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Original article

Chemistry around imidazopyrazine and ibuprofen: Discovery of novel fatty acid amide hydrolase (FAAH) inhibitors

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ABSTRACT

Based on the imidazo-[1,2-a]-pyrazin-3-(7H)-one scaffold, a dual action prodrug has been designed for combining antioxidant and anti-inflammatory activities, possibly unmasked upon oxidation. The construction of the target-molecule requires two building blocks, namely a 2-amino-1,4-pyrazine and a 2-ketoaldehyde. Attempts to synthesize the 2-ketoaldehyde (**5a**) derived from ibuprofen failed, but led to the corresponding 2-ketoaldoxime (**7a**) which could not be condensed with the pyrazine synthons. However, a model compound, i.e. phenylglyoxal aldoxime, reacted well under microwave activation to furnish novel imidazo[1,2-a]-pyrazine-3-(7H)-imine derivatives (**18a**,**b**). These heterobicycles behave as antioxidants by inhibiting the lipid peroxidation, and one compound (**18b**) is endowed with a significant anti-inflammatory effect in a cellular test. Unexpectedly, all the synthetic intermediates derived from ibuprofen are good inhibitors of FAAH, the most active compound (**4a**) featuring the 1,3-dithian-2-yl motif.

1. Introduction

The search of dual action drugs is a field of growing interest in medicinal chemistry [1,2]. In the particular case of dual action prodrugs, two entities featuring synergic activities are covalently linked. This bond can be chemically or enzymatically transformed *in vivo*, leading to the simultaneous release of two active molecules at the same site. In the course of previous works dedicated to antioxidant compounds belonging to the coelenteramine and coelenterazine families [3,4], we have disclosed an oxidative degradation pathway of imidazopyrazinones **A** (mother antioxidant) producing a 2-amino-1,4-pyrazine **C** (daughter antioxidant) and a carboxylic acid **D**. This route occurs in parallel to the oxidative degradation into 2-amido-1,4-pyrazine **B** producing light (chemiluminescence pathway) (Fig. 1) [5]. This offers a unique opportunity to possibly combine in the same molecule **A** an antioxidant activity with an anti-inflammatory activity by choosing adequately the nature of the substituent R. Indeed, upon oxidation, this fragment will be released as the R–CO_2H molecule **D** and a lot of nonsteroidal anti-inflammatory drugs (NSAID) are in fact carboxylic acids [6]. Moreover, the advantages of molecules combining antioxidant and anti-inflammatory activities have been already demonstrated in the case of modified NSAIDs such as ibuprofen [7,8].

Hence our target-molecule is the so-called "ibuprofen prodrug" shown in Fig. 2, where R′, R″ could be simply H, or preferably a phenolic group for increasing the antioxidant activity [3,4]. Classically, the synthesis of imidazo-[1,2-a]-pyrazin-3-(7H)-one (**E**) makes use of two building blocks, namely the α-ketoaldehyde (**F**) and the aminopyrazine (**C**) (Fig. 2). Their condensation is performed in aqueous acidic solutions [9,10].

In this article, we describe our persistent attempts to prepare the α-ketoaldehyde **F** derived from ibuprofen. Since the corresponding oxime was made available, we have also examined the condensation of α-ketoaldoximes with 2-amino-1,4-pyrazines. Unexpected bicycles have been obtained featuring modest antioxidant and anti-inflammatory activities. Finally, a series of synthetic intermediates (toward the target **F**), endowed with poor anti-inflammatory activity, has been evaluated for another activity

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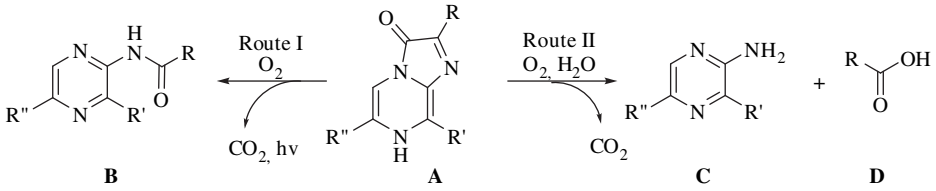


Fig. 1. Two pathways of oxidative degradation of imidazopyrazinone **A** (R = Me, Ph). Route I is preferred in lipophilic media and route II is preferred in hydrophilic media [5]. **A** is the "mother anti-oxidant"; **B** is devoid of antioxidative properties; **C** is the "daughter anti-oxidant" (R″ = C_6H_4-*p*OH) [3,4].

related to the endocannabinoid system. This led to the unexpected discovery of novel "hits" for the inhibition of fatty acid amide hydrolase (FAAH).

2. Results and discussion

2.1. Synthesis of the α-ketoaldehyde synthon (*F*)

Racemic ibuprofen **1a** was used as starting material. Indeed, this anti-inflammatory drug is given as the (R) + (S) mixture because *in vivo*, the inactive (R)-isomer is enzymatically transformed into the active (S)-isomer [11]. Several approaches toward our target α-ketoaldehyde were considered. Aryl α-ketoaldehydes are usually obtained by oxidation of methylketones and diazoketones, or from α-(α′)-(di)haloketones [12–15]. Few methods are dedicated to benzyl α-ketoaldehydes [9,10,16] and none to the α-(methyl)benzyl analogs.

In a first route (Scheme 1), we prepared the 1,3-dithianyl precursor **4a** by substitution of the corresponding Weinreb amide **3a** with 1,3-dithiane anion [17]. Previously, ibuprofen **1a** was activated into **2a** with *N*-(methanesulfonyl)benzotriazole [18,19]. Unfortunately, all attempts to "hydrolyze" the dithiane moiety into an aldehyde function failed: neither compound **5a** (or **5′a**) nor the corresponding hydrate or dialkyl acetal were identified in the crude mixtures. Typical conditions investigated were [20]: $Hg(ClO_4)_2$/$CaCO_3$ in alcohol, HgO/$CaCO_3$ in wet acetone, CAN (cerium ammonium nitrate) in CH_3CN-borate buffer, NBS (N-bromosuccinimide) in MeOH or wet acetone, NBS/TMP in CH_3CN–H_2O, Selectfluor in CH_3CN, MCPBA (*m*-chloroperbenzoic acid) then H_3O^+, Meerwein salt or MeTf in CH_3CN, then H_2O, etc. Similar disappointing results were recorded for the deprotection of analogs **4b** and **4c** (Scheme 1). It is worth noting that the hydrolysis of α-carbonyl(dithio)acetal derivatives has not been yet described in the literature.

The second route (Scheme 2) was based on the α-nitroketone **6a** as key intermediate [21], easily obtained by reaction of the activated acid **2a** with nitromethane in basic conditions. The conversion of a nitromethylene moiety into a carbonyl group is known as the Nef reaction; beside the traditional use of very strong acids, smooth conditions have been developed, making this reaction compatible with a large variety of functionalities [22]. However, to our knowledge, the Nef reaction was never applied to α-nitromethylketones. The precursor **6a** was submitted to a set of representative conditions, namely: 10% aq. H_2SO_4 in alcohol, oxone in acetone, $SnCl_2$/thiophenol in EtOH–H_2O, $NaNO_2$/DBU in AcOH, $TiCl_3$ reduction, etc. The desired compound **5a** was not obtained, but the oxime intermediate **7a** could be isolated after reduction with tin chloride [23,24]. Attempts to further hydrolyze **7a** into **5a** were unsuccessful ($SnCl_2$, $NaHCO_3$, (L)-tartric acid, $NaHSO_3$, H_2O).

Lastly, we turned to the method using a α-bromomethylketone as key intermediate (Scheme 3) [25]. Sodium salt of ibuprofen was transformed in acid chloride **8a** with oxalyl chloride, and then

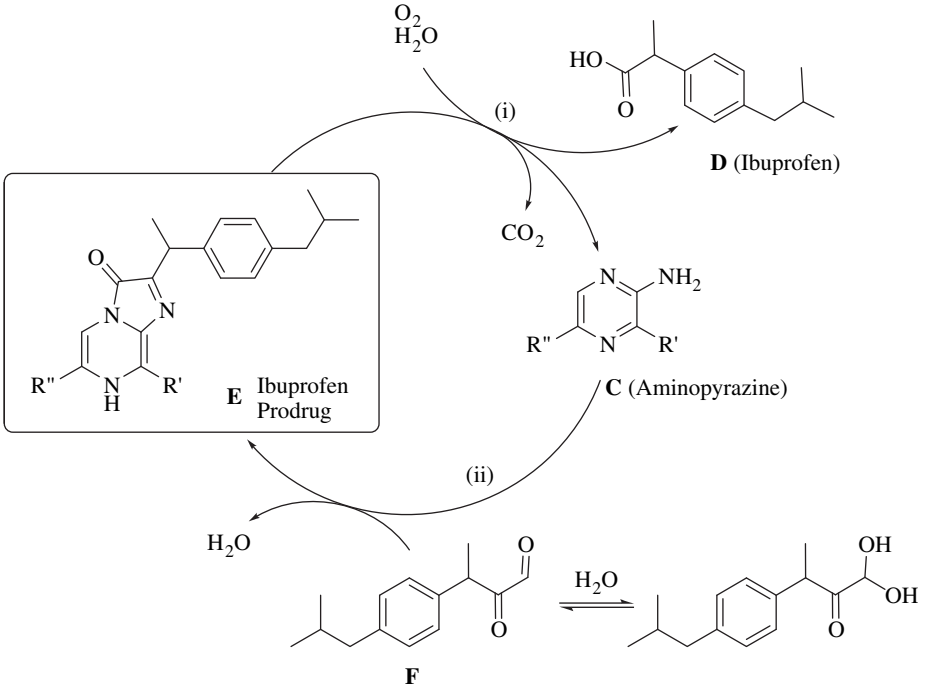
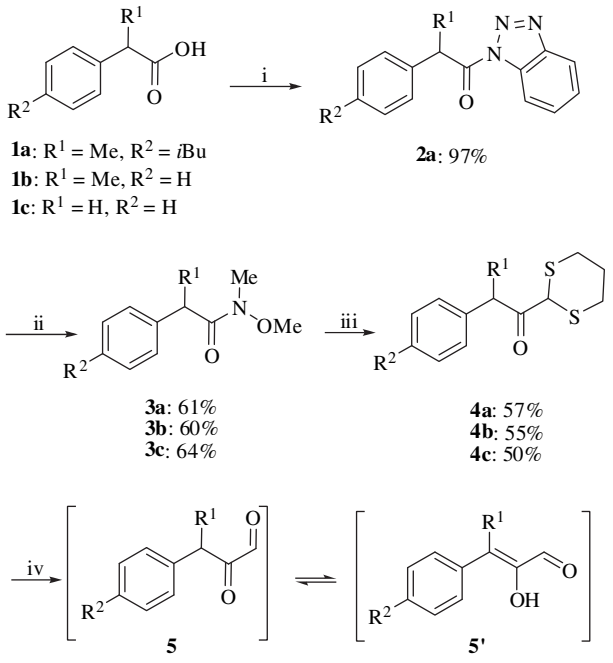


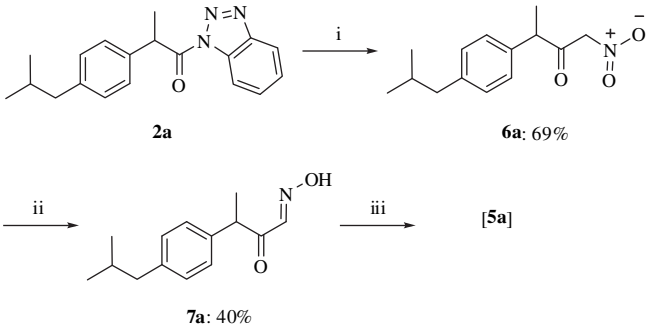
Fig. 2. Design of potential dual action prodrug endowed with antioxidant and anti-inflammatory activities. Route (i) shows the expected oxidative degradation liberating ibuprofen and an aminopyrazine antioxidant (R″ = C_6H_4-*p*OH). Route (ii) shows the prodrug **E** synthesis from the aminopyrazine **C** and the α-ketoaldehyde synthon **F**.



Reagents and conditions: (i) BtMs, Et_3N, THF, reflux, 17 h; (ii) MeONHMe.HCl, Et_3N, THF, 20 °C, 17 h; (iii) Li–(S,S) , THF, -78 °C, 4 h; (iv) hydrolysis (see text).

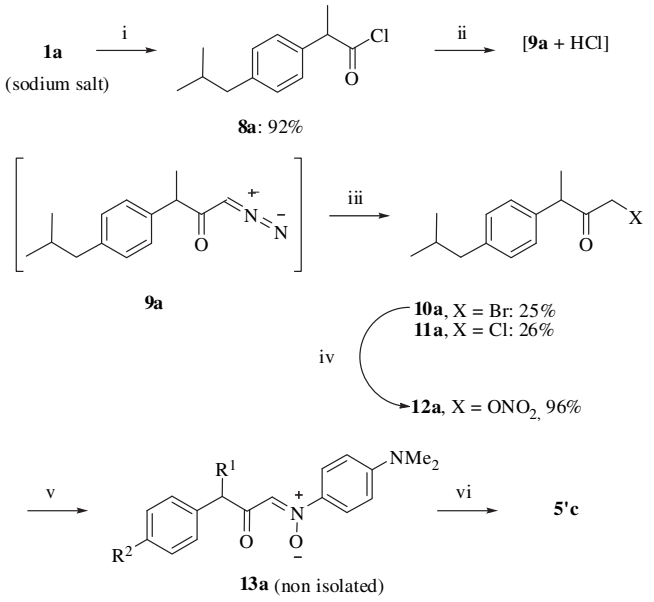
Scheme 1. First synthetic approach toward ketoaldehyde **5**.

reacted with diazomethane in ether solution. The diazo intermediate **9a** was treated *in situ* with HBr in acetic acid. Two products were formed in almost equivalent amounts (NMR of the crude mixture): the expected α-bromomethylketone **10a** and the α-chloromethylketone **11a** resulting from the reaction of **9a** with HCl formed during its synthesis. The halogenated compounds were separated by column chromatography with difficulty. The bromoketone **10a**, but not the chloroketone **11a**, furnished the nitrate **12a** by substitution with silver nitrate. Unfortunately, nitrite elimination under basic conditions (AcONa 10% in DMSO) [10,26] failed to deliver pure α-ketoaldehyde **5a**. We recovered an untractable mixture, showing only a weak signal at 9.21 ppm (for the aldehyde proton) in ^1H NMR. Treatment of **10a** with pyridine (reflux, CH_2Cl_2), followed by *N,N′*-dimethyl-nitrosoaniline in the presence of aq. NaOH gave a nitrone intermediate [27,28] (**13a**) which acid hydrolysis (10% H_2SO_4) also furnished an untractable mixture. Nevertheless, the same sequences of reactions (see Scheme 3)



Reagents and conditions: (i) CH_3NO_2, *t*BuOK, DMSO, 10 °C to 20 °C, 4 h; (ii) $SnCl_2.H_2O$, PhSH, Et_3N, CH_3CN, 20 °C, 1 h; (iii) hydrolysis (see text).

Scheme 2. Second synthetic approach toward **5**.



Reagents and conditions: (i) $(COCl)_2$, ether, 0 °C to 20 °C, 4 h; (ii) CH_2N_2, ether, 20 °C, 2 h; (iii) HBr 47%, AcOH, 20 °C, 1 h; (iv) $AgNO_3$, CH_3CN, 20 °C, 40 h; (v) pyridine, then nitrosoaniline, NaOHaq; (vi) 10% H_2SO_4.

Scheme 3. Third synthetic approach toward **5**.

applied to phenylacetyl chloride ($R^1 = R^2 = H$) resulted in the known 2-hydroxy-3-phenyl-2-propenal **5′c** (tautomer of **5c**) [29]. This positive control confirmed that the reactivity of 2-methylarylacetyl is quite different from the unsubstituted arylacetyl, and we abandoned any effort toward the synthesis of ibuprofen-derived aldehyde **5a** (or **5′a**).

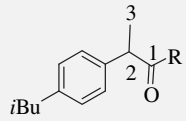
The ibuprofen derivatives (**2**–**4**; **6**–**8** and **10**–**12**) have been purified and characterized as usual (see Experimental protocols). Typical NMR data are collected in Table 1.

2.2. Condensation of the aminopyrazine synthon (**C**) with α-ketoaldoximes

Since we succeeded to obtain the oxime derivative **7a** instead of the target-aldehyde **5a** (see Scheme 2), we decided to examine the reaction of α-ketoaldoximes with 2-amino-1,4-pyrazines under acidic conditions, as used to synthesize imidazopyrazinones.

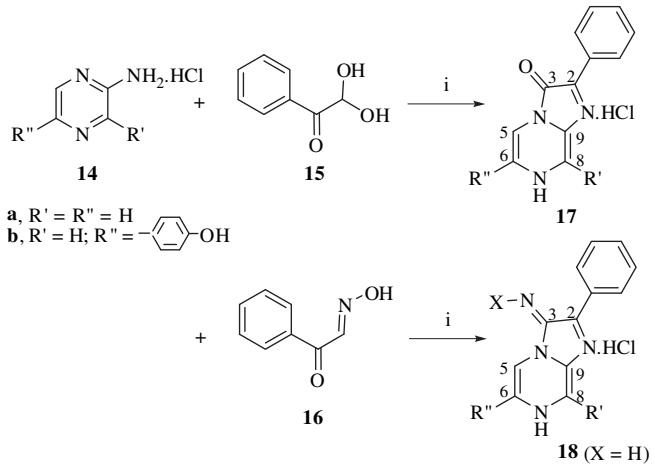
The experimental conditions were first established considering simple systems, namely the commercial aminopyrazine **14a** ($R' = R'' = H$), phenylglyoxal hydrate (**15**) and phenylglyoxal aldoxime (**16**) (Scheme 4). Under the classical conditions (HCl_{aq} (4 equiv.), EtOH, reflux overnight [30,31]), the condensations of **14a** with **15** and **16** led respectively to the bicycles **17a** and **18a**, in 70% and 40% yields. The chemical structure of **18**, which is different from the expected one, will be discussed later on. Similarly, the reactions of 2-amino-5-*p*-(hydroxy)phenyl-1,4-pyrazine (**14b** [32]) with **15** and **16** furnished the imidazopyrazine derivatives **17b** and **18b**, in 66% and 15% yields respectively. We could optimize the production of the novel heterocycles **18** by using the pre-formed hydrochloride salts of the aminopyrazines **14** as starting materials and an excess of **16**, and by activating the condensation under microwave (MW) heating. Thus a 1:2 mixture **14**·HCl:**16** in ethanol was heated at 80 °C for 4 h in an MW oven (Scheme 4); compounds **18a** and **18b** are now recovered in about 60% and 30% yield. The same protocol applied also readily to the synthesis of the known imidazopyrazinones **17a**,**b** [31,33] (Scheme 4). Unfortunately, we

Table 1
Typical NMR data of ibuprofen derivatives.

Entry		^{1}H NMR (ppm)				^{13}C NMR (ppm)			
		H-2	H-3	$J_{2,3}$ (Hz)	*R*	C-1	C-2	C-3	*R*
1	**2a**	5.40 (q)	1.74 (d)	7.1	7.48 (dd); 7.63 (dd); 8.08 (d); 8.29 (d)	173.9	44.7	18.9	114.9; 120.4; 126.4; 131.7; 131.8; 146.5
2	**3a**	4.10 (q)	1.42 (d)	7.0	3.16 (s); 3.40 (s)	175.9	41.9	19.8	32.6; 61.4
3	**4a**	4.19 (q)	1.44 (d)	6.9	1.99 (m); 2.09 (m); 2.50 (m); 2.59 (m); 3.04 (m); 3.51 (m)	203.1	44.6	18.7	25.4; 26.1; 26.3
4	**6a**	3.80 (q)	1.48 (d)	6.9	5.12 (d_{AB}, 14.7 Hz); 5.20 (d_{AB}, 14.7 Hz)	196.6	51.8	17.2	82.1
5	**7a**	4.55 (q)	1.43 (d)	7.1	7.50 (s)	198.8	47.0	18.0	149.1
6	**8a**	4.09 (q)	1.59 (d)	7.0	/	176.0	57.4	19.0	/
7	**10a**	4.10 (q)	1.43 (d)	6.9	3.78 (d_{AB}, 12.7 Hz); 3.92 (d_{AB}, 12.7 Hz)	202.0	49.8	17.8	33.5
8	**11a**	3.89 (q)	1.43 (d)	6.9	4.02 (d_{AB}, 15.4 Hz); 4.09 (d_{AB}, 15.4 Hz)	202.3	49.9	17.7	47.4
9	**12a**	3.78 (q)	1.45 (d)	7.0	4.82 (d_{AB}, 17.5 Hz); 4.91 (d_{AB}, 17.5 Hz)	201.6	49.8	17.0	73.0

were unable to identify any product from the reaction of aminopyrazine **14a** with the α-ketoaldoxime **7a** derived from ibuprofen, using a number of experimental conditions (**14a**·HCl, EtOH, 80 °C, 18 h; dioxane-EtOH, 85 °C, 24 h; dioxane-H_2O, 100 °C, 70 h; dioxane-H_2O + 2 equiv. HClaq, 100 °C, 24 h; EtOH, 80 °C, MW, 4 h; dioxane-H_2O, 100 °C, MW, 5 h). Under mild conditions, the reagents are mainly recovered. In the presence of an excess of HCl, the aldoxime **7a** was degraded and under MW activation, the starting materials were accompanied with increasing amounts of degradation products in function of the reaction time.

The structure of the heterobicycles **18a**,**b** resulting from the condensation of **14a**,**b** with the commercial α-ketoaldoxime **16** is very intriguing. Formation of either the imidazopyrazinoxime (structure **18** with X = OH) or the imidazopyrazinone (structure **17** resulting from the *in situ* oxime hydrolysis) was expected. But both the ^{1}H and ^{13}C NMR data collected for compounds **18a**,**b** show tenuous differences as compared to the corresponding known imidazopyrazinones **17a**,**b** [4] (see Table 2). From the HRMS data, the related oximes (see Scheme 4, structure **18** with X = OH) can be excluded: m/e = 211.0983 (**18a**) for M + 1 corresponding to the formula $C_{12}H_{10}N_4$, and m/e = 303.1244 (**18b**) for M + 1 corresponding to the formula $C_{18}H_{14}N_4O$. Also the IR and UV spectra of **17** and **18** are different (see Experimental protocols). We tried to obtain crystals for X-ray diffraction analysis, but without success: **18a**,**b** (hydrochloride salts) precipitated as amorphous powders.



Scheme 4. Condensation of aminopyrazine hydrochloride with α-ketoaldehyde hydrate and α-ketoaldoxime under microwave heating.

Attempts to crystallize other salts (oxalate, fumarate, nitrate, perchlorate, tetrafluoroborate) also failed: small crystals formed but they flopped under filtration. Lastly, we tried to derivatize the heterocycles **18a**,**b** by silylation, alkylation or acylation reactions. Under the usual conditions of such reactions, only untractable mixtures were recovered. In the absence of X-ray data (for **18** or derivatives), the proposed imidazopyrazinimine structures **18a**, **b** (see Scheme 4, X = H) could not be definitively established, but they are fitting at best with all the spectral data. Their formation implies a reductive step, somewhere in the reaction cascade, for explaining the formal loss of one oxygen atom. The reducing agent is actually unknown (auto-redox system in the presence of air [34], aldoxime in excess,…).

The alleged imidazopyrazinimines (**18** with X = H) have been evaluated for their capacity to inhibit the lipid peroxidation, by using a standard test [35]. Briefly, a micellar solution of linoleic acid is incubated with AAPH (2,2′-azo-*bis*-(2-aminopropane)dihydrochloride) as free-radical generator. In the presence of air, the substrate radicalar oxidation produces conjugated dienes, which can be monitored at 234 nm as a function of time. Added antioxidants delay this oxidation process. At 10 μM, the lag-time induced by **18a** and **18b** is respectively of 92 min and 140 min; in the same test, the reference values of **17a** and **17b** are 142 min and 133 min. The imidazopyrazinimines (**18**) behave thus as antioxidant reagents, like the known imidazopyrazinones (**17**). This could confirm the structural similarity between **17** and **18**. However, **18** are devoid of the chemiluminescence properties characteristic of coelenterazine-like compounds (results not shown).

2.3. Biological evaluations

Antioxidant agents could be possibly endowed with anti-inflammatory properties [36]. Indeed, since the reactive oxygen species (ROS) exhibit a pro-inflammatory activity, their capture by

Table 2
Comparative NMR data ($7 \cdot 10^{-2}$ M, CD_3OD).[a]

	17a	18a	17b	18b
H-5	8.23 (d)	8.30 (d)	8.42 (s)	8.50 (s)
H-6	7.64 (d)	7.64 (d)	/	/
H-8	8.79 (s)	8.79 (s)	8.89 (s)	8.79 (s)
C-2	138.5	141.9	128.6[b]	140.2
C-3	146.7	137.4	147.8[b]	136.6
C-9	130.0	133.4	129.4[b]	132.7

[a] ^{1}H NMR at 500 MHz and ^{13}C NMR at 125 MHz; δ values in ppm; d = doublet and s = singlet.
[b] Spectrum recorded in DMSO-d_6.

an antioxidant agent should lead to a decrease of the amount of pro-inflammatory species, and therefore, to the inflammation abatement. Accordingly, both the imidazopyrazine and ibuprofen derivatives have been assayed in a cellular test for anti-inflammatory activity based on Caco-2 cells as a widely used model of the human intestinal barrier [37,38]. Briefly, the secretion of interleukin-8 (IL-8), a pro-inflammatory cytokine, upon stimulation by IL-1β, was measured in the presence of the tested molecule at 50 μM. Results are expressed in % *vs.* the control (no product) and EGCG ((−)-epigallocatechin-3-gallate) was considered as the anti-inflammatory active reference [39]. The molecules were tested 2 or 3 times, each in triplicate. Despite the rather poor reproducibility inherent to such a cellular assay, the Fig. 3A shows that compounds **17b** and **18b**, two phenol-substituted imidazopyrazine derivatives, significantly decreased the secretion of IL-8, considered as a hallmark of the intestinal inflammation, to respectively 65% ($p < 0.05$) and 56% ($p < 0.01$) of the control value. Nevertheless, they were less efficient than EGCG (15%, $p < 0.001$). The compound **18b** was still active at 5 μM (75%, data not shown). In the same assay realized with ibuprofen and its derivatives (Fig. 3B), ibuprofen (sodium salt, **1a**) appears modestly active, but its derivatives display either a significant anti-inflammatory effect, *i.e.* **3a** (55%, $p < 0.01$) > **11a** (70%, $p < 0.05$) > **2a** (76%, $p < 0.05$), or pro-inflammatory effect, *i.e.* **7a** (290%, $p < 0.05$).

The production of fatty acid amides (FAAs) is increased under some inflammation conditions [40]. FAAs are endogenous compounds interacting with the cannabinoid receptors, a class of receptors involved in a great number of physiological effects, among which the dampening of inflammation [41,42]. The rate of FAAs *in vivo* is controlled by a hydrolytic enzyme, i.e. FAAH (fatty acid amide hydrolase), responsible of their metabolism.

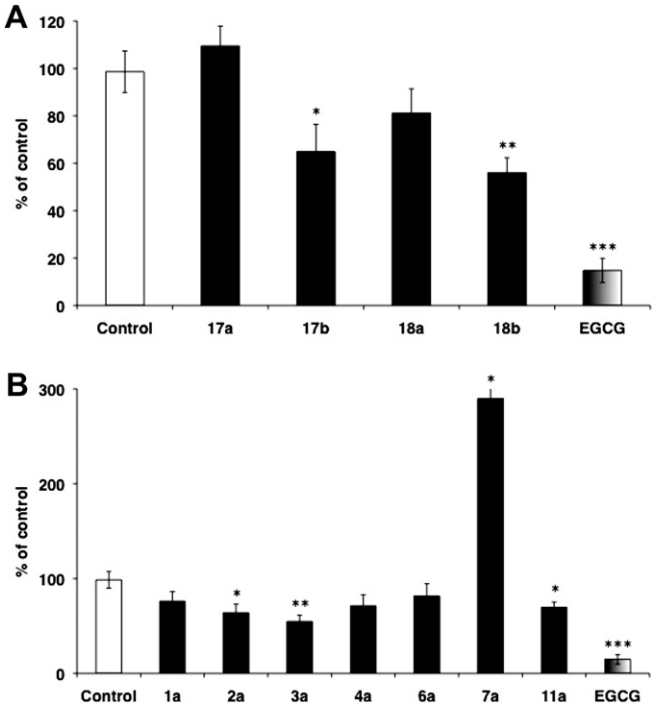


Fig. 3. Anti-inflammatory effect of imidazopyrazine (A) and ibuprofen (B) derivatives. Intestinal Caco-2 cells were concomitantly incubated, for 24 h, with the pro-inflammatory stimulus IL-1β at 25 ng/ml in the absence (control) or presence of tested compound at 50 μM. EGCG, at 50 μM, was used as an anti-inflammatory reference. IL-8 was then quantified in the extracellular media by ELISA assay. Results are expressed as the percentage of IL-8 *vs* the control. Data represent means ± S.E.M. from two or three independent experiments, each with three replicates per condition. *, **, *** indicate, respectively, $p < 0.05$, $p < 0.01$, $p < 0.001$ as compared to the control condition.

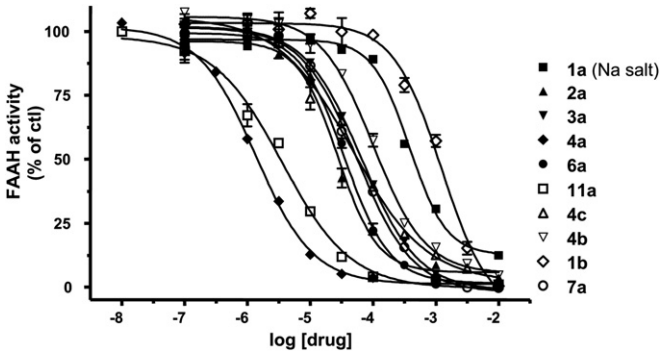


Fig. 4. Dose–response curves.

Accordingly, this enzyme may be a useful therapeutic target for the treatment of certain inflammatory states. Recently, our laboratory became interested in the discovery of novel FAAH inhibitors [43], so we decided to evaluate the series of ibuprofen derivatives herein synthesized (see Schemes 1–3), using our in-house expertise.

It is now well established that ibuprofen inhibits the metabolism of anandamine, an endogenous cannabimimetic agent [44,45]. Both enantiomers of ibuprofen, similarly to other NSAIDs (ketorolac, flurbiprofen, indomethacin and niflumic acid) [46–48], behave as good inhibitors of FAAH, the enzyme processing anandamide into arachidonic acid and ethanolamine [49,50]; a pH dependent activity has been established (pI_{50} of (R)-Ibu. = 4.26 ± 0.07 at pH 6 and 3.69 ± 0.04 at pH 8, against rat brain FAAH) [46]. The clinical effects of NSAIDs result from the inhibition of cylcooxygenase enzymes (COX-1 and COX-2), causing gastrointestinal toxicity as injurious side effect [6]. Derivatives of ibuprofen such as esters and amides have been shown to retain the analgesic activity of the parent acid, while reducing the gastric mucosal damages [51]. Interestingly, amide derivatives of ibuprofen featuring pyridinyl heterocycles are also excellent inhibitors of FAAH [52].

The activity of the novel ibuprofen derivatives was assayed according to a protocol already validated in our group for testing,

Table 3
pI_{50} values of racemic ibuprofen derivatives (from three independent experiments).

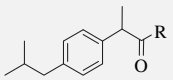
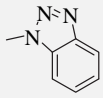
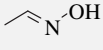
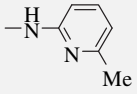
Entry			pI_{50}	
	Cmpd	R	*h*FAAH	*h*MGL
1	**1a**	$-O^- Na^+$	3.42 ± 0.03	n.d
2	**2a**		4.56 ± 0.03	3.71 ± 0.12
3	**3a**	−N(Me)OMe	4.19 ± 0.02	3.19 ± 0.10
4	**4a**		5.86 ± 0.03	n.d
5	**6a**		4.45 ± 0.02	3.31 ± 0.05
6	**7a**		4.25 ± 0.04	n.d
7	**11a**		5.45 ± 0.05	3.70 ± 0.10

Table 4
pI_{50} values of 1,3-dithiane derivatives and references (from three independent experiments).

Entry	Cmpd	R^1	R^2	R	pI_{50} (*h*FAAH)
1	**1a**	Me	*i*-Bu	OH	3.55 [48]
2	**1b**	Me	H	OH	2.91 ± 0.04
3	**4a**	Me	*i*-Bu		5.86 ± 0.03
4	**4b**	Me	H		4.00 ± 0.03
5	**4c**	H	H		4.33 ± 0.06
6	Ibu-am5	Me	*i*-Bu		5.08 ± 0.08 [52]

among other templates, substrate analogues, lipophilic β-lactams (azetidin-2-ones), and heterocycle-based inhibitors [43,53–55]. The assay is a competitive hydrolytic experiment using the radiolabelled substrate (anandamide = arachidonoylethanolamide, AEA) to measure FAAH activity. Briefly, recombinant human FAAH in buffer (pH 7.4) is added to glass tubes that contain either the drug in DMSO or DMSO alone (control). Hydrolysis is initiated by adding [^{3}H]-AEA solution and the tubes are incubated at 37 °C. Tubes containing buffer only are used as controls for chemical hydrolysis (blank) and this value is systematically subtracted. Reactions are stopped by adding ice-cold MeOH/$CHCl_3$, the radiolabelled ethanolamine is extracted and the radioactivity is measured by liquid scintillation counting (LSC). The selectivity *vs.* monoacylglycerol lipase (MGL) was similarly assayed, using the [^{3}H]-labelled 2-oleoylglycerol (2-OG) as competitive substrate [43,53–55].

The inhibition results expressed as FAAH activity (% of control) *vs.* drug concentration are shown in Fig. 4. From those experimental dose-response curves, the pI_{50} (*i.e.* $-\log IC_{50}$) values were calculated (Table 3). The results clearly show that all tested ibuprofen derivatives are more active than the parent carboxylate (sodium salt) and behave as selective inhibitors of FAAH *vs.* MGL, another hydrolytic enzyme of the endocannabinoid system. We recorded the following decreasing order of activity: **4a** (entry 4, *R* = 1,3-dithian-2-yl), **11a** (entry 7, R = chloromethyl), **2a** (entry 2, R = benzotriazolyl), **6a** (entry 5, R = nitromethyl), **7a** (entry 6, R = *N*-(hydroxy)imino), **3a** (entry 3, R = *N*-(methoxy)methylamino), and **1a** (entry 1, ibuprofen from commercial source). The most active compound **4a** (1-(1,3-dithian-2-yl)-2-(4-isobutylphenyl)-propan-1-one) was compared to related dithiane derivatives featuring fewer alkyl substituents, namely **4b** (1-(1,3-dithian-2-yl)-2-phenylpropan-1-one) and **4c** (1-(1,3-dithian-2-yl)-2-phenylethan-1-one). We found that the ibuprofen core significantly enhances the activity compared to the simple benzyl derivatives (Table 4, compare entry 3 with entries 4 and 5). The same effect was visible when comparing ibuprofen **1a** (acid, entry 1) with 2-methyl-phenylacetic acid (**1b**, entry 3).

3. Conclusion

Our idea to construct an original ibuprofen prodrug based on the *in situ* oxidation of an imidazopyrazinone precursor (**E**) could not be applied because the required ibuprofen-synthon (**F**) was synthetically inaccessible. However during our Holy Grail quest, we discovered a novel imidazopyrazine framework, presumably the imine compounds **18** (X = H) (and not the corresponding oximes (X = OH)). The *p*-hydroxyphenyl derivative **18b** is endowed with antioxidant and anti-inflammatory activities and appears even more active than the related imidazopyrazinone **17b**. Nevertheless, this result is rather difficult to exploit further due to the uncertainty about the structural assignment and the high synthetic challenge.

Ibuprofen (**1a**) showed only a poor anti-inflammatory effect on the IL-8 secretion by the inflamed intestinal cells. This can be explained as the anti-inflammatory mechanism of NSAIDs implicates another target of the inflammation cascade *i.e.* the inhibition of pro-inflammatory enzymes, the cyclooxygenases (COX-1 and COX-2) [56]. However, some ibuprofen derivatives with masked acid function (**2a**, **3a**) exhibited a significant activity.

Knowing that the cannabinoid system can contribute to the pharmacological effects of NSAIDs [52], we evaluated the ibuprofen derivatives against FAAH. The loss of activity of this enzyme has been shown to provide beneficial effects in models of inflammation due to the increased levels of N-acyl-ethanolamines [57]. Interestingly, we disclosed novel FAAH inhibitors among the series of ibuprofen derivatives synthesized as precursors of the synthon **F**. In particular, the best FAAH inhibitor **4a** is more active than *N*-(6-(methyl)pyridin-2-yl)-ibuprofen-amide (Table 4, entry 6, Ibu-am5) recently selected by Fowler *et al.* [52] as "lead" to explore the potential analgesic effect of compounds combining COX- and FAAH-inhibitory properties. Even if **4a** is devoid of anti-inflammatory action in our cellular test, its unique activity against FAAH is of interest. Indeed, the recognized therapeutic promise of FAAH inhibition for the treatment of anxiety, eating and sleep disorders, among others [50], nowadays, stimulates the research of novel inhibitors by structure-based design or high-throughput screening [58]. Our present work provides an unexpected entry in this field with the 1,3-dithian-2-yl ketone derived from ibuprofen as a novel "hit".

4. Experimental protocols

4.1. Materials and methods

Anhydrous solvents were provided by Fluka. All reagents were obtained from Aldrich–Fluka, Acros or ROCC and used as received.

^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a BRUCKER AVANCE-300 spectrometer. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a BRUCKER AM-500 spectrometer. The attributions were established by homonuclear and heteronuclear correlations or by selective decoupling experiments. Chemical shifts are reported as δ values (in ppm) downfield from TMS for ^{1}H and ^{13}C or from $CFCl_3$ for ^{19}F. The mass spectra were obtained using a Finnigan-MAT TSQ-7000 instrument (FAB, EI and CI modes) or an LCQ Finnigan-MAT LCQ instrument (APCI and ESI modes). HRMS analyses were obtained at the Université Mons-Hainaut, Mass spectroscopy laboratory, Pr. Flammang or at the University College of London (UK), chemistry department, Dr. L. D. Harris laboratory. UV spectra were recorded on a UV-vis-NIR Varian-Cary spectrophotometer (λ given in nm). IR spectra were determined using a Shimadzu FTIR-8400S apparatus. Thin-layer chromatography was carried out on silica gel 60 plates F254 (Merck, 0.2 mm); product visualization was effected with UV light (λ = 254 nm), $KMnO_4$ or I_2. For flash chromatography we used Merck silica gel 60 of 230–400 mesh ASTM. Melting points were determined on an electro-thermal apparatus BUCHI Melting Point B-540.

4.2. Organic chemistry

4.2.1. 1-(Benzotriazol-1-yl)-2-(4-isobutylphenyl)propan-1-one (**2a**)

To a solution of ibuprofen (398 mg, 1.93 mmol) in dry THF (4 mL, 2 mL/mmol) were added BtMs (420 mg, 2.13 mmol) and TEA (300 μL, 2.14 mmol) under Ar atmosphere. The solution was heated overnight to reflux. The mixture was cooled to RT and evaporated to dryness. The residue was dissolved in ethyl acetate, washed with HCl 0.33 M, Na_2CO_3 (sat.), brine, dried over $MgSO_4$, filtered and evaporated under vacuum. We obtained 573 mg (97%) of a white solid.

R_f 0.73 (hexane/AcOEt 5:2); m.p. 52–55 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (d, $^3J = 6.6$ Hz, 6H), 1.74 (d, $^3J = 7.1$ Hz, 3H), 1.80 (m, 1H), 2.40 (d, $^3J = 7.2$ Hz, 2H), 5.40 (q, $^3J = 7.1$ Hz, 1H), 7.09 (d, $^3J = 8.1$ Hz, 2H), 7.41 (d, $^3J = 8.1$ Hz, 2H), 7.48 (dd, $^3J = 7.2$ Hz, $^3J = 8.2$ Hz, 1H), 7.63 (dd, $^3J = 7.2$ Hz, $^3J = 8.2$ Hz, 1H), 8.08 (d, $^3J = 8.2$ Hz, 1H), 8.29 (d, $^3J = 8.2$ Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.9, 22.7, 30.4, 44.7, 45.3, 114.9, 120.4, 126.4, 128.1, 129.9, 131.7, 131.6, 136.7, 141.4, 146.5, 173.9; FT-IR (ATR-SeZn, cm^{-1}) ν 2954 (s), 1734 (s), 1450(m), 1380 (s), 1166 (m), 948 (s), 750 (m); MS (APCI) m/z: 308 $[M+H]^+$, 120 $[Bt+H]^+$; HRMS calcd for $C_{19}H_{21}N_3ONa$: 330.1582, found: 330.1571.

4.2.2. N-Methoxy-N-methyl-2-(4-isobutylphenyl)propanamide (**3a**)

To a solution of N-methoxy-N-methylamine hydrochloride (350 mg, 3.59 mmol) and TEA (500 μL, 3.56 mmol) in dry THF (5 mL) was added 1-(benzotriazol-1-yl)-2-(4-isobutylphenyl) propan-1-one (1 g, 3.25 mmol) under Ar atmosphere. The mixture was stirred overnight at RT, then evaporated to dryness and the residue was diluted in ethyl acetate (25 mL). The organic layer was washed with HCl (0.33 M), Na_2CO_3 (sat.), brine, dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by flash chromatography (hexane/ethyl acetate 7:1) and 491 mg (61%) of pale yellow oil were obtained.

R_f 0.54 (hexane/ethyl acetate 5:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (d, $^3J = 6.6$ Hz, 6H), 1.42 (d, $^3J = 7.0$ Hz, 3H), 1.83 (m, 1H), 2.43 (d, $^3J = 7.2$ Hz, 2H), 3.16 (s, 3H), 3.40 (s, 3H), 4.10 (q, $^3J = 7.0$ Hz, 1H), 7.07 (d, $^3J = 8.1$ Hz, 2H), 7.20 (d, $^3J = 8.1$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.8, 22.6, 30.4, 32.6, 41.9, 45.3, 61.4, 127.6, 129.6, 139.4, 140.4, 175.9; FT-IR (ATR-SeZn, cm^{-1}) ν 2954 (s), 1660 (s), 1510 (m), 1462 (s), 1379 (s), 1173 (m), 1119 (m), 1069 (m), 987 (s), 848 (m), 806 (w), 773 (w); MS (ESI) m/z: 521 $[2M+Na]^+$, 272 $[M+Na]^+$, 250 $[M+H]^+$, 161 $[M-C_3H_6NO_2]^+$; HRMS calcd for $C_{15}H_{24}NO_2$: 250.1807, found: 250.1814.

4.2.3. N-Methoxy-N-methyl-2-phenylpropionamide (**3b**)

To a solution of 2-phenylpropionic acid (2.14 mL, 14.3 mmol) in dry THF (60 mL) were added dropwise TEA (6.3 mL, 45.2 mmol) and mesyl chloride (1.3 mL, 16.5 mmol) under Ar atmosphere at 0 °C. After 10 min, N-methoxy-N-methyl ammonium chloride (2.25 g, 23.1 mmol) was added and the mixture was stirred 1 h at RT. The mixture was diluted with water (30 mL) and diethyl ether (30 mL). The two layers were separated and the aqueous layer was extracted (2 ×) with diethyl ether. The combined organic layers were washed with HCl (1 M), NaOH (1 M) and brine, dried over $MgSO_4$, filtered and concentrated under vacuum to obtain 1.653 g (60%) of yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (d, $^3J = 6.9$ Hz, 3H), 3.16 (s, 3H), 3.40 (s, 3H), 4.12 (m, 1H), 7.29 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.9, 32.7, 42.3, 61.4, 127.0, 127.9, 128.9, 142.2, 177.3; FT-IR (NaCl, cm^{-1}) ν 2935 (m), 1662 (s), 1452 (m), 1380 (m), 1176 (w), 986 (m), 754 (w), 700 (m); MS (ESI) m/z: 194 $[M+H]^+$.

4.2.4. N-Methoxy-N-methyl-2-phenylacetamide (**3c**)

To a solution of 2-phenylacetyl chloride (870 μL, 6.45 mmol) in dry CH_2Cl_2 (10 mL) were added N-methoxy-N-methyl ammonium chloride (690 mg, 7.07 mmol) and dropwise TEA (2 mL, 14.3 mmol) under Ar atmosphere at 0 °C. The mixture was stirred 1 h at RT. The mixture was diluted with water (5 mL) and acidified with HCl 2 M (5 mL). The two layers were separated, the organic layer was washed with $NaHCO_3$ (sat.) and brine, dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by flash chromatography (cyclohexane/ethyl acetate 5:1) and 740 mg (64%) of yellow oil were obtained.

R_f 0.17 (cyclohexane/ethyl acetate 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.18 (s, 3H), 3.59 (s, 3H), 3.77 (s, 2H), 7.29 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.2, 39.4, 61.2, 127.1, 128.9, 129.7, 134.8, 172.2; FT-IR (NaCl, cm^{-1}) ν 2939 (m), 1645 (s), 1455 (w), 1246 (w), 1187 (w), 1032 (w), 730 (w); MS (APCI) m/z: 180 $[M+H]^+$, 91 $[C_7H_7]^+$.

4.2.5. 1-(1,3-Dithian-2-yl)-2-(4-isobutylphenyl)-propan-1-one (**4a**)

To a solution of 1,3-dithiane (135 mg, 1.12 mmol) in dry THF (2 mL, ± 2 mL/mmol) was added dropwise n-BuLi (440 μL, 2.5 M in hexane) at −35 °C under Ar atmosphere. The mixture was stirred vigorously for 1 h between −30 °C and −20 °C and was added dropwise to a solution of N-methoxy-N-methyl-2-(4-isobutylphenyl)propanamide (245 mg, 0.98 mmol) in dry THF (3 mL) at −78 °C under Ar atmosphere. The mixture was warmed to RT in 4 h and NH_4Cl (sat., 5 mL) was added to quench the reaction. The mixture was extracted with diethyl ether (3 × 10 mL), the organic layers were combined, dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by flash chromatography (hexane/ethyl acetate 10:1) and 174 mg (57%) of yellow oil were obtained.

R_f 0.75 (hexane/ethyl acetate 10:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (d, $^3J = 6.6$ Hz, 6H), 1.44 (d, $^3J = 6.9$ Hz, 3H), 1.84 (m, 1H), 1.99 (m, 1H), 2.09 (m, 1H), 2.44 (d, $^3J = 7.1$ Hz, 2H), 2.50 and 2.59 (m, 2H), 3.04 (ddd, $^3J = 14.0$ Hz, $^3J = 11.5$ Hz, $^3J = 2.6$ Hz, 1H), 3.51 (ddd, $^3J = 13.8$ Hz, $^3J = 11.8$ Hz, $^3J = 2.9$ Hz, 1H), 4.19 (s, 1H), 4.19 (q, $^3J = 6.9$ Hz, 1H), 7.09 (d, $^3J = 8.3$ Hz, 2H), 7.19 (d, $^3J = 8.3$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.7, 22.7, 25.4, 26.1, 26.3, 30.4, 44.6, 45.3, 49.7, 128.0, 130.1, 137.4, 141.2, 203.1; FT-IR (ATR-SeZn, cm^{-1}) ν 2956 (s), 1709 (s), 1423 (s), 1242 (m), 1029 (s), 848 (w); MS (ESI) m/z: 347 $[M+K]^+$, 331 $[M+Na]^+$, 309 $[M+H]^+$; HRMS calcd for $C_{17}H_{24}ONaS_2$: 331.1166, found: 331.1155.

4.2.6. 1-(1,3-Dithian-2-yl)-2-phenylpropan-1-one (**4b**)

The protocol described for **4a** was applied.

Yield: 55%; R_f 0.23 (cyclohexane/ethyl acetate 95:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (d, $^3J = 6.9$ Hz, 3H), 2.02 (m, 2H), 2.53 (m, 2H), 3.04 (ddd, $^3J = 14.4$ Hz, $^3J = 11.4$ Hz, $^3J = 2.1$ Hz, 1H), 3.48 (ddd, $^3J = 14.4$ Hz, $^3J = 11.2$ Hz, $^3J = 2.7$ Hz, 1H), 4.18 (s, 1H), 4.23 (q, $^3J = 6.9$ Hz, 1H), 7.27 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.3, 25.0, 25.7, 25.9, 44.4, 49.8, 127.3, 127.9, 128.9, 139.9, 202.6; FT-IR (NaCl, cm^{-1}) ν 2929 (m), 1708 (s), 1493 (m), 1452 (m), 1423 (m), 1261 (w), 1066 (w), 1028 (m), 912 (w), 742 (w), 700 (s); MS (APCI, positive mode) m/z: 253 $[M+H]^+$, 217, 215; MS (APCI, negative mode) m/z: 251 $[M-H]^-$, 209, 179, 175.

4.2.7. 1-(1,3-Dithian-2-yl)-2-phenylethan-1-one (**4c**)

The protocol described for **4a** was applied.

Yield: 50%; R_f 0.32 (cyclohexane/ethyl acetate 95:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02 (m, 2H), 2.57 (m, 2H), 3.24 (ddd, $^2J = 14.3$ Hz, $^3J = 11.1$ Hz, $^3J = 2.7$ Hz, 2H), 3.96 (s, 2H), 4.26 (s, 1H), 7.27 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.0, 25.8, 45.3, 46.7, 127.1, 128.6, 129.4, 133.6, 199.8; FT-IR (NaCl, cm^{-1}) ν 2916 (m), 1708 (s), 1496 (w), 1425 (w), 1315 (m), 1257 (m), 1049 (m), 754 (m), 704 (s); MS (APCI) m/z: 239 $[M+H]^+$, 163 $[C_6H_{11}OS_2]^+$, 149 $[C_5H_9OS_2]^+$.

4.2.8. 3-(4-Isobutylphenyl)-1-nitrobutan-2-one (**6a**)

To a vigorously stirred solution of nitromethane (36 μL, 0.66 mmol) in dry DMSO (3.5 mL, 5 mL/mmol) at 10 °C was added *t*-BuOK (164 mg, 1.46 mmol) under Ar atmosphere. The mixture

was raised to RT in 10 min. Then, a solution of 1-(benzotriazol-1-yl)-2-(4-isobutylphenyl)propan-1-one (204 mg, 0.66 mmol) in dry DMSO (3.5 mL, 5 mL/mmol) was added dropwise to the reaction mixture. After 4 h of stirring at RT, the mixture was poured into water (15 mL, ± 4 × 5 mL/mmol), acidified with acetic acid 10% and extracted with ethyl acetate (3 × 15 mL). Organic layers were combined, washed with brine (3 × 40 mL), dried over $MgSO_4$, filtered and concentrated under vacuum. After purification by flash chromatography (hexane/ethyl acetate 10:1), 122 mg (69%) of a yellow oil were obtained.

R_f 0.15 (hexane/ethyl acetate 10:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90 (d, ^3J = 6.6 Hz, 6H), 1.48 (d, ^3J = 6.9 Hz, 3H), 1.85 (m, 1H), 2.47 (d, ^3J = 7.2 Hz, 2H), 3.80 (q, ^3J = 6.9 Hz, 1H), 5.12 (d, ^2J = 14.7 Hz, 1H), 5.20 (d, ^2J = 14.7 Hz, 1H), 7.10 (d, ^3J = 8.1 Hz, 2H), 7.16 (d, ^3J = 8.1 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.2, 22.6, 30.4, 45.3, 51.8, 82.1, 127.9, 130.7, 135.2, 142.3, 196.6; FT-IR (ATR-SeZn, cm^{-1}) ν 2956 (s), 1735 (s), 1562 (s), 1382 (m), 1035 (w), 671 (w); MS (APCI) *m/z*: 248 $[M - H]^-$; HRMS calcd for $C_{14}H_{19}NO_3Na$: 272.1263, found: 272.1259.

4.2.9. 1-(N-Hydroxyimino)-3-(4-isobutylphenyl)butan-2-one (**7a**)

To a stirred solution of $SnCl_2.H_2O$ (230 mg, 1.21 mmol), PhSH (370 μL, 3.59 mmol) and NEt_3 (510 μL, 3.61 mmol) in acetonitrile (4 mL) was added a solution of 3-(4-isobutylphenyl)-1-nitrobutan-2-one (200 mg, 0.80 mmol) in 1 mL of acetonitrile. After 1 h the solvent was removed under reduced pressure, water (10 mL) was added and the resulting solution was extracted with diethyl ether (3 × 10 mL). The combined organic layers were washed with $NaHCO_3$ (sat.), brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/ethyl acetate 10:1); 75 mg (40%) of yellow oil were obtained.

R_f 0.22 (hexane/ethyl acetate 10:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (d, ^3J = 6.6 Hz, 6H), 1.43 (d, ^3J = 7.1 Hz, 3H), 1.83 (m, 1H), 2.42 (d, ^3J = 7.2 Hz, 2H), 4.55 (q, ^3J = 7.1 Hz, 1H), 7.07 (d, ^3J = 8.1 Hz, 2H), 7.14 (d, ^3J = 8.1 Hz, 2H), 7.50 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.0, 22.7, 30.4, 45.3, 47.0, 128.1, 129.8, 137.3, 140.9, 149.1, 198.8; FT-IR (ATR-SeZn, cm^{-1}) ν 3315 (m), 2952 (s), 1674 (s), 1604 (w), 1510 (m), 1456 (s), 1382 (w), 1267 (w), 1169 (m), 1069 (m), 1004 (s), 927 (w), 849 (m), 796 (w), 767 (w), 690 (m); MS (APCI) *m/z*: 234 $[M + H]^+$, 216 $[M - OH]^+$, 201 $[M - OH - CH_3]^+$, 161 $[M - C_2H_2NO_2]^+$; HRMS calcd for $C_{14}H_{19}NO_2Na$: 256.1313, found: 256.1307.

4.2.10. 2-(4-Isobutyl-phenyl)-propionyl chloride (**8a**)

To a suspension of ibuprofen sodium salt (1.47 g, 6.4 mmol) in dry diethyl ether (20 mL) was added dropwise oxalyl chloride (605 μL, 7.05 mmol) at 0 °C under Ar atmosphere and the mixture was stirred for 4 h. The mixture was filtered, concentrated under vacuum, diluted with hexane, filtered again and evaporated under vacuum to obtain 1.32 g (92%) of colourless liquid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90 (d, 6H), 1.59 (d, 3H), 1.86 (m, 1H), 2.47 (d, 2H), 4.09 (q, 1H), 7.15 (d, 2H), 7.20 (d, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.0, 22.7, 30.4, 45.3, 57.4, 127.9, 130.1, 134.8, 142.1, 176.0.

4.2.11. 3-(4-Isobutylphenyl)-1-bromo- and 1-chloro-butan-2-one (**10a**) and (**11a**)

To a solution of 2-(4-isobutyl-phenyl)-propionyl chloride (1.32 g, 5.87 mmol) in diethyl ether (50 mL) at 0 °C was added dropwise a diethyl ether solution of diazomethane (see Arndt, F. *Organic Synthesis Coll.* 1943, *2*, 165) until acyl chloride was perfectly consumed (disappearance of the yellow colour of CH_2N_2 solution). After removing the solvent under vacuum, acetic acid (6 mL) was added. The solution was cooled to 0 °C and aqueous HBr (47%, 1.3 mL) was added dropwise slowly. The mixture was allowed to warm to RT and was stirred for 1 h. Then the solution was poured into cold water (10 mL), neutralized with Na_2CO_3 to pH 7. The solution was extracted with ethyl acetate (3 × 30 mL), the combined organic layers were washed with water, brine then dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by flash chromatography (hexane/diethylether 97:3) to obtain 352 mg (25%) of 3-(4-isobutylphenyl)-1-bromobutan-2-one as a colourless oil and 243 mg (26%) of 3-(4-isobutylphenyl)-1-chlorobutan-2-one as a colourless oil (CAUTION: protect from light for storage).

10a: R_f 0.24 (hexane/diethylether 97:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (d, ^3J = 6.6 Hz, 6H), 1.43 (d, ^3J = 6.9 Hz, 3H), 1.85 (m, 1H), 2.45 (d, ^3J = 7.2 Hz, 2H), 3.78 (d, ^2J = 12.7 Hz, 1H), 3.92 (d, ^2J = 12.7 Hz, 1H), 4.10 (q, ^3J = 6.9 Hz, 1H), 7.12 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.8, 22.5, 30.3, 33.5, 45.1, 49.8, 127.7, 130.1, 136.8, 141.4, 202.0; FT-IR (ATR-SeZn, cm^{-1}) ν 2953 (s), 1732 (s), 1651 (w), 1510 (m), 1464 (m), 1454 (m), 1385 (m), 1282 (w), 1167 (m), 1018 (s), 848 (s), 796 (m); MS (CI/CH_4) *m/z*: 285 [M (^{81}Br) + H]$^+$, 283 [M (^{79}Br) + H]$^+$, 161 [M − $C_2H_2OBr]^+$; MS (ESI) *m/z*: 590 [2 × M (^{81}Br) + Na]$^+$, 588 [M (^{81}Br) + M (^{79}Br) + Na]$^+$, 586 [2 × M (^{79}Br) + Na]$^+$, 307 [M (^{81}Br) + Na]$^+$, 305 [M (^{79}Br) + Na]$^+$; HRMS calcd for $C_{14}H_{19}ONaBr$: 305.0517, found: 305.0517.

11a: R_f 0.19 (hexane/diethylether 97:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (d, ^3J = 6.6 Hz, 6H), 1.43 (d, ^3J = 6.9 Hz, 3H), 1.85 (m, 1H), 2.45 (d, ^3J = 7.2 Hz, 2H), 3.98 (q, ^3J = 6.9 Hz, 1H), 4.02 (d, ^2J = 15.4 Hz, 1H), 4.09 (d, ^2J = 15.4 Hz, 1H), 7.12 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.7, 22.5, 30.3, 45.1, 47.4, 49.9, 127.7, 130.1, 136.6, 141.4, 202.3; FT-IR (ATR-SeZn, cm^{-1}) ν 2955 (s), 1732 (s), 1510 (m), 1464 (m), 1454 (m), 1382 (m), 1333 (w), 1284 (w), 1166 (m), 1111 (m), 1064 (m), 1020 (m), 848 (s), 796 (s), 781 (m); MS (FAB) *m/z*: 240 [M (^{37}Cl)]$^{\bullet+}$, 238 [M (^{35}Cl)]$^{\bullet+}$, 161 [M − $C_2H_2OCl]^+$; HRMS calcd for $C_{14}H_{19}ONaCl$: 261.1022, found: 261.1020.

4.2.12. 3-(4-Isobutylphenyl)-1-nitrate-butan-2-one (**12a**)

To a solution of 3-(4-isobutylphenyl)-1-bromobutan-2-one (268 mg, 0.95 mmol) in dry acetonitrile (5 mL) was added $AgNO_3$ (340 mg, 2.0 mmol) at RT and the mixture was stirred for 40 h. The mixture was filtered on a flash silice pad and concentrated under vacuum to obtain 241 mg (96%) of yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (d, ^3J = 6.6 Hz, 6H), 1.45 (d, ^3J = 7.0 Hz, 3H), 1.85 (m, 1H), 2.46 (d, ^3J = 7.2 Hz, 2H), 3.78 (q, ^3J = 7.0 Hz, 1H), 4.82 (d, ^2J = 17.5 Hz, 1H), 4.91 (d, ^2J = 17.5 Hz, 1H), 7.13 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.0, 22.5, 30.3, 45.1, 49.8, 73.0, 127.7, 130.1, 135.8, 141.7, 201.6; FT-IR (ATR-SeZn, cm^{-1}) ν 2955 (s), 1736 (s), 1645 (s), 1512 (m), 1454 (m), 1404 (m), 1365 (m), 1283 (s), 1130 (w), 1058 (w), 1008 (m), 970 (m), 941 (w), 847 (s), 800 (m), 752 (w); MS (CI/CH_4) *m/z*: 265 [M]$^{\bullet-}$, 218 [M − $HNO_2]^-$, 161 [M − $C_2H_2OBr]^+$; MS (ESI) *m/z*: 288 [M + Na]$^+$; HRMS calcd for $C_{14}H_{19}NO_4Na$: 288.1212, found: 288.1210.

4.2.13. 1-Bromo-3-phenylpropan-2-one (**10c**)

To a solution of 2-phenylacetyl chloride (670 μL, 5.06 mmol) in diethyl ether (15 mL) at 0 °C was added a diethyl ether solution of diazomethane until acyl chloride was perfectly consumed. After removing the solvent under vacuum, acetic acid (6 mL) was added. The solution was cooled to 0 °C and aqueous HBr (47%, 1 mL) was added dropwise slowly. The mixture was allowed to warm to RT and was stirred for 1 h. Then the solution was poured into cold water (10 mL), neutralized with Na_2CO_3 to pH 7. The solution was extracted with ethyl acetate (3 × 30 mL), the combined organic layers were washed with water, brine then dried over $MgSO_4$, filtered and concentrated under vacuum to obtain 818 mg of a colourless oil. As the NMR spectrum showed only two products, 1-bromo-3-phenylpropan-2-one as major product (∼90%) and 1-chloro-3-phenylpropan-2-one as minor product (<10%), the crude material was used without any purification.

10c: Yield: 69%; R_f 0.41 (hexane/diethylether 97:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.92 (s, 2H), 3.95 (s, 2H), 7.22–7.38 (m, 5H); ^{13}C

NMR (75 MHz, $CDCl_3$) δ 33.4, 46.7, 127.4, 129.4, 128.9, 133.1, 199.3; FT-IR (NaCl, cm^{-1}) ν 3059 (m), 3030 (m), 2936 (s), 1725 (s), 1495 (m), 1391 (m), 1181 (m), 1045 (s), 739 (m); MS (CI/CH_4) *m/z*: 215 [M (^{81}Br) + H]$^+$, 213 [M (^{79}Br) + H]$^+$.

11c: Yield: 8%; R_f 0.36 (hexane/diethylether 97:3); ^1H NMR (300 MHz, $CDCl_3$) δ 3.90 (s, 2H), 4.12 (s, 2H), 7.22–7.38 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 45.1, 52.2, 127.4, 128.7, 129.9, 132.2, 194.1; MS (CI/CH_4) *m/z*: 171 [M (^{37}Cl) + H]$^+$, 169 [M (^{35}Cl) + H]$^+$.

4.2.14. 2-Hydroxy-3-phenyl-2-propenal (*5′c*)

A solution of 1-bromo-3-phenylpropan-2-one (810 mg, 3.8 mmol) in DCM (40 mL) was refluxed in the presence of pyridine (1.3 mL) under Ar atmosphere for 2 h. Concentration of the solution under vacuum afforded the pyridinium salt as a solid. The solid was dissolved in water (40 mL) and N,N-dimethyl-4-nitrosoaniline (600 mg, 4.00 mmol) was added at 0 °C followed by 1 M aqueous NaOH (4 mL). The resulting suspension was allowed to reach RT and stirred for 2 h with sonicating occasionally. The suspension was filtered and the solid was suspended into 10% aqueous H_2SO_4 (50 mL) at 0 °C. The suspension was warmed to RT and stirred 2 h with sonicating occasionally. The suspension was extracted with diethyl ether (2 × 50 mL) and the combined organic layers were washed with water (2×) and brine. The solution was dried with Na_2SO_4, filtered and concentrated under vacuum to obtain 347 mg (62%) of a brown solid.

^1H NMR (300 MHz, $CDCl_3$) δ 6.18 (s, 1H), 6.60 (s, 1H, OH), 7.40 (m, 3H), 7.85 (d, ^3J = 7.8 Hz, 1H), 9.25 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 122.9, 128.9, 129.5, 130.6, 133.7, 148.8, 188.4; MS (APCI, positive mode) *m/z*: 181, 167, 149 [M + H]$^+$, 131 [M − OH]$^+$, 120 [M − CO]$^+$; MS (APCI, negative mode) *m/z*: 179, 147 [M − H]$^-$, 117; MS (ESI) *m/z*: 181, 167, 149 [M + H]$^+$.

4.2.15. 2-Phenylimidazo[1,2-a]pyrazin-3-(7H)-one (*17a*)

2-Aminopyrazine hydrochloride (**14a**) was prepared from commercial 2-aminopyrazine (solution in ether) and 2 M HCl (5 eq.). The precipitated salt was filtered off and washed with ether. A solution of 2-aminopyrazine hydrochloride (133 mg, 1.02 mmol) and phenylglyoxal (308 mg, 2.02 mmol) in ethanol (4 mL) was heated (at 80 °C) in Microwave (Mw) oven at 150 W for 5 min, then at 75 W of 4 h. After concentration under vacuum, the residue was dissolved in methanol (0.5 mL) and the product was precipitated by addition of diethyl ether to furnish 165 mg of a dark red solid (66%).

^1H NMR (500 MHz, CD_3OD) δ 7.49 (m, 3H), 7.64 (d, ^3J = 5.6 Hz, 1H), 8.23 (d, ^3J = 5.6 Hz, 1H), 8.25 (d, ^3J = 6.9 Hz, 2H), 8.79 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 116.1, 119.4, 128.2, 129.9, 130.0, 130.6, 130.9, 132.0, 138.5, 146.7; FT-IR (ATR-SeZn, cm^{-1}) ν 3096 (s), 2923 (s), 1668 (m), 1573 (s), 1497 (s), 1448 (m), 1367 (w), 1288 (w), 1230 (s), 1124 (s), 1088 (w), 1070 (w), 1000 (w), 906 (m), 775 (s), 696 (s); MS (ESI) *m/z*: 212 [M + H]$^+$; HRMS calcd for $C_{12}H_9N_3ONa$: 234.0643, found: 234.0638; UV–vis (MeOH, 10^{-4} M): λ_m (nm) 466, 287.

4.2.16. 2-Phenyl-6-p-hydroxyphenylimidazo[1,2-a]pyrazin-3(7H)-one (*17b*)

To a stirred solution of 2-amino-5-(*p*-hydroxyphenyl)-1,4-pyrazine (180 mg, 0.96 mmol) and phenylglyoxal (306 mg, 1.97 mmol) in ethanol (3 mL) was added concentrated HCl (36%) (310 μL) and the mixture was heated at reflux overnight. The reaction mixture was concentrated under vacuum. The product was precipitated by addition of diethyl ether to the residue dissolved in a minimum of methanol. After two precipitations, 225 mg (69%) of a dark red solid were obtained.

^1H NMR (500 MHz, DMSO-d_6) δ 6.89 (d, ^3J = 8.6 Hz, 2H), 7.36 (t, ^3J = 7.9 Hz, 1H), 7.47 (t, ^3J = 7.9 Hz, 2H), 7.65 (d, ^3J = 8.6 Hz, 2H), 7.99 (s, 1H), 8.38 (d, ^3J = 7.9 Hz, 2H), 8.41 (s, 1H); ^1H NMR (300 MHz, CD_3OD) δ 6.94 (d, ^3J = 8.4 Hz, 2H), 7.49–7.55 (m, 3H), 7.71 (d, ^3J = 8.4 Hz, 2H), 8.12 (d, ^3J = 7.2 Hz, 2H), 8.42 (s, 1H), 8.89 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 108.1, 115.8, 122.7, 126.1, 127.5, 127.6, 128.4, 128.5, 128.6, 129.2, 129.4, 132.5, 147.8, 158.5; ^{13}C NMR (75 MHz, CD_3OD) δ 111.9, 117.2, 123.7, 128.2, 128.8, 129.5, 129.9, 130.2, 131.2, 133.0, 137.2, 161.2 (two quaternary carbons not detected); FT-IR (ATR-SeZn, cm^{-1}) ν 3149 (m), 2964 (m), 1662 (m), 1608 (s), 1516 (s), 1448 (m), 1346 (w), 1218 (s), 1155 (s), 1016 (w), 920 (w), 837 (m), 769 (m), 690 (w); MS (ESI) *m/z*: 304 [M + H]$^+$.

4.2.17. 2-Phenylimidazo[1,2-a]pyrazin-3(7H)-imine (*18a*)

A solution of 2-aminopyrazine chlorohydrate (134 mg, 1.02 mmol) and phenylglyoxal aldoxime (315 mg, 2.11 mmol) in ethanol (4 mL) was heated (at 80 °C) in a Mw oven at 150 W for 5 min, then at 75 W overnight. The reaction mixture was concentrated under vacuum. The product was precipitated by adding diethyl ether to the residue dissolved in a minimum of methanol. After two precipitations, 159 mg (64%) of a dark red solid were obtained.

M.p.: >225 °C, decomposition; ^1H NMR (300 MHz, CD_3OD) δ 7.51 (m, 3H), 7.65 (d, ^3J = 5.6 Hz, 1H), 7.93 (d, ^3J = 7.8 Hz, 2H), 8.30 (d, ^3J = 5.6 Hz, 1H), 8.79 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 116.1, 119.2, 128.6, 130.2, 130.8, 131.8, 132.6, 133.4, 137.4, 141.9; FT-IR (ATR-SeZn, cm^{-1}) ν 3120 (s), 1633 (s), 1556 (m), 1467 (s), 1404 (s), 1296 (w), 1261 (w), 1217 (m), 1130 (w), 1060 (s), 1022 (w), 905 (w), 775 (m), 696 (m); MS (ESI) *m/z*: 211 [M + H]$^+$; HRMS calcd for $C_{12}H_{11}N_4$: 211.0984, found: 211.0983; UV–vis (MeOH, 10^{-4} M): λ_m (nm) 429, 269.

4.2.18. 2-Phenyl-6-p-hydroxyphenylimidazo[1,2-a]pyrazin-3(7H)-imine (*18b*)

To a solution of 2-amino-5-(*p*-hydroxyphenyl)-1,4-pyrazine (181 mg, 0.97 mmol) and phenylglyoxal aldoxime (301 mg, 2.02 mmol) in ethanol (4 mL) was added concentrated HCl (36%, 310 μL) and the mixture was heated (at 80 °C) in a Mw oven at 150 W for 5 min, then at 100 W for 4 h. The reaction mixture was concentrated under vacuum. The product was precipitated by addition of diethyl ether to the residue dissolved in a minimum of methanol. After two precipitations, 98 mg (30%) of a dark red solid were obtained.

M.p.: >250 °C, decomposition; ^1H NMR (300 MHz, CD_3OD) δ 6.98 (d, ^3J = 8.7 Hz, 2H), 7.49 (t, ^3J = 7.5 Hz, 1H), 7.55 (t, ^3J = 7.5 Hz, 2H), 7.68 (d, ^3J = 8.7 Hz, 2H), 7.93 (d, ^3J = 7.5 Hz, 2H), 8.50 (s, 1H), 8.79 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 111.6, 117.3, 123.2, 128.6, 129.5, 130.3, 130.9, 131.8, 132.0, 132.7, 134.5, 136.6, 140.2, 161.2; FT-IR (ATR-SeZn, cm^{-1}) ν 2927 (s), 2854 (m), 1652 (m), 1612 (m), 1452 (m), 1252 (m), 1111 (s), 1056 (m), 837 (s), 779 (m), 698 (m); MS (ESI, positive mode) *m/z*: 303 [M + H]$^+$; MS (ESI, negative mode) *m/z*: 301 [M − H]$^-$; HRMS calcd for $C_{18}H_{15}N_4O$: 303.1246, found: 303.1244.

4.3. Evaluations

4.3.1. Inhibition of lipid peroxidation

A micellar solution of linoleic acid (4 mM) containing Tween–20 as surfactant in phosphate buffer (50 mM, pH 7.4) and EDTA (0.1 mM) is incubated at 37 °C with 2 mM AAPH (2,2′-azo-*bis* (2-amidinopropane)-dihydrochloride) as the free-radical generator. The production of conjugated dienes by the peroxidation of linoleate is monitored continuously at 234 nm using a wavelength tuneable microplate spectrophotometer reader (SpectraMAX 190, Molecular Devices). Antioxidant efficiency of the tested compound was evaluated by the lag phase duration until onset of peroxidation. Tested compounds were initially dissolved in ethanol (10^{-2}M) then in phosphate buffer (50 mM, pH 7.4); they were assayed at final concentrations of 1.25, 2.5, 5, 10 and 20 μM.

4.3.2. Anti-inflammatory assay

The human intestinal Caco-2 cells (ATCC, Rockville, MD), used between passages 30 and 50 were routinely grown in DMEM containing 4.5 g/l glucose, 25 mM Hepes, 10% (v/v) fetal calf

serum (FCS) (Hyclone Perbio-Sciences, Erembodegem, BE), 2% (v/v) L-glutamine 200 mM and 1% (v/v) nonessential amino acids (NEAA) (Invitrogen, Carlsbad, CA). For the experiments, cells were seeded at a density of 40×10^3 cells/cm^2 in 96-well plates (Nunc, Roskilde, DK) and cultivated until 7 days post-confluence with culture medium changes three times per week. The cells were incubated with the pro-inflammatory stimulus, IL-1β at 25 ng/ml in DMEM containing 0.5% (v/v) FCS, alone (control) or in presence of the tested compound at 50 μM. After 24 h treatment, the extracellular media were collected and processed for IL-8 quantification with the Human IL-8 ELISA kit (BD Biosciences Pharmingen, San Diego, CA), according to the manufacturer's instructions.

4.3.3. FAAH and MGL inhibition

Recombinant human FAAH (in 165 μL of Tris–HCl, 100 mM, pH 7.4) was added on ice to glass tubes (5 μg protein/tube) containing either drugs or DMSO (10 μL). Hydrolysis was initiated by adding 25 μL of [^{3}H]-anandamide (50,000 dpm, 2 μM final concentration) in Tris–HCl containing 0.1% BSA and tubes were incubated for 10 min at 37 °C in a shaking water bath. Reactions were stopped by rapidly placing the tubes on ice and adding 400 μL of ice-cold MeOH–$CHCl_3$ (1:1 v/v). Following centrifugation (1700 g, 5 min, 4 °C) the [^{3}H]-ethanolamine in the aqueous layer was recovered (200 μL) and counted by liquid scintillation. Blanks (controls for chemical hydrolysis) were prepared (buffer instead of FAAH) and the values systematically subtracted. MGL activity was assayed in Tris–HCl pH 8, according to a similar protocol and using [^{3}H]-oleoylglycerol (50,000 dpm, 10 μM final concentration) as substrate.

4.3.4. Data analysis

Results were expressed as means ± S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA) coupled with Scheffe's post-hoc test. The computer program was Systat 5.2.1 (Systat Inc., Evanston, IL). Results with a two-sided p value < 0.05 were considered statistically significant.

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