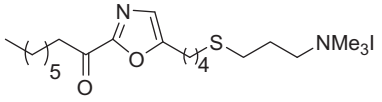
4.1.6.3. *N,N,N*-Trimethyl-3-(6-(2-dodecanoyloxazol-5-yl)hexylthio)propan-1-ammonium iodide (35).

Compound **35** was synthesized following the general procedure for the quaternization using 0.072 mmol of oxazole **17**. Yield = 64% (white solid). R_f = 0.69 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3491 cm^{-1} (quaternary amine), 1695 cm^{-1} (ketone C=O stretch). ^1H NMR (400 MHz, DMSO): δ 7.21 (s, 1H, H–C=C), 3.38–3.28 (m, 2H, –$CH_2NMe_3^+$), 3.05 (s, 9H, N(CH_3)$_3^+$), 2.96 (t, 2H, J = 7.2 Hz, –CH_2CO), 2.74 (t, 2H, J = 7.6 Hz, –CH_2–C=C), 2.57–2.50 (m, 4H, –CH_2S), 2.01–1.90 (m, 2H), 1.67–1.45 (m, 6H), 1.41–1.17 (m, 20H), 0.85 (t, 3H, J = 6.8 Hz, –CH_3). ^{13}C NMR (125 MHz, DMSO): δ 187.8, 156.7, 156.6, 124.8, 64.5, 52.3 (3C), 38.0, 31.3, 30.8, 28.9 (2C), 28.8, 28.7 (2C), 28.7, 28.4, 27.9, 27.8, 27.7, 26.7, 24.8, 23.4, 22.4, 22.1, 13.9. HRMS (ESI) calcd for $C_{27}H_{51}N_2O_2S$ $[M]^+$ 467.3666, found 467.3666. LCMS (ESI) found 467.25 for $[M]^+$.



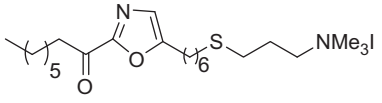
4.1.6.4. *N,N,N*-Trimethyl-3-(2-(2-octanoyloxazol-5-yl)ethylthio)propan-1-ammonium iodide (36).

Compound **36** was synthesized following the general procedure for the quaternization using 0.067 mmol of oxazole **18**. Yield = 72% (yellow oil). R_f = 0.45 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3456 cm^{-1} (quaternary amine), 1695 cm^{-1} (ketone C=O stretch). ^1H NMR (400 MHz, DMSO): δ 7.29 (t, 1H, J = 0.8 Hz, H–C=C), 3.36–3.30 (m, 2H, –$CH_2NMe_3^+$), 3.10–3.06 (m, 2H, –CH_2–C=C), 3.06 (s, 9H, N(CH_3)$_3^+$), 2.98 (t, 2H, J = 7.2 Hz, –CH_2CO), 2.88 (t, 2H, J = 7.4 Hz, –CH_2S), 2.58 (t, 2H, J = 7.0 Hz, –CH_2S), 2.03–1.92 (m, 2H), 1.67–1.58 (m, 2H), 1.34–1.19 (m, 8H), 0.85 (t, 3H, J = 6.8 Hz, –CH_3). ^{13}C NMR (125 MHz, DMSO): δ 187.7, 156.7, 154.8, 125.6, 64.3, 52.3 (3C), 38.0, 31.0, 28.4 (2C), 28.3, 27.4, 25.4, 23.3, 22.3, 21.9, 13.9. HRMS (ESI) calcd for $C_{19}H_{35}N_2O_2S$ $[M]^+$ 355.2414, found 355.2412. LCMS (ESI) found 355.18 for $[M]^+$.



4.1.6.5. *N,N,N*-Trimethyl-3-(4-(2-octanoyloxazol-5-yl)butylthio)propan-1-ammonium iodide (37).

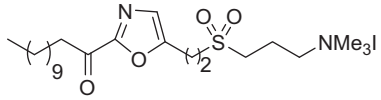
Compound **37** was synthesized following the general procedure for the quaternization using 0.105 mmol of oxazole **20**. Yield = 71% (yellow semi solid). R_f = 0.51 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3468 cm^{-1} (quaternary amine), 1695 cm^{-1} (ketone C=O stretch). ^1H NMR (400 MHz, DMSO): δ 7.22 (t, 1H, J = 0.8 Hz, H–C=C), 3.37–3.31 (m, 2H, –$CH_2NMe_3^+$), 3.06 (s, 9H, N(CH_3)$_3^+$), 2.97 (t, 2H, J = 7.2 Hz, –CH_2CO), 2.78 (dt, 2H, J = 7.4, J = 0.8 Hz, –CH_2–C=C), 2.58 (t, 2H, J = 7.2 Hz, –CH_2S), 2.52 (t, 2H, J = 7.2 Hz, –CH_2S), 2.01–1.90 (m, 2H), 1.76–1.67 (m, 2H), 1.64–1.54 (m, 4H), 1.34–1.17 (m, 8H), 0.85 (t, 3H, J = 7.0 Hz, –CH_3). ^{13}C NMR (125 MHz, DMSO): δ 187.7, 156.7, 156.3, 124.8, 64.3, 52.2 (3C), 37.9, 31.0, 30.3, 28.4 (2C), 28.1, 27.5, 25.9, 24.4, 23.3, 22.3, 21.9, 13.9. HRMS (ESI) calcd for $C_{21}H_{39}N_2O_2S$ $[M]^+$ 383.2727, found 383.2732. LCMS (ESI) found 383.20 for $[M]^+$.



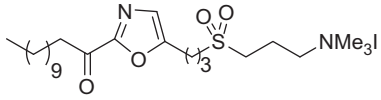
4.1.6.6. *N,N,N*-Trimethyl-3-(6-(2-octanoyloxazol-5-yl)hexylthio)propan-1-ammonium iodide (38).

Compound **38** was synthesized following the general procedure for the quaternization using 0.088 mmol of oxazole **22**. Yield = 60% (white solid). R_f = 0.43 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3456 cm^{-1} (quaternary amine), 1695 cm^{-1} (ketone C=O stretch). ^1H NMR (400 MHz,

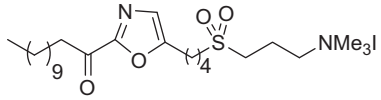
DMSO): δ 7.21 (s, 1H, *H*–C═C), 3.37–3.28 (m, 2H, –C*H*$_2$NMe$_3^+$), 3.05 (s, 9H, N(C*H*$_3$)$_3^+$), 2.96 (t, 2H, *J* = 7.2 Hz, –C*H*$_2$CO), 2.74 (t, 2H, *J* = 7.4 Hz, –C*H*$_2$–C═C), 2.56–2.51 (m, 4H, –C*H*$_2$S), 1.99–1.87 (m, 2H), 1.65–1.48 (m, 6H), 1.43–1.17 (m, 12H), 0.85 (t, 3H, *J* = 6.8 Hz, –C*H*$_3$). ^{13}C NMR (125 MHz, DMSO): δ 187.7, 156.7, 156.5, 124.7, 64.3, 52.2 (3C), 37.9, 31.0, 30.8, 28.7, 28.4 (2C), 27.9, 27.7, 27.6, 26.7, 24.7, 23.3, 22.3, 21.9, 13.9. HRMS (ESI) calcd for $C_{23}H_{43}N_2O_2S$ [M]$^+$ 411.3040, found 411.3037. LCMS (ESI) found 411.26 for [M]$^+$.



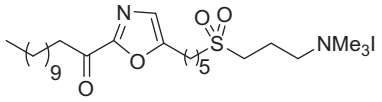
4.1.6.7. *N,N,N*-Trimethyl-3-(2-(2-dodecanoyloxazol-5-yl)ethylsulfonyl)-propan-1-ammonium iodide (39). Compound **39** was synthesized following the general procedure for the quaternization using 0.070 mmol of oxazole **23**. Yield = 45% (yellow salt). R_f = 0.34 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3409 cm^{-1} (quaternary amine), 1694 cm^{-1} (ketone C═O stretch), 1283 cm^{-1} (SO_2 asym. stretch), 1114 cm^{-1} (SO_2 sym. stretch). ^{1}H NMR (400 MHz, DMSO): δ 7.35 (s, 1H, *H*–C═C), 3.65–3.56 (m, 2H, –C*H*$_2$SO$_2$), 3.44–3.34 (m, 2H, –C*H*$_2$NMe$_3^+$), 3.28–3.21 (m, 4H, –C*H*$_2$SO$_2$, –C*H*$_2$–C═C), 3.08 (s, 9H, N(C*H*$_3$)$_3^+$), 2.98 (t, 2H, *J* = 7.4 Hz, –C*H*$_2$CO), 2.23–2.08 (m, 2H), 1.67–1.51 (m, 2H), 1.35–1.11 (m, 16H), 0.85 (t, 3H, *J* = 7.2 Hz, –C*H*$_3$). ^{13}C NMR (125 MHz, DMSO): δ 187.7, 156.8, 152.9, 125.8, 63.3, 52.2 (3C), 48.8, 48.5, 38.0, 31.2, 28.9 (2C), 28.8, 28.7, 28.6, 28.4, 23.3, 22.0, 18.1, 15.8, 13.9. HRMS (ESI) calcd for $C_{23}H_{43}N_2O_4S$ [M]$^+$ 443.2938, found 443.2933. LCMS (ESI) found 443.29 for [M]$^+$.



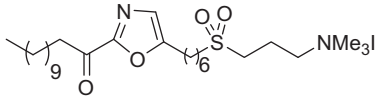
4.1.6.8. *N,N,N*-Trimethyl-3-(3-(2-dodecanoyloxazol-5-yl)propylsulfonyl)-propan-1-ammonium iodide (40). Compound **40** was synthesized following the general procedure for the quaternization using 0.070 mmol of oxazole **24**. Yield = 50% (white solid). R_f = 0.36 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3414 cm−1 (quaternary amine), 1690 cm^{-1} (ketone C═O stretch), 1265 cm^{-1} (SO_2 asym. stretch), 1129 cm^{-1} (SO_2 sym. stretch). ^{1}H NMR (400 MHz, DMSO): δ 7.28 (s, 1H, *H*–C═C), 3.44–3.34 (m, 2H, –C*H*$_2$NMe$_3^+$), 3.28–3.20 (m, 4H, –C*H*$_2$SO$_2$), 3.08 (s, 9H, N(C*H*$_3$)$_3^+$), 3.02–2.91 (m, 4H, –C*H*$_2$CO, –C*H*$_2$–C═C), 2.21–1.97 (m, 4H), 1.66–1.52 (m, 2H), 1.35–1.08 (m, 16H), 0.85 (t, 3H, *J* = 7.0 Hz, –C*H*$_3$). ^{13}C NMR (125 MHz, DMSO): δ 187.7, 156.8, 154.9, 125.2, 63.4, 52.2 (3C), 50.7, 48.2, 38.0, 31.2, 28.9 (2C), 28.8, 28.7, 28.6, 28.4, 23.5, 23.2, 22.0, 19.6, 15.7, 13.9. HRMS (ESI) calcd for $C_{24}H_{45}N_2O_4S$ [M]$^+$ 457.3094, found 457.3086. LCMS (ESI) found 457.36 for [M]$^+$.



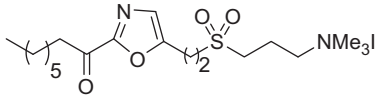
4.1.6.9. *N,N,N*-Trimethyl-3-(4-(2-dodecanoyloxazol-5-yl)butylsulfonyl)-propan-1-ammonium iodide (41). Compound **41** was synthesized following the general procedure for the quaternization using 0.065 mmol of oxazole **25**. Yield = 68% (yellow solid). R_f = 0.34 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3432 cm−1 (quaternary amine), 1701 cm^{-1} (ketone C═O stretch), 1282 cm^{-1} (SO_2 asym. stretch), 1120 cm^{-1} (SO_2 sym. stretch). ^{1}H NMR (400 MHz, DMSO): δ 7.23 (s, 1H, *H*–C═C), 3.43–3.35 (m, 2H, –C*H*$_2$NMe$_3^+$), 3.27–3.12 (m, 4H, –C*H*$_2$SO$_2$), 3.08 (s, 9H, N(C*H*$_3$)$_3^+$), 2.97 (t, 2H, *J* = 7.4 Hz, –C*H*$_2$CO), 2.85–2.77 (m, 2H, –C*H*$_2$–C═C), 2.20–2.08 (m, 2H), 1.82–1.67 (m, 4H), 1.65–1.54 (m, 2H), 1.35–1.14 (m, 16H), 0.85 (t, 3H, *J* = 6.8 Hz, –C*H*$_3$). ^{13}C NMR (125 MHz, DMSO): δ 187.7, 156.8, 155.9, 124.9, 63.4, 52.2 (3C), 51.0, 48.3, 37.9, 31.2, 28.9 (2C), 28.8, 28.7, 28.6, 28.4, 25.5, 24.3, 23.3, 22.0, 20.6, 15.7, 13.9. HRMS (ESI) calcd for $C_{25}H_{47}N_2O_4S$ [M]$^+$ 471.3251, found 471.3250. LCMS (ESI) found 471.28 for [M]$^+$.



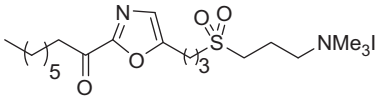
4.1.6.10. *N,N,N*-Trimethyl-3-(5-(2-dodecanoyloxazol-5-yl)pentylsulfonyl)-propan-1-ammonium iodide (42). Compound **42** was synthesized following the general procedure for the quaternization using 0.064 mmol of oxazole **26**. Yield = 44% (yellow solid). R_f = 0.36 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3477 cm−1 (quaternary amine), 1697 cm^{-1} (ketone C═O stretch), 1286 cm^{-1} (SO_2 asym. stretch), 1115 cm^{-1} (SO_2 sym. stretch). ^{1}H NMR (400 MHz, DMSO): δ 7.22 (s, 1H, *H*–C═C), 3.42–3.35 (m, 2H, –C*H*$_2$NMe$_3^+$), 3.21–3.12 (m, 4H, –C*H*$_2$SO$_2$), 3.08 (s, 9H, N(C*H*$_3$)$_3^+$), 2.96 (t, 2H, *J* = 7.2 Hz, –C*H*$_2$CO), 2.76 (t, 2H, *J* = 7.4 Hz, –C*H*$_2$–C═C), 2.20–2.06 (m, 2H), 1.77–1.54 (m, 6H), 1.51–1.40 (m, 2H), 1.35–1.16 (m, 16H), 0.85 (t, 3H, *J* = 6.8 Hz, –C*H*$_3$). ^{13}C NMR (125 MHz, DMSO): δ 187.7, 156.7, 156.4, 124.8, 63.4, 52.2, 51.2 (3C), 48.2, 37.9, 31.2, 28.9 (2C), 28.8, 28.7, 28.6, 28.4, 27.0, 26.2, 24.6, 23.3, 22.0, 20.8, 15.7, 13.9. HRMS (ESI) calcd for $C_{26}H_{49}N_2O_4S$ [M]$^+$ 485.3408, found 485.3408. LCMS (ESI) found 485.30 for [M]$^+$.



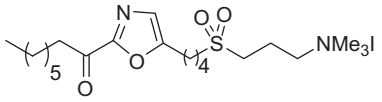
4.1.6.11. *N,N,N*-Trimethyl-3-(6-(2-dodecanoyloxazol-5-yl)hexylsulfonyl)-propan-1-ammonium iodide (43). Compound **43** was synthesized following the general procedure for the quaternization using 0.052 mmol of oxazole **27**. Yield = 62% (yellow solid). R_f = 0.41 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3455 cm−1 (quaternary amine), 1697 cm^{-1} (ketone C═O stretch), 1282 cm^{-1} (SO_2 asym. stretch), 1131 cm^{-1} (SO_2 sym. stretch). ^{1}H NMR (400 MHz, DMSO): δ 7.21 (t, 1H, *J* = 0.8 Hz, *H*–C═C), 3.41–3.35 (m, 2H, –C*H*$_2$NMe$_3^+$), 3.18–3.12 (m, 4H, –C*H*$_2$SO$_2$), 3.08 (s, 9H, N(C*H*$_3$)$_3^+$), 2.96 (t, 2H, *J* = 7.2 Hz, –C*H*$_2$CO), 2.75 (dt, 2H, *J* = 7.4, *J* = 0.8 Hz, –C*H*$_2$–C═C), 2.19–2.04 (m, 2H), 1.73–1.50 (m, 6H), 1.47–1.14 (m, 20H), 0.85 (t, 3H, *J* = 6.8 Hz, –C*H*$_3$). ^{13}C NMR (125 MHz, DMSO): δ 187.7, 156.7, 156.5, 124.8, 63.4, 52.2 (3C), 51.4, 48.2, 37.9, 31.2, 28.9 (2C), 28.8, 28.7, 28.6, 28.4, 27.8, 27.2, 26.4, 24.7, 23.3, 22.0, 20.9, 15.7, 13.9. HRMS (ESI) calcd for $C_{27}H_{51}N_2O_4S$ [M]$^+$ 499.3564, found 499.3565. LCMS (ESI) found 499.29 for [M]$^+$.



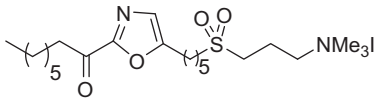
4.1.6.12. *N,N,N*-Trimethyl-3-(2-(2-octanoyloxazol-5-yl)ethylsulfonyl)propan-1-ammonium iodide (44). Compound **44** was synthesized following the general procedure for the quaternization using 0.068 mmol of oxazole **28**. Yield = 79% (yellow semi-solid). R_f = 0.33 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3451 cm−1 (quaternary amine), 1693 cm^{-1} (ketone C═O stretch), 1298 cm^{-1} (SO_2 asym. stretch), 1122 cm^{-1} (SO_2 sym. stretch). ^{1}H NMR (400 MHz, DMSO): δ 7.35 (s, 1H, *H*–C═C), 3.64–3.57 (m, 2H, –C*H*$_2$SO$_2$), 3.43–3.36 (m, 2H, –C*H*$_2$NMe$_3^+$), 3.34–3.20 (m, 4H, –C*H*$_2$SO$_2$, –C*H*$_2$–C═C), 3.09 (s, 9H, N(C*H*$_3$)$_3^+$), 2.98 (t, 2H, *J* = 7.2 Hz, –C*H*$_2$CO), 2.22–2.11 (m, 2H), 1.66–1.55 (m, 2H), 1.35–1.15 (m, 8H), 0.85 (t, 3H, *J* = 6.8 Hz, –C*H*$_3$). ^{13}C NMR (125 MHz, DMSO): δ 187.7, 156.8, 152.9, 125.8, 63.3, 52.2 (3C), 48.8, 48.5, 38.0, 31.0, 28.3 (2C), 23.3, 21.9, 18.1, 15.8, 13.8. HRMS (ESI) calcd for $C_{19}H_{35}N_2O_4S$ [M]$^+$ 387.2312, found 387.2316. LCMS (ESI) found 387.17 for [M]$^+$.



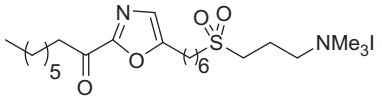
4.1.6.13. *N,N,N*-Trimethyl-3-(3-(2-octanoyloxazol-5-yl)propylsulfonyl)propan-1-ammonium iodide (45). Compound **45** was synthesized following the general procedure for the quaternization using 0.085 mmol of oxazole **29**. Yield = 85% (yellow oil). R_f = 0.34 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3447 cm−1 (quaternary amine), 1695 cm^{-1} (ketone C=O stretch), 1285 cm^{-1} (SO_2 asym. stretch), 1120 cm^{-1} (SO_2 sym. stretch). ^{1}H NMR (400 MHz, DMSO): δ 7.27 (t, 1H, J = 0.8 Hz, H–C=C), 3.42–3.35 (m, 2H, $-CH_2NMe_3+$), 3.28–3.17 (m, 4H, $-CH_2SO_2$), 3.08 (s, 9H, $N(CH_3)_3^+$), 2.97 (t, 2H, J = 7.4 Hz, $-CH_2CO$), 2.93 (dt, 2H, J = 7.2, J = 0.8 Hz, $-CH_2$–C=C), 2.19–2.00 (m, 4H), 1.66–1.56 (m, 2H), 1.35–1.19 (m, 8H), 0.85 (t, 3H, J = 6.8 Hz, $-CH_3$). ^{13}C NMR (125 MHz, DMSO): δ 187.7, 156.8, 154.9, 125.2, 63.4, 52.2 (3C), 50.7, 48.2, 38.0, 31.0, 28.4 (C2), 23.5, 23.3, 22.0, 19.6, 15.7, 13.9. HRMS (ESI) calcd for $C_{20}H_{37}N_2O_4S$ $[M]^+$ 401.2469, found 401.2463. LCMS (ESI) found 401.20 for $[M]^+$.



4.1.6.14. *N,N,N*-Trimethyl-3-(4-(2-octanoyloxazol-5-yl)butylsulfonyl)propan-1-ammonium iodide (46). Compound **46** was synthesized following the general procedure for the quaternization using 0.084 mmol of oxazole **30**. Yield = 87% (brown oil). R_f = 0.35 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3447 cm−1 (quaternary amine), 1694 cm^{-1} (ketone C=O stretch), 1285 cm^{-1} (SO_2 asym. stretch), 1119 cm^{-1} (SO_2 sym. stretch). ^{1}H NMR (400 MHz, DMSO): δ 7.23 (t, 1H, J = 0.8 Hz, H–C=C), 3.43–3.35 (m, 2H, $-CH_2NMe_3^+$), 3.26–3.21 (m, 2H, $-CH_2SO_2$), 3.19 3.13 (m, 2H, $-CH_2SO_2$), 3.08 (s, 9H, $N(CH_3)_3^+$), 2.97 (t, 2H, J = 7.2 Hz, $-CH_2CO$), 2.86–2.78 (m, 2H, $-CH_2$–C=C), 2.20–2.09 (m, 2H), 1.82–1.69 (m, 4H), 1.67–1.54 (m, 2H), 1.35–1.16 (m, 8H), 0.85 (t, 3H, J = 7.0 Hz, $-CH_3$). ^{13}C NMR (125 MHz, DMSO): δ 187.7, 156.8, 155.9, 124.9, 63.4, 52.2 (3C), 51.0, 48.3, 37.9, 31.0, 28.4 (2C), 25.5, 24.3, 23.3, 21.9, 20.6, 17.7, 13.9. HRMS (ESI) calcd for $C_{21}H_{39}N_2O_4S$ $[M]^+$ 415.2625, found 415.2621. LCMS (ESI) found 415.22 for $[M]^+$.



4.1.6.15. *N,N,N*-Trimethyl-3-(5-(2-octanoyloxazol-5-yl)pentylsulfonyl)propan-1-ammonium iodide (**47**). Compound **47** was synthesized following the general procedure for the quaternization using 0.060 mmol of oxazole **31**. Yield = 66% (brown oil). R_f = 0.34 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3448 cm^{-1} (quaternary amine), 1696 cm^{-1} (ketone C=O stretch), 1282 cm^{-1} (SO_2 asym. stretch), 1118 cm^{-1} (SO_2 sym. stretch). ^{1}H NMR (400 MHz, DMSO): δ 7.22 (s, 1H, H–C=C), 3.41–3.34 (m, 2H, $-CH_2NMe_3^+$), 3.21–3.11 (m, 4H, $-CH_2SO_2$), 3.07 (s, 9H, $N(CH_3)_3^+$), 2.96 (t, 2H, J = 7.2 Hz, $-CH_2CO$), 2.76 (t, 2H, J = 7.2 Hz, $-CH_2$–C=C), 2.19–1.96 (m, 2H), 1.78–1.55 (m, 6H), 1.51–1.40 (m, 2H), 1.34–1.19 (m, 8H), 0.85 (t, 3H, J = 6.8 Hz, $-CH_3$). ^{13}C NMR (125 MHz, DMSO): δ 187.7, 156.7, 156.5, 124.9, 64.3, 53.0, 52.9, 49.3, 38.7, 31.6, 29.0, 28.9, 27.6, 26.8, 25.3, 23.9, 22.5, 21.4, 21.3, 17.4, 16.7, 14.0. HRMS (ESI) calcd for $C_{22}H_{41}N_2O_4S$ $[M]^+$ 429.2782, found 429.2778. LCMS (ESI) found 429.23 for $[M]^+$.



4.1.6.16. *N,N,N*-Trimethyl-3-(6-(2-octanoyloxazol-5-yl)hexylsulfonyl)propan-1-ammonium iodide (48). Compound **48** was synthesized following the general procedure for the quaternization using 0.056 mmol of oxazole **32**. Yield = traces (white solid). R_f = 0.34 (EtOAc/AcOH/MeOH/H_2O 3/3/3/2). IR: 3441 cm−1 (quaternary amine), 1695 cm^{-1} (ketone C=O stretch), 1281 cm^{-1} (SO_2 asym. stretch), 1111 cm^{-1} (SO_2 sym. stretch). ^{1}H NMR (400 MHz, DMSO): δ 7.21 (s, 1H, H–C=C), 3.35–3.25 (m, 2H, $-CH_2NMe_3^+$), 3.19–3.09 (m, 4H, $-CH_2SO_2$), 3.08 (s, 9H, $N(CH_3)_3^+$), 2.96 (t, 2H, J = 7.4 Hz, $-CH_2CO$), 2.75 (t, 2H, J = 7.4 Hz, $-CH_2$–C=C), 2.10–1.98 (m, 2H), 1.73–1.54 (m, 6H), 1.47–1.16 (m, 12H), 0.85 (t, 3H, J = 7.0 Hz, $-CH_3$). ^{13}C NMR (125 MHz, DMSO): δ 187.7, 156.7, 156.5, 124.7, 64.3, 52.2 (3C), 37.9, 35.6, 31.0, 30.7, 28.7, 28.4, 27.9, 27.7, 27.6, 26.7, 24.7, 23.3, 22.3, 21.9, 13.9. HRMS (ESI) calcd for $C_{23}H_{43}N_2O_4S$ $[M]^+$ 443.2938, found 443.2930. LCMS (ESI) found 443.25 for $[M]^+$.

4.2. FAAH inhibition assay

4.2.1. Reagents and materials

Brains of female Sprague Dawley rats (Harlan Winkelmann); Triton X-100, dimethyl sulfoxide (DMSO), 6-pyren-1-ylhexanoic acid (Fluka); potassium dihydrogenphosphate, potassium hydrogenphosphate, EDTA, methanol HPLC-grade (VWR); phosphate buffer saline tablets, acetonitrile HPLC-grade (Sigma); cyclohexylcarbamic acid 3′-carbamoylbiphenyl-3-ylester (URB 597), 1-oxazolo[4,5-*b*]pyridin-2-yl-6-phenylhexan-1-one (PHOP) (Cayman Chemical); *N*-(2-hydroxyethyl)-4-pyren-1-ylbutanamide and (6-(2-methyl-4,5-diphenyl-1*H*-imidazol-1-yl)hexyl)carbamic acid phenyl ester were prepared according to the published procedure.[25]

4.2.2. Preparation of rat brain microsomes

Two rat brains were homogenized for 3 min under ice-cooling with a fivefold volume potassium phosphate buffer (pH 7.4, 0.1 M) containing EDTA (1 mM) using a Potter-Elvehjem homogenizer at 1000–1200 rpm. The homogenate was centrifuged (1000×*g*, 4 °C, 10 min) and the resulting supernatant was isolated and was centrifuged again (10,000×*g*, 4 °C, 30 min). Finally, the supernatant was centrifuged (40,000×*g*, 4 °C, 60 min) and the obtained supernatant was discharged. The pellet was resuspended in 2 ml of ice-cold potassium phosphate buffer (pH 7.4, 0.1 M) containing EDTA (1 mM) and stored in 50 μl aliquots at −80 °C. Immediately before each incubations, a 50 μl aliquot was diluted with 350 μl potassium phosphate buffer (pH 7.4, 0.1 M) and homogenized shortly (2 × 5 s) at 0 °C with a Branson sonifier B15.

4.2.3. Incubation procedure and HPLC analysis

The substrate *N*-(2-hydroxyethyl)-4-pyren-1-ylbutanamide was dissolved in methanol at a concentration of 2.5 mg/ml. An aliquot of this solution was thoroughly dried under a stream of nitrogen. The residue was resuspended by intense vortexing and sonication (sonication bath) in a phosphate buffer (0.01 M, pH 7.4 at 20 °C) containing 0.2% Triton X-100, EDTA (1 mM), so that the concentration of the substrate was 114 μM. Next, an aliquot (88 μl) of the obtained mixture was added to 2 μl of a 10 mM compound DMSO stock solution, or to 2 μl of DMSO in case of the controls, and the obtained mixture was pre-incubated for

10 min at 37 °C. The enzymatic reaction was initiated by adding 10 μl of rat brain microsome preparation and was subsequently incubated for 60 min at 37 °C. The final incubation volume of 100 μl contained a pyrenylbutanamide substrate concentration of 100 μM. The enzyme reaction was terminated by the addition of 200 μl acetonitrile/methanol (1:1, v/v) including the internal standard 6-pyren-1-ylhexanoic acid (0.025 μg/200 μl). After cooling in an ice bath for 10 min, samples were centrifuged (2000×*g*, 4 °C, 5 min). Blank incubations in the absence of the enzyme were carried out in parallel. HPLC analysis of the supernatants was carried out as described recently applying a Nucleosil 100 C18 analytical column (3 mm inside diameter × 125 mm, particle size 3 μm) (Macherey & Nagel) protected with a Phenomenex C18 guard column (3 mm inside diameter × 4 mm).

References and notes

1. Cravatt, B. F.; Giang, D. K.; Mayfield, S. P.; Boger, D. L.; Lerner, R. A.; Gilula, N. B. *Nature* **1996**, *384*, 83.
2. Lambert, D. M.; Fowler, C. J. *J. Med. Chem.* **2005**, *48*, 5059.
3. Jhaveri, M. D.; Richardson, D.; Chapman, V. *Br. J. Pharmacol.* **2007**, *152*, 624.
4. Labar, G.; Michaux, C. *Chem. Biodivers.* **2007**, *4*, 1882.
5. Cravatt, B. F.; Lichtman, A. H. *Curr. Opin. Chem. Biol.* **2003**, *7*, 469.
6. Vandevoorde, S. *Curr. Top. Med. Chem.* **2008**, *8*, 247.
7. Deng, H. *Expert Opin. Drug Disc.* **2010**, *5*, 961.
8. Seierstad, M.; Breitenbucher, J. G. *J. Med. Chem.* **2008**, *51*, 7327.
9. Otrubova, K.; Ezzili, C.; Boger, D. L. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4674.
10. Bracey, M. H.; Hanson, M. A.; Masuda, K. R.; Stevens, R. C.; Cravatt, B. F. *Science* **2002**, *298*, 1793.
11. Patricelli, M. P.; Lovato, M. A.; Cravatt, B. F. *Biochemistry* **1999**, *38*, 9804.
12. Kathuria, S.; Gaetani, S.; Fegley, D.; Valino, F.; Duranti, A.; Tontini, A.; Mor, M.; Tarzia, G.; La, R. G.; Calignano, A.; Giustino, A.; Tattoli, M.; Palmery, M.; Cuomo, V.; Piomelli, D. *Nat. Med.* **2003**, *9*, 76.
13. Boger, D. L.; Miyauchi, H.; Du, W.; Hardouin, C.; Fecik, R. A.; Cheng, H.; Hwang, I.; Hedrick, M. P.; Leung, D.; Acevedo, O.; Guimaraes, C. R. W.; Jorgensen, W. L.; Cravatt, B. F. *J. Med. Chem.* **2005**, *48*, 1849.
14. Lichtman, A. H.; Leung, D.; Shelton, C. C.; Saghatelian, A.; Hardouin, C.; Boger, D. L.; Cravatt, B. F. *J. Pharmacol. Exp. Ther.* **2004**, *311*, 441.
15. Hardouin, C.; Kelso, M. J.; Romero, F. A.; Rayl, T. J.; Leung, D.; Hwang, I.; Cravatt, B. F.; Boger, D. L. *J. Med. Chem.* **2007**, *50*, 3359.
16. Romero, F. A.; Du, W.; Hwang, I.; Rayl, T. J.; Kimball, F. S.; Leung, D.; Hoover, H. S.; Apodaca, R. L.; Breitenbucher, J. G.; Cravatt, B. F.; Boger, D. L. *J. Med. Chem.* **2007**, *50*, 1058.
17. Mileni, M.; Garfunkle, J.; DeMartino, J. K.; Cravatt, B. F.; Boger, D. L.; Stevens, R. C. *J. Am. Chem. Soc.* **2009**, *131*, 10497.
18. Hedberg, C.; Dekker, F. J.; Rusch, M.; Renner, S.; Wetzel, S.; Vartak, N.; Gerding-Reimers, C.; Bon, R. S.; Bastiaens, P. I.; Waldmann, H. *Angew. Chem., Int. Ed.* **2011**, *50*, 9832.
19. Wenkert, D.; Chen, T.-F.; Ramachandran, K.; Valasinas, L.; Weng, L.-l.; McPhail, A. T. *Org. Lett.* **2001**, *3*, 2301.
20. Vedejs, E.; Naidu, B. N.; Klapars, A.; Warner, D. L.; Li, V.-s.; Na, Y.; Kohn, H. *J. Am. Chem. Soc.* **2003**, *125*, 15796.
21. Ohba, M.; Izuta, R.; Shimizu, E. *Tetrahedron Lett.* **2000**, *41*, 10251.
22. Pippel, D. J.; Mapes, C. M.; Mani, N. S. *J. Org. Chem.* **2007**, *72*, 5828.
23. Veleiro, A. S.; Pecci, A.; Monteserin, M. C.; Baggio, R.; Garland, M. T.; Lantos, C. P.; Burton, G. *J. Med. Chem.* **2005**, *48*, 5675.
24. Tamayo, A.; Lodeiro, C.; Escriche, L.; Casabo, J.; Covelo, B.; Gonzalez, P. *Inorg. Chem.* **2005**, *44*, 8105.
25. Forster, L.; Schulze, E. A.; Lehr, M. *Anal. Bioanal. Chem.* **2009**, *394*, 1679.
26. Zahov, S.; Drews, A.; Hess, M.; Schulze, E. A.; Lehr, M. *Chem. Med. Chem.* **2011**, *6*, 544.
27. Boger, D. L.; Sato, H.; Lerner, A. E.; Hedrick, M. P.; Fecik, R. A.; Miyauchi, H.; Wilkie, G. D.; Austin, B. J.; Patricelli, M. P.; Cravatt, B. F. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5044.
28. Sit, S. Y.; Conway, C.; Bertekap, R.; Xie, K.; Bourin, C.; Burris, K.; Deng, H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3287.
29. Boger, D. L.; Sato, H.; Lerner, A. E.; Austin, B. J.; Patterson, J. E.; Patricelli, M. P.; Cravatt, B. F. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 265.
30. Jonsson, K.-O.; Vandevoorde, S.; Lambert, D. M.; Tiger, G.; Fowler, C. J. *Br. J. Pharmacol.* **2001**, *133*, 1263.
31. Mileni, M.; Kamtekar, S.; Wood, D. C.; Benson, T. E.; Cravatt, B. F.; Stevens, R. C. *J. Mol. Biol.* **2010**, *400*, 743.
32. Schmitt, M.; Lehr, M. *J. Pharm. Biomed. Anal.* **2004**, *35*, 135.
33. Trost, B. M.; Lee, C. *J. Am. Chem. Soc.* **2001**, *123*, 12191.
34. Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815.