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Article

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Discovery of Novel Nonsteroidal Anti-Inflammatory Drugs and Carbonic Anhydrase Inhibitors Hybrids (NSAIDs−CAIs) for the Management of Rheumatoid Arthritis

Ozlem Akgul,[1,4] Lorenzo Di Cesare Mannelli,[2] Daniela Vullo,[3] Andrea Angeli, [4] Carla Ghelardini,[2] Gianluca Bartolucci,[4] Abdulmalik Saleh Alfawaz Altamimi,[5] Andrea Scozzafava,[3] Claudiu T. Supuran[4*] and Fabrizio Carta[4*]

[1] Ege University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry Bornova-İzmir-Turkey 35100

[2] Dept. of Neuroscience, Psychology, Drug Research and Child Health - NEUROFARBA - Pharmacology and Toxicology Section, University of Florence, 50139 Florence, Italy.

[3] University of Florence, Polo Scientifico, Laboratorio di Chimica Bioinorganica, Rm. 188, Via della Lastruccia 3, 50019 Sesto Fiorentino (Florence), Italy.

[4] University of Florence, NEUROFARBA Dept., Sezione di Scienze Farmaceutiche, Via Ugo Schiff 6, 50019 Sesto Fiorentino (Florence), Italy

[5] Department of Pharmaceutical Chemistry, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173, Alkharj, 11942, Saudi Arabia.

Keywords: Carbonic Anhydrase Inhibitors (CAIs), Non Steroidal Anti-Inflammatory Drugs (NSAIDs), Metalloenzymes, Rheumatoid Arthritis (RA), Hybrids.

Abstract. Herein we report the design as well as the synthesis of a new series of dual hybrid compounds consisting of the therapeutically used Nonsteroidal-Anti-Inflammatory Drugs (NSAIDs ;i.e. indometacin, sulindac, ketoprofen, ibuprofen, diclofenac, ketorolac, etc., cyclooxygenase inhibitors) and the Carbonic Anhydrase Inhibitor (CAIs) fragments of the sulfonamide type. Such compounds are proposed as new tools for the management of ache symptoms associated to rheumatoid arthritis (RA) and related inflammation diseases. The majority of the hybrids reported were effective in inhibiting the ubiquitous human (h) CA I and II as well as the RA over-expressed hCAs IX and XII isoforms, with K_I values comprised in the low-medium nanomolar ranges. The antihyperalgesic activity of selected compounds was assessed by means of the paw-pressure and incapacitance tests using an *in vivo* RA model, and among them the hybrids **6B** and **8B** showed potent antinociceptive effects lasting up to 60 min after administration.

Introduction.

We recently reported as *proof-of-concept* study that hybrid small molecules composed of the Nonsteroidal-Anti-Inflammatory-Drugs and Carbonic-Anhydrase-Inhibitors (NSAIDs−CAIs) fragments were more effective, in terms of potency and time efficacy when compared to the reference drug ibuprofen, for the management of ache symptoms associated to inflammatory diseases such as rheumatoid arthritis (RA).[1] This study was mainly supported by the data obtained in an *in vivo* model of RA, which in turn was based on preliminary reports accounting for the contribution of several Carbonic Anhydrases (CAs, EC 4.2.1.1) to the inflammatory processes.[2-5] The serum of RA affected patients exhibited abnormal expressions of the human (h) CAs I, III and IV isoforms.[2-5] Our group recently demonstrated the over-expression of CA IX and XII isoforms in the synovium obtained from patients affected by Juvenile Idiopathic Arthritis (JIA).[6]

The main reaction catalyzed by CAs is the reversible hydration of carbon dioxide (CO_2 + $H_2O \Leftrightarrow H^+ + HCO_3^-$; eq. 1).[7] The involvement of this reaction in a plethora of physiological events (both at the cellular as well as tissue level) is well established.[7] However this transformation when uncatalyzed is not able to meet the physiological needs of the cells, which is the reason why many isoforms of the CAs have evolved.[7a-c] Thus, it is not surprising that any disruption of this equilibrium is often associated to diseases, including RA.[1,7a] In this context the CAs over-expression is expected to increase the ionic species concentrations (H^+ and HCO_3^-) at cellular level, and since the bicarbonate ions are essential components in cells and are immediately recovered, a local extracellular acidosis is immediately established. Pioneer studies demonstrated that intensity of inflammation processes and the ache-related symptoms in RA affected patients were inversely correlated with tissutal pH values.[8,9] In addition tissue acidosis, was found to be unfavorable for the progression of both humoral and cellular immunity processes. [10]

Despite the enormous progresses achieved in the treatment of RA, still no effective cure for the disease was obtained. To date the pharmacological approaches against RA are based on the use of *i*) Disease-Modifying-Antirheumatic-Drugs (DMARDs), JAK-inhibitors, corticosteroids and biologics

which act slowing down the course of the disease, and *ii*) the NSAIDs which act to ease the symptoms of the inflammation. [11, 12] As a result, many scientific reports account for the discovery of novel diagnostic tools, biological targets, as well as for a variety of lead compounds.[13,14] In this context herein we report a large series of new small molecule hybrids of the NSAID-CAI type, which differ from the previously ones for the CAI fragment (of the sulfonamide type in this article, versus the coumarin chemotype in the previous report)[1] as well as for the length of the linker connecting the two fragments. We assessed the ability of the compounds reported to inhibit the activity of the most relevant hCAs as well as to reduce the RA ache related symptoms by means of *in vitro* enzymatic assays and in *in vivo* animal models of the disease, respectively. Our aim is to further support our previous studies on hCAs as valid and robust pharmacological targets for the treatment of RA inflammatory diseases, and for this reason no cyclooxygenase (COX) activities of the hybrids were investigated in this study, considering the fact that the COOH moiety of the NSAID is essential for their inhibition of COX (and in our reported compounds is transformed in an amide).[15]

Results and Discussion.

Design and Synthesis of Compounds. In analogy to our earlier article,[1] we report the design and synthetic procedures of NSAID-CAI small molecule hybrids consisting of a clinically used NSAID fragment connected to a CAI of the sulfonamide type, by means of a linear alkyl spacer (**Figure 1)**.

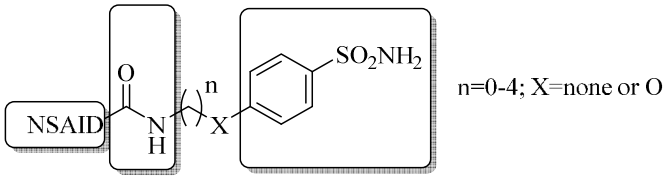
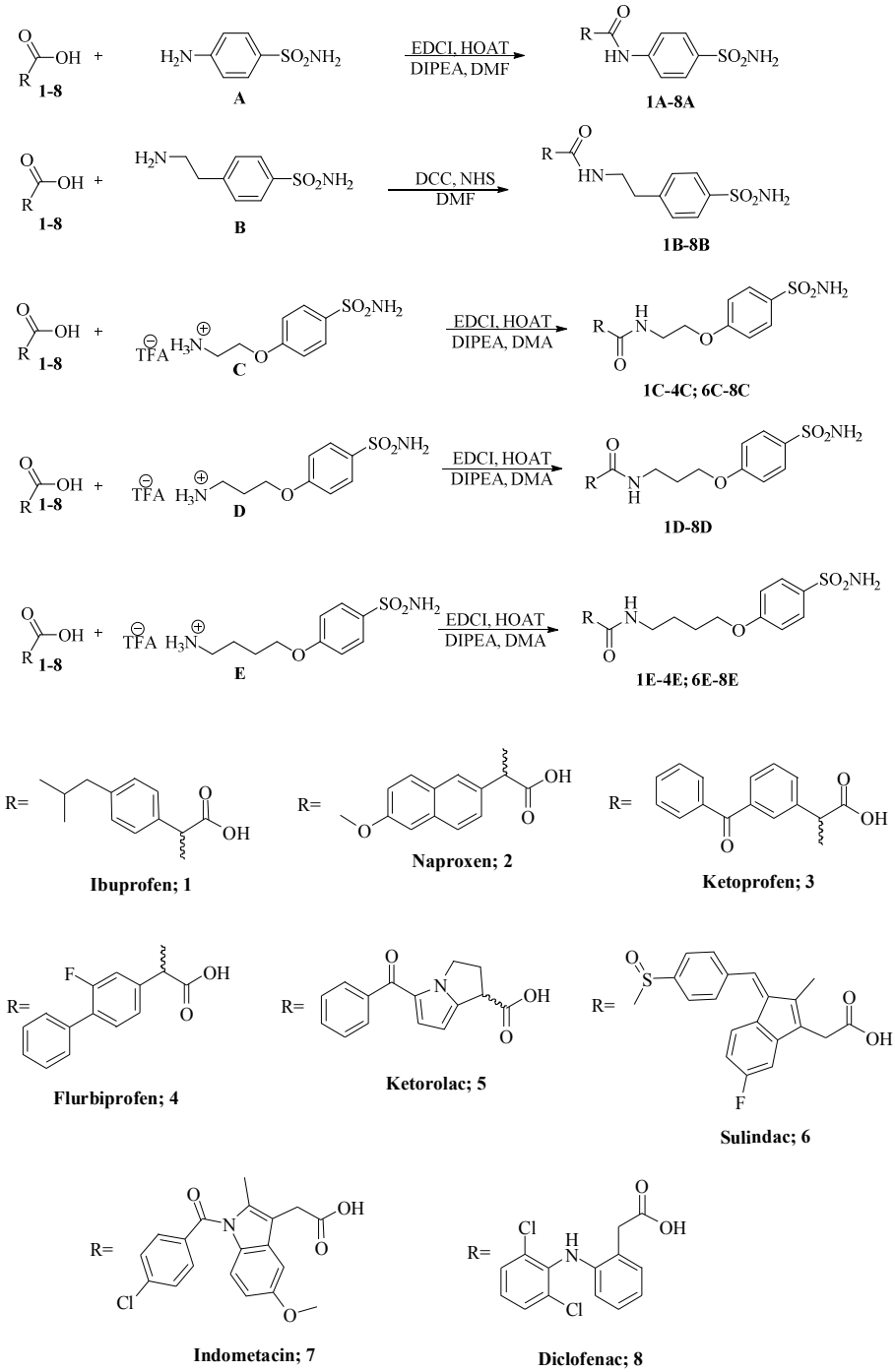


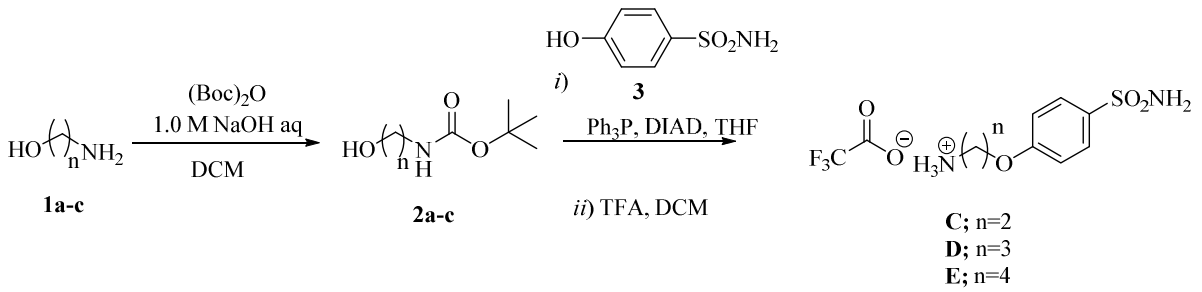
Figure 1. Design of the NSAID-CAI hybrids.

In our synthetic strategy we made use of the NSAID carboxylic acid moiety in order to efficiently install an amide bond, which also represents a virtually unique single break point within the entire hybrid molecules. As for the linker, we focused on linear full carbon or ether type fragments which are biocompatible and of easy access from the synthetic view point (**Scheme 1**).



Scheme 1. General synthetic procedure for the synthesis of NSAID-CAIs.

As above reported the compound series was obtained by means of well established amide coupling reactions using the sulfanilamide **A** and 4-(2-amino-ethyl)-benzenesulfonamide **B** for the **1A-8A** and **1B-8B** series respectively, whereas the intermediates **C-D** and **E** were obtained according to the synthetic procedure depicted in **Scheme 2**. [16]



Scheme 2. Synthetic procedure for the synthesis of **C, D** and **E** intermediates. [16]

All final compounds obtained and their intermediates were properly characterized by means of ^{1}H, ^{13}C, ^{19}F-NMR spectroscopy, HRMS and were ≥ 95% HPLC pure (see Experimental Section for details).

Carbonic Anhydrase Inhibition Studies. All compounds herein reported and the standard CAI acetazolamide (**AAZ**) were assessed for their inhibition properties against the relevant hCA isoforms (i.e. I, II, IV, IX and XII) using the stopped-flow carbon dioxide hydration assay (Table 1). [17]

Table 1. hCA I, II, IV, IX, and XII *in vitro* inhibition data with NSAID-CAI compounds by the Stopped-Flow CO_2 hydrase assay. [17]

	K_I (nM)*

Compound	hCA I	hCA II	hCA IV	hCA IX**	hCA XII
1A	40.4	32.4	218.1	13.9	33.0
2A	7.9	4.2	653.2	12.6	28.5
3A	19.4	4.6	49.1	5.1	2.9
4A	58.5	5.5	717.7	9.2	40.0
5A	16.1	3.6	1495	49.1	24.5
6A	73.3	23.3	519.1	61.3	27.2
7A	208.6	92.5	4375	58.7	40.8
8A	68.2	89.2	179.3	5.3	7.0
1B	79.3	9.2	7298	72.2	93.5
2B	19.1	5.2	6028	12.9	91.9
3B	8.1	6.5	3005	39.3	54.0
4B	42.5	7.8	3053	38.1	92.8
5B	534.4	78.9	3877	94.9	90.3
6B	64.9	27.3	6857	62.5	94.4
7B	494.0	95.8	8365	62.5	92.7
8B	68.4	43.7	8654	43.3	90.2
1C	267.9	7.4	59.9	26.2	7.0
2C	73.8	7.8	73.4	21.2	7.5
3C	21.3	6.7	77.7	76.5	8.6

4C	233.8	7.0	354.7	244.4	68.6
6C	87.0	7.5	5996	103.2	65.0
7C	375.9	4.2	1598	177.2	44.0
8C	769.7	186.8	5483	144.4	57.6
1D	59.4	25.2	63.7	10.4	6.5
2D	57.6	5.8	219.8	18.3	19.4
3D	7.1	5.0	77.2	12.5	5.2
4D	54.2	6.3	301.3	17.5	43.3
5D	44.4	5.0	295.9	18.3	7.3
6D	4.9	5.4	811.0	141.0	35.0
7D	81.7	84.3	522.6	202.4	84.1
8D	63.4	46.2	672.8	137.1	59.2
1E	48.4	284.7	2804	14.0	6.6
2E	71.4	47.1	2693	16.3	4.2
3E	20.9	6.1	92.8	8.0	4.1
4E	242.7	86.7	7264	45.5	94.1
6E	93.8	66.4	7737	22.0	5.0
7E	86.5	8.1	8136	7.7	64.0
8E	341.8	78.8	8023	51.9	93.8
AAZ	250.0	12.1	74.0	25.8	5.7

[*]Means from three diverse assays. The errors were comprised within ±5−10% of the values reported (data not shown).[**] Catalytic domain.

On the basis of the kinetic data reported in Table 1 on the NSAID-CAIs, the following Structure-Activity-Relationships (SARs) can be drawn.

Compounds **1A-8A**.

i) The in vitro kinetic data showed that the Naproxen derivative **2A** was the most potent within the series against the hCA I isoform (K_I 7.9 nM), followed by the ketorolac **5A** and ketoprofen **3A** (K_Is 16.1 and 19.4 nM respectively). Medium inhibition potencies, with K_I values spanning between 40.4-73.3 nM, were obtained for the ibuprofen **1A**, flurbiprofen **4A**, sulindac **6A** and diclofenac **8A**, whereas the indometacin benzenesulfonamide **7A** resulted far the less potent (K_I 208.6 nM) and similar to the standard CAI **AAZ** (K_I 250 nM).

ii) As for the ubiquitous hCA II isoform, better inhibition results were obtained for the **1A-8A** series when compared to the hCA I. As reported in Table 1, compounds **2A-5A** showed K_I values comprised between 5.5 and 3.6 nM, thus up to 3.4 fold stronger when compared to **AAZ** (K_I 12.1 nM). The ibuprofen **1A** and sulindac **6A** derivatives were far less potent (K_Is 32.4 and 23.3 nM respectively) followed by the indometacin **7A** and diclofenac **8A** derivatives (K_Is 92.5 and 89.2 nM respectively).

iii) The hCA IV isoform, which is of particular interest as it is widely expressed in the kidneys and heart tissues, showed that only the ketoprofen derivative **3A** was a valuable inhibitor, with a K_I value 1.51 fold lower when compared to the reference CAI **AAZ** (K_Is 49.1 and 74.0 nM respectively). Conversely all the other compounds of the series showed weak inhibitory properties (K_I comprised between 179.3 and 4375 nM).

iv) Compounds **3A**, **4A** and **8A** were very potent inhibitors of the tumor associated hCA IX isoform with K_I values in the low nanomolar range (K_Is 5.1, 9.2 and 5.3 nM respectively), thus more potent

when compared to **AAZ** (K_I 25.8 nM). Weaker inhibition potencies were reported for the ibuprofen **1A** and naproxen **2A** derivatives (K_Is 13.9 and 12.6 nM respectively), followed by the ketorolac **5A**, sulindac **6A** and indometacin **7A** (K_Is 49.1, 61.3 and 58.7 nM respectively). The latter were also weaker in inhibiting the hCA IX isoform when compared to the standard **AAZ** (K_I 25.8 nM).

v) Among the **1A-8A** series, the ketoprofen **3A** and the diclofenac **8A** derivatives showed low nanomolar inhibition values against the second tumor associated hCA (i.e. isoform XII) and comparable to **AAZ**. As reported in Table 1 the first resulted 1.97 fold stronger when compared to **AAZ** (K_Is 2.9 and 5.7 nM respectively), whereas the latter showed a K_I inhibition value slightly higher (K_I 7.0 nM). As for the other compounds, similar inhibition values and ranging between 27.2 and 33.0 nM were obtained for the ibuprofen **1A**, naproxen **2A**, ketorolac **5A** and the sulindac **6A**. Finally the flurbiprofen **4A** and the indometacin **7A** derivatives resulted the less potent inhibiting the hCA XII, and again with close-matching K_Is (40.0 and 40.8 nM respectively).

Compounds **1B-8B**.

i) Among the ethylaminobenzene sulfonamide series, the hCA I isoform resulted potently inhibited from the ketoprofen **3B** derivative (K_I 8.1 nM) followed by the naproxen **2B** compound (K_I 19.1 nM) and thus they resulted more effective in comparison to the CAI **AAZ** (K_I 250.0 nM). Medium inhibition values were obtained for the ibuprofen **1B**, flurbiprofen **4B**, sulindac **6B** and diclofenac **8B** derivatives (K_Is 79.3, 42.5, 64.9 and 68.4 nM respectively). The ketorolac **5B** and indometacin **7B** compounds resulted ineffective against the hCA I (K_Is 534.4 and 494.0 nM respectively).

ii) Better inhibition results were obtained for the hCA II isoform. As reported in Table 1, compounds **1B-4B** were all low nanomolar inhibitors of this isoform with K_Is spanning between 5.2 and 9.2 nM, and among them the naproxen **3B** derivative was the most potent as being 2.33 fold more potent as compared to the CAI **AAZ** (K_I 12.1 nM). Within the **1B-8B** series the sulindac **6B** and the diclofenac **8B** resulted medium potency inhibitors (K_Is 27.3 and 43.7 nM respectively), whereas the remaining ketorolac **5B** and indometacin **7B** were the less potent.

iii) Compared to the **1A-8A** compounds previously discussed, the series **1B-8B** was ineffective in inhibiting the hCA IV isoform. The corresponding K_I values are reported in Table 1 and were all comprised between 3.0 and 8.7 μM.

iv) The transmembrane and tumor related hCA IX was efficiently inhibited only from the naproxen derivative **2B** being 2 fold more potent of the standard CAI **AAZ** (K_Is 12.9 and 25.8 nM respectively). Then the ketoprofen **3B**, the flurbiprofen **4B** and the diclofenac **8B** containing compounds showed medium nanomolar inhibition values (K_Is 39.3, 38.1 and 43.3 nM respectively), whereas all remaining hybrids in the series resulted low active in inhibiting this isoform (K_Is comprised between 62.5 and 94.9 nM).

v) Among the compound series **1B-8B**, the ketoprofen **3B** derivative was the most potent as hCA XII inhibitor and also resulted 9.5 fold less potent when compared to the reference **AAZ** (K_Is 54.0 and 5.7 nM respectively). The remaining derivatives were all high nanomolar hCA XII inhibitors with K_Is spanning between 90.2 and 94.4 nM.

Compounds **1C-4C** *and* **6C-8C**.

i) Data in table 1 showed that only the ketoprofen **3C** derivative was potent against the hCA I isoform with a K_I value 10 fold higher when compared to **AAZ** (K_Is 21.3 and 250 nM respectively), followed by the naproxen **2C** and the sulindac **6C** which resulted both high nanomolar inhibitors of this isoform (K_Is 73.8 and 87.0 nM). All the remaining compounds in the series were inactive in inhibiting the hCA I with K_I values comparable or higher to the reference **AAZ**.

ii) In analogy to the compound series previously discussed, also in this case the hCA II resulted particularly inhibited. Data in table 1 showed that among this series only the diclofenac **8C** was ineffective against the hCA II (K_I 186.8 nM) whereas all the others were nanomolar inhibitors with K_Is ranging between 4.2 and 7.8 nM, thus slightly more potent when compared to the **AAZ** (K_I 12.1 nM).

iii) As for hCA IV the Ibuprofen **1C** resulted the most potent inhibitor within the series with a K_I being slightly more potent (1.24 fold) when compared to the reference **AAZ** (K_Is 59.9 and 74.0 nM respectively). The naproxen **2C** and the ketoprofen **3C** derivatives (73.4 and 77.7 nM respectively) showed each other similar inhibition potencies whose values were analogous to the reference compound **AAZ** (K_I 74.0 nM). Finally, all the other compounds in the series resulted inactive against the hCA IV (K_Is comprised between 354.7 and 5996 nM).

iv) The in vitro kinetic data reported in Table 1 showed that the hCA IX isoform was efficiently inhibited from the ibuprofen **1C** and naproxen **2C** derivatives whose K_I values were quite similar to the reference **AAZ** (K_Is 26.2, 21.2 and 25.8 nM respectively). Then the ketoprofen derivative **3C** resulted 2.97 fold less potent in inhibiting the hCA IX isoform when compared to the standard CAI (K_Is 76.5 and 25.8 nM respectively), followed by all the remaining hybrids which were weak hCA IX inhibitors with K_I values ranging between 103.2 and 244.4 nM.

v) The second tumor associated hCA isoform (XII) in analogy to the previously discussed one resulted potently inhibited from the ibuprofen **1C** and the naproxen **2C** derivatives with K_I values quite similar each other (7.0 and 7.5 nM) and slightly less effective when compared to the reference **AAZ** (K_I 5.7 nM). Again the Ketoprofen **3C** showed lower inhibition potency (K_I 8.6 nM) followed by the remaining compounds in the series with K_I values comprised between 44.0 and 68.6 nM.

Compounds **1D-8D**.

i) Within the **1D-8D** series tested, the ketoprofen **3D** and the sulindac **6D** were highly potent in inhibiting the hCA I isoform with K_Is values comprised within the low nanomolar range (7.1 and 4.9 nM respectively), and thus more potent when compared to the reference **AAZ** (K_I 250 nM). All the other compounds in the series resulted medium potency inhibitors of this isoform. Among them the ketorolac **5D** derivative was the most potent (K_I 44.4 nM) followed by the flurbiprofen **4D**, the naproxen **2D** and the ibuprofen **1D** (K_Is 54.2, 57.6 and 59.4 nM respectively). The least potent inhibitors of the hCA I isoform were the dichlofenac **8D** and the indometacin **7D** which showed K_Is in the high nanomolar (K_Is 63.4 and 81.7 nM respectively).

ii) The highly abundant hCA II isoform was efficiently inhibited from the naproxen **2D**, the ketoprofen **3D**, the flurbiprofen **4D**, the ketorolac **5D** and the sulindac **6D** derivatives with low nanomolar K_Is and spanning between 5.4 and 6.3 nM, thus being in the average of 2 fold higher potent compared to **AAZ** (K_I 12.1 nM). Significant worsening of the hCA I inhibition was observed for the ibuprofen **1D** (K_I 25.2 nM), the diclofenac **8D** (K_I 46.2 nM) and the indometacin **7D** (K_I 84.3 nM) derivatives.

iii) As reported in Table 1 the hCA IV isoform was efficiently inhibited from the ibuprofen **1D** and the ketoprofen **3D** derivatives. In particular the first one resulted 1.16 fold more potent of the reference CAI **AAZ** (K_Is of 63.7 and 74.0 nM respectively), whereas the latter was slightly less potent (K_I 77.2 nM). All the remaining compounds in the series resulted ineffective in inhibiting the hCA IV isoform with K_I values comprised in within 219.8 and 811.0 nM.

iv) As for the tumor associated hCA IX, the sulindac **6D**, the indometacin **7D** and the diclofenac **8D** derivatives (K_Is 141.0, 202.4 and 137.1 nM respectively) resulted lower potent inhibitors in comparison to the standard CAI (K_I 25.8 nM). More interesting results were obtained for the remaining compounds **1D-5D**, which showed low nanomolar inhibition values and almost flat SAR. Among them, the ibuprofen **1D** and the ketoprofen **3D** derivatives resulted the most potent CAIs with K_Is of 10.4 and 12.5 nM respectively, thus being 2.5 and 2.0 fold respectively more potent when compared to the reference **AAZ**.

v) Remarkable data were obtained for the second tumor correlated hCA (the XII). The ketoprofen **3D** derivative was the most potent within the series and being also slightly more potent of the CAI **AAZ** (K_Is of 5.2 and 5.7 nM respectively). Similarly, the ibuprofen **1D** and the ketorolac **5D** resulted quite effective inhibitors of the hCA XII (K_I values of 6.5 and 7.3 nM respectively). As reported in table 1, a definite SAR was obtained for the remaining compounds in the series as increasing K_I values against the hCA XII were obtained for the naproxen **2D** (19.4 nM), the sulindac **6D** (35.0 nM), the flurbiprofen **4D** (43.3 nM), the diclofenac **8D** (59.2 nM) and for the indometacin **7D** (84.1 nM) derivatives.

Compounds **1E-4E** *and* **6E-8E**

i) In analogy to the three compound series previously discussed, also in this case the ketoprofen **3E** derivative resulted the most effective inhibitors of the hCA I isoform with K_I value of 20.9 nM, thus being 12.0 fold more potent of the reference CAI **AAZ** (K_I 250 nM). Increasing K_I values were observed for the other compounds in the series such as the ibuprofen **1E**, the naproxen **2E**, the indometacin **7E** and the sulindac **6E** which showed K_I values comprised between 48.4 and 93.8 nM. Finally the flurbiprofen **4E** and the diclofenac **8E** were the least active in the series (K_Is 242.7 and 341.8 nM).

ii) The hCA II isoform was efficiently inhibited from the ketoprofen **3E** and indometacin **7E** derivatives being 1.98 and 1.50 fold respectively more potent when compared to the standard **AAZ** (K_Is 6.1, 8.1 and 12.1 nM respectively). Medium potency inhibition against the hCA II was shown from the naproxen **2E** and the sulindac **6E** (K_Is of 47.1 and 66.6 nM) followed by the diclofenac **8E** and the flurbiprofen **4E** derivatives which were high nanomolar K_I values (K_Is 78.8 and 86.7 nM). Finally the ibuprofen **1E** derivative was ineffective (K_I 284.7 nM).

iii) In analogy to the compounds series previously discussed the hCA IV was the least inhibited among those considered in the present study. As reported in table 1 the ketoprofen **3E** derivative was the only compound in the series which showed inhibition potency, although of modest entity, being 1.25 fold less potent when compared to the reference CAI **AAZ** (K_Is of 92.8 and 74.0 nM respectively). All the remaining derivatives in the series resulted not active with K_Is values comprised within the low micromolar range.

iv) Conversely to the hCA IV, the IX isoform was potently inhibited from the majority of the compounds herein reported. In particular, the indometacin **7E** and the ketoprofen **3E** derivatives were the most potent, having K_I values 3.35 and 3.23 fold respectively lower when compared to the reference CAI **AAZ** (table 1). Slight higher inhibition values were obtained for the ibuprofen **1E** and for the naproxen **2E** derivatives (K_Is of 14.0 and 16.3 nM respectively), followed by the sulindac **6E** whose inhibition value was comparable to the **AAZ** (K_Is of 22.0 and 25.8 nM

respectively). The remaining compounds in the series, the flurbiprofen **4E** and the diclofenac **8E** derivatives, were the least potent with K_Is of 45.5. and 51.9 nM respectively.

v) Interesting results were also obtained for the second tumor associated hCA (the XII isoform). The flurbiprofen **4E** and the diclofenac **8E** derivatives were both ineffective in inhibiting the enzyme being 16.5 fold less potent when compared to the reference CAI **AAZ** (K_Is of 94.1, 93.8 and 5.7 nM respectively), followed by the indometacin **7E** derivative which was a medium potency inhibitor (K_I value of 64.0 nM). Better results were obtained for the remaining compounds which showed comparable inhibition values to the standard CAI (such as the ibuprofen **1E** and the sulindac **6E** derivatives whose K_Is were of 6.6 and 5.0 nM) or even lower as in the case of the naproxen **2E** and the ketoprofen **3E** derivatives (K_Is 4.2 and 4.1 nM respectively).

In summary the effects on the in vitro kinetic activity due to the linker lengths connecting the NSAID and the CAI fragments within the compound series here reported, afforded quite complex SARs which are not clearly defined. Among each CA isoform we observed: *i*) the naproxen **2A**, the ketoprofen **3B** and its longer derivative **3D**, were the most potent inhibitors of the ubiquitous hCA I isoform (K_Is of 7.9, 8.1 and 7.1 nM respectively); the hCA II isoform was potently inhibited by many compounds, with K_Is in the low nanomolar range regardless the nature of the NSAID or the linker. Among them the ketorolac **5A**, the naproxen **2A**, the ketoprofen **3A** and the indometacin **7C** derivatives resulted the most effective hCA II inhibitors (K_Is of 3.6, 4.2, 4.6 and 4.2 nM respectively); *iii*) The membrane associated hCA IV was potently inhibited by the ketoprofen **3A** (K_I 49.1 nM) followed by the ibuprofen **1C** and **1D** derivatives (K_Is of 59.9 and 63.7 nM) which were slightly more potent when compared to **AAZ** (K_I 74.0 nM); *iv*) The hCA XII isoform was potently inhibited from the ketoprofen **3A** and the diclofenac **8A** with K_Is of 5.1 and 5.3 nM respectively, thus being 5.1 and 4.9 fold respectively more potent of the reference CAI **AAZ** (K_I of 25.8 nM); amongst all compounds tested the ketoprofen **3A** derivative was also the most potent inhibitor the last hCA here considered (XII) with a K_I value of 2.9 nM, thus being nearly 2 fold more potent of the standard CAI **AAZ** (K_I of 5.7 nM). Slightly less potent inhibition values against

this isoform were obtained for the naproxen **2E**, the ketoprofen **3E**, the sulindac **6E** and the ketoprofen **3D** derivatives (K_Is of 4.2, 4.1, 5.0 and 5.2 nM respectively), which behaved anyhow as very potent inhibitors.

Pain relieving tests. The acute pain relieving effect of hybrids **2B**, **3B**, **6B** and **8B** (0.1–10.0 mg kg^{-1} p.o.) was assessed in an *in vivo* model of RA which was induced by complete Freund's adjuvant (CFA) i.a. treatment. Efficacy was compared with NSAIDs contained in the different hybrids (naproxen, ketoprofen, sulindac and diclofenac). For this purpose, NSAIDs were administered p.o. using a dosage equimolar to the highest dose of composite compounds (10.0 mg/kg).

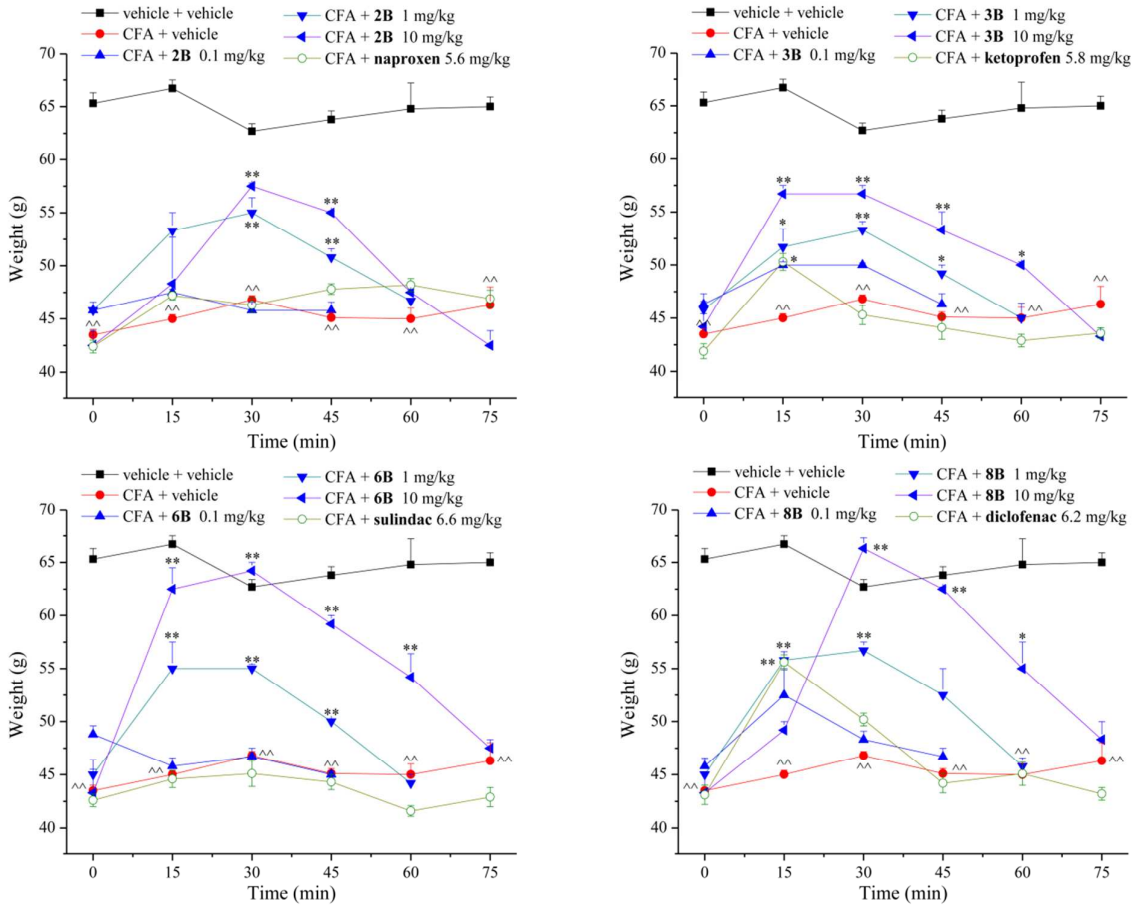


Figure 2. Acute pain reliever effect of **2B**, **3B**, **6B** and **8B** in a CFA i.a. injection induced RA model in rats. The Paw pressure test was used to assess the hypersensitivity towards noxious mechanical stimuli. NSAIDs (naproxen, ketoprofen, sulindac and diclofenac) contained in the different hybrids

were used as references tested at a dosage equimolar to 10 mg/kg of the corresponding NSAID–CAI. The measurements were accomplished on day 14 after CFA injection. The hybrids were suspended in 1% carboxymethyl cellulose (CMC) and administered orally. The values reported are the mean of 8 rats performed in 2 distinct experiments. ^^P<0.01 vs vehicle + vehicle treated animals. *P<0.05 and **P<0.01 *vs* time 0 min of the same group.

In Figure 2, the hypersensitivity to a mechanical noxious stimulus (Paw pressure test) is shown. After 14 days from i.a. CFA injection, the tolerated weight on the ipsilateral paw decreased to 43.5 ± 0.5 g (CFA + vehicle group in figure 2), thus considerably lower to the value of 63.3 ± 1.0 g relative to vehicle treated animals (1% CMC), which in turn displayed a stable threshold to nociceptive stimuli over the time course of the tests. Among the compounds tested, the naproxen derivative **2B** at 0.1 mg/kg concentration resulted ineffective in determining any antinociceptive effect, whereas at 1.0 mg/kg concentration was able to increase the pain threshold in a dose dependent manner. As reported in Figure 2 it was observed a sensible effect after 15 min administration, peaking at 30 min and lasting up to 60 min. The administration of **2B** at 10.0 mg/kg resulted only in a slight increase of the observed values at 30 and 45 min after administration when compared to the previous experiment at 10 fold lower concentration. Interestingly no extension of the antinociceptive effect over the time was observed. The naproxen alone (5.6 mg/kg) resulted completely ineffective. As for the ketoprofen **3B,** a dosage concentration of 0.1 mg/kg determined a slight pain relieving effect at 15 min which remained constant up to 30 min (50.0 ± 0.3 and 50.0 ± 0.2 g respectively) and lasted for 45 min. An increase of the of the dosage of **3B** to 1.0 mg kg^{-1}, showed higher effects. As reported in Figure 1 at 15 minutes the antinociceptive response evoked from **3B** was significative (51.7 ± 1.7 g), picking at 30 min (53.3 ± 0.8 g) and lasting up to 60 min after administration. The higher dosage of 3B (10 mg/kg) resulted in further increase of the pain relieve effects, which reached the highest value at 15 min and remained constant up to 30 (56.7 ± 0.8 g). In this case the antinociceptive effect was also prolonged up to 75 min from the treatment.

The ketoprofen alone (5.8 mg/kg) increased the pain threshold only at 15 min with value comparable to its corresponding hybrid **3B** at the lowest concentration (Figure 2).

In analogy to **3B** also the sulindac **6B** hybrid, evoked in a dose dependent manner the anti-hypersensitive effect. As reported in Figure 2 the compound resulted completely ineffective at 0.1 mg/kg^{-1} dosage, whereas a significant efficacy at 1.0 mg/kg^{-1} dosage was reported and lasted up to 60 min after administration. As shown in Figure 2 the maximum effect was reached at 15 min and was constant up to 30 min (55.0 ± 2.5 and 55.0 ± 0.4 g respectively). Finally, the treatment with **6B** at 10 fold higher concentrations (10 mg/kg^{-1}) fully counteracted the CFA-induced hypersensitivity of the ipsilateral paw at 15 min (62.5 ± 2.0 g) and picked at 30 min (64.2 ± 0.8 g) and the effect lasted up to 75 min after administration. Again the sulindac *di per se* (6.6 mg/kg) resulted inactive when considered in our experiments.

The last compound tested (i.e. the diclofenac derivative **8B**) at a dosage of 0.1 mg/kg^{-1} was able to induce partial antinociceptive effect which lasted up to 45 min after administration and peaked at 15 min with a value of 52.5 ± 2.5 g. The antinociceptive response for compound **8B** resulted further enhanced at the dose of 1.0 mg kg^{-1}. In this case the maximum effect was reached at 30 min (56.7 ± 0.8 g) and completely vanished at 60 min. The administration of **8B** at 10.0 mg kg^{-1} dose resulted both in increase of the pain threshold of the ipsilateral paw (peaking at 30 min with a value of 66.3 ± 1.0 g) as well as of the efficacy time frame (extended up to 75 min). The NSAID diclofenac alone (6.2 mg/kg^{-1}) induced a significant pain relief only at 15 min after administration with a value perfectly matching with the hybrid **8B** at 1.0 mg/kg^{-1} dosage.

Interestingly any of the tested compounds changed the pain threshold of the contralateral paw, thus suggesting that such molecules do not influence the normal pain sensitivity (data not shown).

The efficacy of hybrids **2B**, **3B**, **6B** and **8B** was also evaluated against the spontaneous pain, which was measured as the hind limb weight bearing alterations originated by unilateral damage (Incapacitance test, Figure 3).

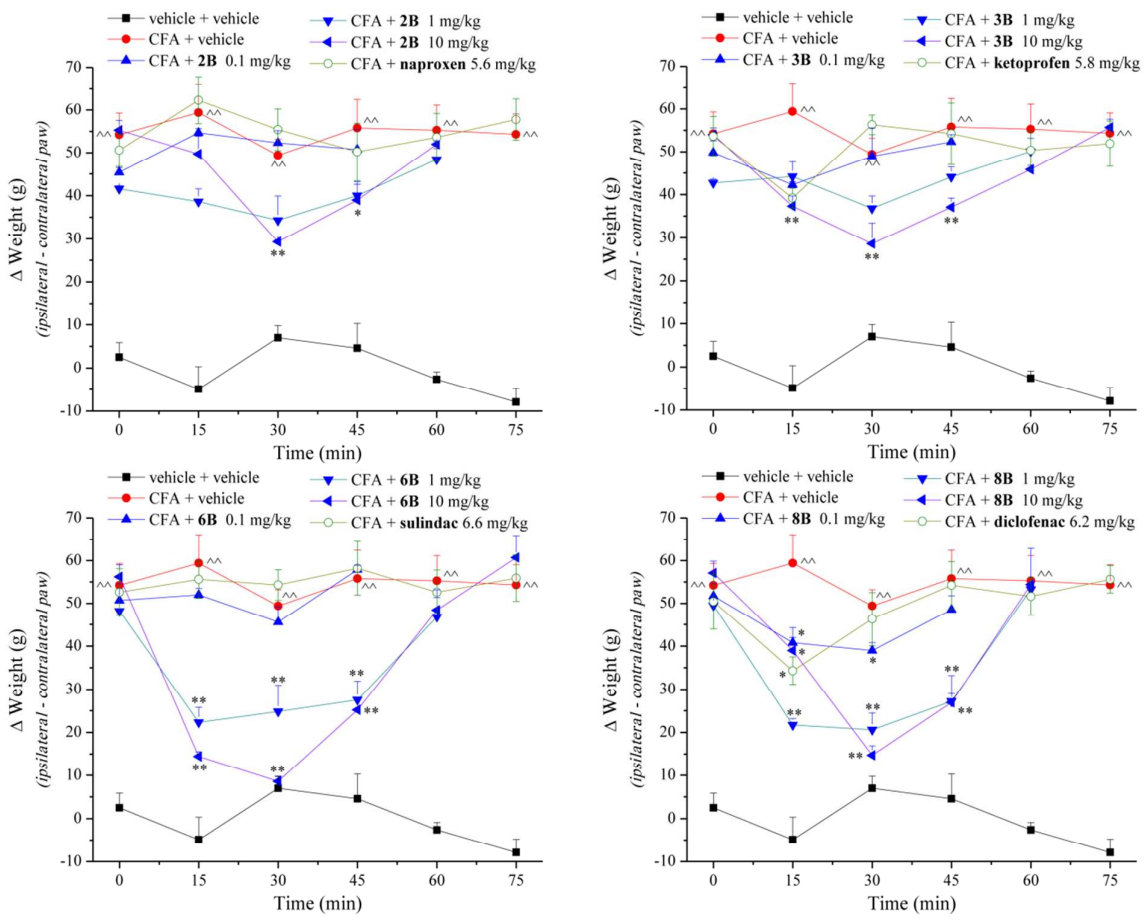


Figure 3. Acute pain reliever effect of **2B**, **3B**, **6B** and **8B** in a CFA i.a. injection induced RA model in rats. The incapacitance test was performed in order to assess the hind limb weight bearing alterations, which were measured as postural imbalance related to pain stimuli. The data values are the mathematical difference between the weight applied on the contralateral and ipsilateral limb (Δ Weight). NSAIDs (naproxen, ketoprofen, sulindac and diclofenac) contained in the different hybrids were used as references tested at a dosage equimolar to 10.0 mg/kg^{-1} of the corresponding NSAID–CAI. The measurements were done 14 days after CFA injection. All hybrids were suspended in 1% CMC and administered orally. The values are the mean of 8 rats and obtained in 2 different experiments. ^^P<0.01 vs vehicle + vehicle treated animals. *P<0.05 and **P<0.01 vs time 0 min of the same group.

The difference values between the weight burdened on the contralateral and the ipsilateral paw (Δ weight) was increased in CFA treated animals when compared to the values of the control group (54.2 ± 5.1 g *vs* 2.5 ± 3.4 g respectively). Compound **2B** was inactive at the lowest concentration (0.1 mg/kg^{-1}), whereas it showed activity when administered at higher dosages of 1.0 and 10.0 mg/kg^{-1}. In both cases the maximum effect was reached at 30 min after administration (34.3 ± 5.6 g and 29.3 ± 0.4 g respectively) and lasted up to 60 min. As for the hybrid **3B,** its administration at the lowest concentration slightly reduced the Δ weight with a peaking value at 15 min of 42.3 ± 0.7 g. Significative effects were observed when **3B** was administered at 1.0 and 10.0 mg/kg^{-1}. In both cases both the intensity as well as the duration of the effect were dose dependent. As shown in figure 3, the compound **3B** at 1.0 mg/kg^{-1} peaked at 30 min with a value of 36.8 ± 2.9 g and lasted up to 60 min. A 10 fold increase of the dose administered (10.0 mg/kg^{-1}) resulted in a maximum Δ weight value at 30 min of 28.6 ± 4.8 g and enhancement of the effect up to 75 min. The experiments conducted on the sulindac hybrid **6B** revealed a complete ineffectiveness on reducing the postural imbalance when administered at the lowest dosage (0.1 mg/kg^{-1}). Conversely significant effects were observed when **6B** was administered at the concentrations of 1.0 and 10.0 mg/kg^{-1}. As reported in figure 3, the acute pain reliever effect followed a dose dependent trend after a single administration, and peaked at 15 and 30 min respectively (22.3 ± 3.5 g and 8.6 ± 1.1 g). In both cases the effect lasted up to 60 min. The final compound considered (i.e. the diclofenac hybrid **8B**) showed a weak effect at the lowest concentration (0.1 mg/kg^{-1}) with a peaking value of 39.0 ± 1.8 g and lasting up to 45 min after single dose administration. More intense effects were observed when the compound **8B** was administered at 1.0 and 10.0 1 mg/kg^{-1} dosages. As reported in figure 3 the dosage increase resulted both in widening of the time frame efficacy as well as of the magnitude with peaking values of 20.6 ± 3.9 g and 14.7 ± 2.2 g respectively at 30 min after administration. The acute pain reliever effects of the hybrid compounds **2B**, **3B**, **6B** and **8B** tested were compared with their relative NSAIDs counterparts (i.e. naproxen, ketoprofen, sulindac and diclofenac). As reported in figure 3, only the ketoprofen and diclofenac were the able to evoke significant relief effects even

if their efficacy and potency were significantly lower when compared to their hybrid **3B** and **8B** respectively (Figure 3).

Conclusions. We report new low molecular weight NSAID-CAI hybrid molecules as potential drug lead compounds for the treatment of ache-related symptoms associated to inflammatory diseases such as the RA. All compounds in the obtained series consisted of a clinically used NSAID fragment (acting as COX inhibitors) linked to a sulfonamide based CAI moiety. Unlike our previously reported NSAID-CAI hybrids, which showed remarkable inhibition properties of the hCA IV, moderate K_Is against the IX and XII isoforms and no activity on the ubiquitous hCAs I and II, the ones herein reported were ineffective inhibitors of hCA IV and effective inhibitors of the remaining enzymatic isoforms considered here (hCA I, II, IX and XII). Such a complementary *in vitro* kinetic activity between the two hybrid series is certainly due to the nature of the CAI fragment, which is of the coumarin type in the first series [1] and of the benzenesulfonamide in the latter one. This has been demonstrated by comparison of analogous derivatives between the two series having the same tether length. Even if it was not possible to draw a straightforward SAR from the obtained *in vitro* kinetic data, we were able to demonstrate that the most potent hybrid derivatives acting as efficient inhibitors against the hCAs herein considered showed remarkable in vivo efficacy. More importantly, in this study we carried out for the first time a complete kinetic profiling of a large series of NSAID-CAI hybrids potentially useful for the development of drug leads for the treatment of RA related diseases. Such a data which will be particularly useful for the selection of the compounds out of the pool having the proper selectivity index against the hCA isoforms involved in RA. The *in vivo* data obtained by using a CFA induced arthritis model in rats with selected NSAID-CAI hybrids, showed potent antihyperalgesic effects in a dose dependent manner, starting already at 1 mg kg^{-1}. In particular, compounds **6B** and **8B** resulted to be the best performing ones in terms of intensity and long-lasting effects of the antihyperalgesic action, both in the *in vivo* tests considered.

In conclusion we gave further evidence to our previous hypothesis that NSAID−CAI hybrid compounds are indeed valuable leads for the management of ache symptoms typical of inflammatory pathologies such as the RA. Moreover, the high affinity showed by these new hybrid molecules on the ubiquitous and erythrocyte expressed hCAs I and II represents an interesting feature which can be of particular advantage both for the systemic distribution of the drugs into the organism as well as for enhancement of their half-life, since their binding to blood present CA isoforms may lead to a depot effect. In this context it is worth mentioning that RA symptoms highly affect the life quality of patients, which are progressively unable to carry out any activities such as at work, in social relations and leisures.[30] Therefore an ideal pharmacological approach should consist of long lasting drugs or with extended time clearances. Studies in this direction are currently being developed in our laboratories.

Experimental protocols.

Chemistry. All anhydrous solvents and reagents used in this study were purchased from Alfa Aesar, TCI and Sigma-Aldrich. The synthetic reactions involving air- or moisture-sensitive chemicals were carried out under a nitrogen atmosphere using dried glassware and syringes techniques in order to transfer the solutions. Nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR) spectra were recorded using a Bruker Advance III 400 MHz spectrometer using DMSO-d_6 as solvent. The chemical shifts are reported in parts per million (ppm) and the coupling constants (*J*) are expressed in Hertz (Hz). The splitting patterns are designated as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; brs, broad singlet; dd, doublet of doublets. The correct assignment of exchangeable protons (i.e. O*H* and N*H*) was carried out by means of the addition of D_2O. Analytical thin-layer chromatography (TLC) was done on Merck silica gel F-254 plates. Flash chromatography was performed on Merck Silica gel 60 (230-400 mesh ASTM) as the stationary phase and appropriate mixtures of ethyl acetate/*n*-hexane were the eluents. Melting points (m.p.) were measured in open capillary tubes with a Gallenkamp MPD350.BM3.5 apparatus and are

uncorrected. The HPLC was performed by using a Waters 2690 separation module coupled with a photodiode array detector (PDA Waters 996) using a Nova-Pak C18 4 μm 3.9 mm × 150 mm (Waters) silica-based reverse phase column. The sample was dissolved in 10% acetonitrile/H_2O and an injection volume of 45 μL. The mobile phase (flow rate 1.0 mL/min) was a gradient of H_2O + trifluoroacetic acid (TFA) 0.1% (A) and acetonitrile + TFA 0.1% (B), with steps as follows: (A%:B%), 0−10 min 90:10, 10−25 min gradient to 60:40, 26:28 min isocratic 20:80, 29−35 min isocratic 90:10. TFA 0.1% in water as well in acetonitrile was used as counterion. All compounds reported here were ≥ 95% HPLC pure. The solvents used in MS measures were acetone, acetonitrile (Chromasolv grade) and mQ water 18 MΩ. The high resolution mass spectrometry (HRMS) analysis were performed with a Thermo Finnigan LTQ Orbitrap mass spectrometer coupled with an electrospray ionization source (ESI). Analysis were carried out in positive ion mode $[M+H]^+$, and it was used a proper dwell time acquisition to achieve 60,000 units of resolution at Full Width at Half Maximum (FWHM). Elemental composition of compounds were calculated on the basis of their measured accurate masses, accepting only results with an attribution error less than 5 ppm and a not integer RDB (double bond/ring equivalents) value. [31] Stock solutions of analytes were prepared using acetone (1.0 mg $mL^{-1)}$ and stored at 4 °C. Then working solutions of each analyte were prepared by dilution of the stock solutions using mQ H_2O/ACN 1/1 (*v/v)* up to a concentration of 1.0 μg mL^{-1} The HRMS analysis was performed by introducing the analyte working solution via syringe pump at 10 μL min^{-1}.

a) General Procedure for synthesis of compounds 1A-8A, 1C-4C and 6C-8C, 1D-8D, 1E-8E. [18]

The appropriate NSAID **1-8** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), were dissolved in dry DMF or DMA solvents (3.0 ml) and stirred for 10 minutes at r.t., followed by addition of the amine **A**, **C**, **D** or **E** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in the

same solvent system (1.5 ml). The reaction was stirred until the reaction stops (TLC monitoring), quenched with a 3.0 M aqueous HCl solution at 0 °C and extracted with ethyl acetate (3 x 15 ml). The combined organic layers were washed with 3.0 M aqueous HCl solution, H_2O (3 x 20 ml), sat. aqueous $NaHCO_3$ (3 x 20 ml), dried over Na_2SO_4 and evaporated under *vacuo* to give a residue which was purified by silica gel column chromatography eluting with ethyl acetate/*n*-hexane or MeOH/DCM, crystallization or trituration from the appropriate solvent to afford the titled compounds.

b) General Procedure for synthesis of compounds 1B-8B. [18]

The appropriate NSAID **1-8** (1.0 eq.) and *N,N'*-dicyclohexylcarbodiimide (DCC, 1.1 eq.) were dissolved in dry dimethylformamide (DMF, 3.0 ml) and stirred at r.t. under a nitrogen atmosphere for 10 minutes until a white precipitate occurs, then *N*-hydroxysuccinimide (NHS, 1.1 eq.) dissolved in dry DMF (1.5 ml) was added and the reaction mixture was stirred at r.t. until starting material was consumed (TLC monitoring) followed by addition of the 4-aminoethylbenzenesulfonamide **B** (2.0 eq.) dissolved in the same solvent. The reaction mixture was stirred until the reaction stops (TLC monitoring), quenched with a 3.0 M aqueous HCl solution at 0-5 °C and extracted with ethyl acetate (3 x 15 ml). The precipitate formed during the extraction was filtered-off and the combined organic layers were washed with H_2O (5 x 15 ml), sat. aqueous $NaHCO_3$ (3 x 20 ml), dried over Na_2SO_4 and evaporated under *vacuo* to give a deposit that was purified by silica gel chromatography eluting with MeOH/DCM 5% *v/v* followed by crystallization from ethyl acetate/petroleum ether to afford the title compounds.

2-(±)-(4-Isobutylphenyl)-*N*-(4-sulfamoylphenyl)propanamide 1A. Ibuprofen **1** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), sulfonilamide **A** (2.0 eq.) and *N,N*-diisopropylethylamine

(DIPEA, 4.0 eq.) in DMF (4.5 ml) were treated according to the **general procedure a** previously reported. The crude product was purified by silica gel column chromatography eluting with ethyl acetate/*n*-hexane 50% *v/v* and crystallized from ethyl acetate/petroleum ether to afford the title compound **1A** as a white solid in 19 % yield, silica gel TLC R_f 0.68 (Ethyl acetate/*n*-hexane 50% *v/v*); mp 194.8 ºC; δ_H (400 MHz, DMSO-d_6) 0.88 (6H, d, *J* 6.6, -CH($\underline{CH_3}$)$_2$), 1.43 (3H, d, *J* 7.0, -CH$\underline{CH_3}$), 1.83 (1H, p, *J* 6.8, $\underline{CH}$(CH$_3$)$_2$), 2.43 (2H, d, *J* 7.1, -$\underline{CH_2}$CH(CH$_3$)$_2$), 3.85 (1H, p, *J* 6.8, -$\underline{CH}$CH$_3$), 7.14 (2H, d, *J* 8.0, Ar-H), 7.26 (2H, s, SO_2NH_2, exchange with D_2O), 7.32 (1H, d, *J* 8.0, Ar-H), 7.77 (4H, s, 4 x Ar-H), 10.39 (1H, s, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 174.0 (C=O), 143.1, 140.7, 139.8, 139.3, 130.0 (2 x Ar-C), 128.0 (2 x Ar-C), 127.7 (2 x Ar-C), 119.7 (2 x Ar-C), 46.7, 45.3, 30.6, 23.2 (-CH($\underline{CH_3}$)$_2$), 19.7.

Experimental in agreement with reported data.[19]

2-(±)-(4-Isobutylphenyl)-*N*-(4-sulfamoylphenethyl)propanamide 1B. Ibuprofen **1** (1.0 eq.), *N,N'*-dicyclohexylcarbodiimide (DCC, 1.1 eq.), *N*-hydroxysuccinimide (NHS, 1.1 eq.) and 4-aminoethylbenzenesulfonamide **B** (2.0 eq.), dissolved in dry DMF (1.5 ml), were treated according to the **general procedure b** previously reported to afford the titled compound **1B** as a white solid in 23% yield, silica gel TLC R_f 0.19 (MeOH/DCM 5 % *v/v*); mp 149.3 ºC; δ_H (400 MHz, DMSO-d_6) 0.90 (6H, d, *J* 6.6, -CH($\underline{CH_3}$)$_2$), 1.32 (3H, d, *J* 7.0, -CH$\underline{CH_3}$), 1.85 (1H, p, *J* 6.8, -$\underline{CH}$(CH$_3$)$_2$), 2.46 (2H, d, *J* 7.1, -$\underline{CH_2}$CH(CH$_3$)$_2$), 2.77 (2H, t, *J* 7.1, -CONHCH$_2\underline{CH_2}$-), 3.33-3.26 (2H, m, -CONH$\underline{CH_2}$CH$_2$-), 3.55 (1H, d, *J* 7.2, -$\underline{CH}$CH$_3$), 7.12 (2H, d, *J* 8.1, Ar-H), 7.22(2H, d, *J* 8.1, Ar-H), 7.30 (2H, d, *J* 8.3, Ar-H), 7.32 (2H, s, SO_2NH_2, exchange with D_2O), 7.72 (2H, d, *J* 8.3, Ar-H), 8.03 (1H, t, *J* 5.6, CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 174.4 (C=O), 144.7, 143.0, 140.4, 140.2, 130.2 (2 x Ar-C), 129.8 (2 x Ar-C), 128.0 (2 x Ar-C), 126.6 (2 x Ar-C), 45.7, 45.2, 40.8 (-CONH$\underline{CH_2}\underline{CH_2}$-), 35.7 (-CONH$\underline{CH_2}\underline{CH_2}$-), 30.7, 23.2(-CH($\underline{CH_3}$)$_2$), 19.4; ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{21}H_{29}N_2O_3S$ = 389.1893, found 389.1894.

2-(±)-(4-Isobutylphenyl)-*N*-(2-(4-sulfamoylphenoxy)ethyl)propanamide 1C. Ibuprofen **1** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxyethyl)amino)benzenesulfonamide **C** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was purified by silica gel column chromatography using Ethyl acetate/n-hexane 66% *v/v* and crystallized from EtOH/H_2O to afford the title compound **1C** as a white solid in 25% yield, silica gel TLC R_f 0.36 (Ethyl acetate/n-hexane 66% *v/v*); mp 152 ºC; δ_H (400 MHz, DMSO-d_6) 0.88 (6H, d, *J* 6.4, -CH($\underline{CH_3}$)$_2$), 1.34 (3H, d, *J* 7.2, -CH$\underline{CH_3}$), 1.82 (1H, p, *J* 6.8, -$\underline{CH}$(CH$_3$)$_2$), 2.43 (2H, d, *J* 7.2, -$\underline{CH_2}$CH(CH$_3$)$_2$), 3.45 (2H, m, -CONH$\underline{CH_2}$CH$_2$), 3.59 (1H, q, *J* 6.8, -$\underline{CH}$CH$_3$), 4.08 (2H, t, *J* 5.6, -CONHCH$_2\underline{CH_2}$), 7.08 (4H, d, *J* 8.8, Ar-H), 7.24-7.23(4H, m, SO_2NH_2, exchange with D_2O, 2 x Ar-H), 7.76 (2H, d, *J* 8.8, Ar-H), 8.21 (1H, t, *J* 5.6, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 174.7 (C=O), 161.7, 140.3, 140.1, 137.2, 129.6 (2 x Ar-C), 128.5 (2 x Ar-C), 127.8 (2 x Ar-C), 115.4 (2 x Ar-C), 67.6 (-CONHCH$_2\underline{CH_2}$), 45.5, 45.0, 39.0, 30.5, 23.1, 23.1, 19.5; ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{21}H_{29}N_2O_4S$ = 405.1842, found 405.1841.

2-(±)-(4-Isobutylphenyl)-*N*-(3-(4-sulfamoylphenoxy)propyl)propanamide 1D. Ibuprofen **1** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxypropyl)amino)benzenesulfonamide **D** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product purified by silica gel column chromatography using Ethyl acetate/n-hexane 50% *v/v* and crystallized from ethyl acetate/petroleum ether to yield the title compound **1D** as a white solid in 28% yield, silica gel TLC R_f 0.10 (Ethyl acetate/n-hexane 50% *v/v*); mp 127.1 ºC; δ_H (400 MHz, DMSO-d_6) 0.87-0.85 (8H,

m, -CH($\underline{CH_3}$)$_2$, -CONHCH$_2\underline{CH_2}$CH$_2$), 1.32 (3H, d, *J* 7.0, -CH$\underline{CH_3}$), 1.88-1.77 (1H, m, -$\underline{CH}$(CH3)2), 2.41 (2H, d, *J* 7.1, -$\underline{CH_2}$CH(CH3)2), 3.23-3.18 (2H, m, -CONH$\underline{CH_2}$CH$_2$CH$_2$), 3.56 (1H, d, *J* 7.0, -$\underline{CH}$CH3), 4.00-3.97 (2H, m, -CONHCH$_2$CH$_2\underline{CH_2}$), 7.02 (2H, d, *J* 8.9, Ar-H), 7.08 (2H, d, *J* 8.0, Ar-H), 7.23(2H, d, *J* 7.6, Ar-H), 7.23 (2H, s, SO_2NH_2, exchange with D_2O), 7.75 (2H, d, *J* 8.9, Ar-H), 8.05 (1H, m, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 173.9 (C=O), 161.3, 140.1, 139.6, 136.6, 129.2 (2 x Ar-C), 128.0 (2 x Ar-C), 127.3 (2 x Ar-C), 114.8 (2 x Ar-C), 66.0 (-CONHCH$_2$CH$_2\underline{CH_2}$), 45.2, 44.6, 35.8, 30.0, 29.0, 22.6, 19.0; ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{22}H_{31}N_2O_4S$ = 419.1999, found 419.2006.

2-(±)-(4-Isobutylphenyl)-*N*-(4-(4-sulfamoylphenoxy)butyl)propanamide 1E. Ibuprofen **1** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxybuthyl)amino)benzenesulfonamide **E** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was purified by silica gel column chromatography using Ethyl acetate/n-hexane 50% *v/v* and crystallized from ethyl acetate/petroleum ether to afford the title compound **1E** as a white solid in 23% yield, silica gel TLC R_f 0.5 (Ethyl acetate/n-hexane 66% *v/v*); mp 101.8 ºC; δ_H (400 MHz, DMSO- d_6) 0.88 (6H, d, *J* 6.8, -CH($\underline{CH_3}$)$_2$), 1.34 (3H, d, *J* 7.2, -CH$\underline{CH_3}$), 1.55 (2H, p, *J* 7.1, -CONHCH$_2\underline{CH_2}$CH$_2$CH$_2$), 1.70 (2H, p, *J* 7.0, -CONHCH$_2$CH$_2\underline{CH_2}$CH$_2$), 1.84 (1H, sep., *J* 6.8, -$\underline{CH}$(CH$_3$)$_2$), 2.43 (2H, d, *J* 6.8, -$\underline{CH_2}$CH(CH$_3$)$_2$), 3.12 (2H, q, *J* 6.4, -CONH$\underline{CH_2}$CH$_2$CH$_2$CH$_2$), 3.58 (1H, q., *J* 6.8, -$\underline{CH}$CH$_3$), 4.03 (2H, t, *J* 6.4, -CONHCH$_2\underline{CH_2}$CH$_2$CH$_2$), 7.11-7.06 (4H, m, 4 x Ar-H), 7.22 (2H, s, SO_2NH_2, exchange with D_2O), 7.24 (2H, d, *J* 8.0, 2 x Ar-H), 7.77 (2H, d, *J* 8.8, 2 x Ar-H), 7.96 (1H, t, *J* 5.4, -CONH, exchange with D_2O), δ_C (100 MHz, DMSO- d_6) 174.2 (C=O), 161.9, 140.6, 140.0, 137.0, 129.6 (2 x Ar-C), 128.5 (2 x Ar-C), 127.8 (2 x Ar-C), 115.3 (2 x Ar-C), 68.5, 45.7, 45.1, 39.0, 30.5,

26.8, 26.5, 23.1 (-CH($\underline{CH_3}$)$_2$), 19.5; ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{23}H_{33}N_2O_4S$ = 433.2155, found 433.2163.

2-(±)-(6-Methoxynaphthalen-2-yl)-*N*-(4-sulfamoylphenyl)propanamide 2A. Naproxen **2** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), sulfonilamide **A** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in DMF (4.5 ml) were treated according to the **general procedure a** previously reported to afford the title compound **2A** as a white solid in 8% yield, silica gel TLC R_f 0.17 (Ethyl acetate/n-hexane 50% *v/v*); mp 202.5 ºC; δ_H (400 MHz, DMSO-d_6) 1.54 (3H, d, *J* 7.0, -CH$\underline{CH_3}$), 3.89 (3H, s,-OCH_3), 4.02 (1H, d, *J* 7.0, -$\underline{CH}CH_3$), 7.18 (1H, dd, *J* 2.6/8.9, naphtalen-H), 7.26 (2H, s, SO_2NH_2, exchange with D_2O), 7.32 (1H, d, *J* 2.5, naphtalen-H), 7.54 (1H, dd, *J* 1.7/8.6, naphtalen-H), 7.77-7.85 (7H, m, 3 x naphtalen-H, 4 x Ar-H), 10.46 (1H, s, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 173.5 (C=O), 157.7, 142.7, 138.9, 137.2, 133.9, 129.7, 129.0, 127.5 (2 x Ar-C), 127.2, 126.8, 126.1, 119.4 (2 x Ar-C), 119.3, 106.3, 55.8 (-OCH_3), 46.6 (-$\underline{CH}CH_3$), 19.3 (-CH$\underline{CH_3}$); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{20}H_{21}N_2O_4S$ = 385.1216, found 385.1209.

2-(±)-(6-Methoxynaphthalen-2-yl)-*N*-(4-sulfamoylphenethyl)propanamide 2B. Naproxen **2** (1.0 eq.), *N,N'*-dicyclohexylcarbodiimide (DCC, 1.1 eq.), *N*-hydroxysuccinimide (NHS, 1.1 eq.) and 4-aminoethylbenzenesulfonamide **B** (2.0 eq.) dissolved in dry DMF (1.5 ml) were treated according to the **general procedure b** previously reported to afford the titled compound **2B** as a white solid in 55% yield, silica gel TLC R_f 0.14 (MeOH/DCM 5 % *v/v*); mp 161.3 °C; δ_H (400 MHz, DMSO-d_6) 1.42 (3H, d, *J* 7.0, -CH$\underline{CH_3}$), 2.79-2.76 (2H, m, -CONHCH$_2\underline{CH_2}$), 3.32-3.28 (2H, m, -CONH$\underline{CH_2}CH_2$), 3.72 (1H, q, *J* 7.0, -$\underline{CH}CH_3$), 3.90 (3H, s,-OCH_3), 7.18(1H, dd, *J* 2.6/8.9, naphtalen-H), 7.31-7.30 (5H, m, naphtalen-H, 2 x Ar-H, SO_2NH_2, exchange with D_2O), 7.43 (1H,

dd, *J* 1.8/8.5, naphtalen-H), 7.69 (2H, d, *J* 8.3, 2 x naphtalen-H), 7.82-7.72 (3H, m, naphtalen-H, 2 x Ar-H), 8.09 (1H, t, *J* 5.6, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 174.0 (C=O), 157.6, 144.3, 142.6, 138.0, 133.8, 129.7 (2 x Ar-C), 129.0, 127.22, 127.1, 126.2 (2 x Ar-C), 125.9, 119.2, 106.3, 55.8 ($-OCH_3$), 45.7 ($-\underline{CH}CH_3$), 40.8 ($-CONH\underline{CH_2}CH_2$), 35.3 ($-CONHCH_2\underline{CH_2}$), 19.0 ($-CH\underline{CH_3}$);

Experimental in agreement with reported data. [20]

2-(±)-(6-Methoxynaphthalen-2-yl)-*N*-(2-(4-sulfamoylphenoxy)ethyl)propanamide 2C. Naproxen **2** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxyethyl)amino)benzenesulfonamide **C** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was crystallized from $EtOH/H_2O$ to afford the title compound **2C** as a white solid in 19 % yield, silica gel TLC R_f 0.23 (EtOAc/n-hexane 66% v/v); mp 170.3 ºC; δ_H (400 MHz, DMSO-d_6) 1.44 (3H, d, *J* 6.8, $-CH\underline{CH_3}$), 3.47 (2H, m, $-CONH\underline{CH_2}CH_2$), 3.80 (1H, d, *J* 6.8, $-\underline{CH}CH_3$), 3.89 (3H, s, $-OCH_3$), 4.09 (2H, t, *J* 5.6, $-CONHCH_2\underline{CH_2}$), 7.07 (2H, d, *J* 8.8, 2Xnaphtalen-H), 7.16 (1H, dd, *J* 2.4/8.8, naphtalen-H), 7.24(2H, s, SO_2NH_2, exchange with D_2O), 7.29 (1H, d, *J* 2.8, naphtalen-H), 7.46 (1H, dd, *J* 1.6/8.8, naphtalen-H), 7.77-7.73 (5H, m, naphtalen-H, 4 x Ar-H), 8.30-8.27 (1H, t, *J* 5.4, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 174.7 (C=O), 161.7, 158.0, 138.2, 137.2, 134.0, 130.0, 129.3, 128.6 (2 x Ar-C), 127,5, 127.3, 126.1, 119.4, 115.4 (2 x Ar-C), 106.6, 67.6 ($-CONHCH_2\underline{CH_2}$), 56.1 ($-OCH_3$), 45.8 ($-\underline{CH}CH_3$), 39.1 ($-CONHCH_2\underline{CH_2}$), 19.4 ($-CH\underline{CH_3}$); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{22}H_{25}N_2O_5S$ = 429.1479 found 429.1484.

2-(±)-(6-Methoxynaphthalen-2-yl)-*N*-(3-(4-sulfamoylphenoxy)propyl)propan amide 2D. Naproxen **2** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxypropyl)amino)benzenesulfonamide **D** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product purified by silica gel column chromatography using Ac/DCM (14.3 % *v/v*) and crystallized from EtOH/H_2O (5 % *v/v*) to yield the title compound **2D** as a white solid in 52% yield, silica gel TLC R_f 0.5 (Ac/DCM 14.3 % *v/v*); mp 164 ºC; δH (400 MHz, DMSO-d_6) 1.44 (3H, d, *J* 7.0, -CH$\underline{CH_3}$), 1.87 (2H, p, *J* 6.7, -CONHCH$_2\underline{CH_2}$CH$_2$), 3.26-3.23 (2H, m, -CONH$\underline{CH_2}$CH$_2$CH$_2$), 3.75 (1H, d, *J* 7.0, -$\underline{CH}$CH$_3$), 3.89 (3H, s, -OCH$_3$), 4.00 (2H, t, *J* 6.4, -CONHCH$_2$CH$_2\underline{CH_2}$), 7.00 (2H, dd, *J* 2.0/7.0, 2 x naphtalen-H), 7.17 (1H, dd, *J* 2.6/8.9, naphtalen-H), 7.23(2H, s, SO_2NH_2, exchange with D_2O) 7.38 (1H, d, *J* 2.5, naphtalen-H), 7.47 (1H, dd, *J* 2.8/10.48, naphtalen-H), 7.80-7.73 (5H, m, naphtalen-H, 4 x Ar-H), 8.11-8.09 (1H, t, *J* 5.6, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 174.1 (C=O), 161.6, 157.7, 138.2, 136.8, 133.9, 129.8, 129.1, 128.4 (2 x Ar-C), 127.3, 127.1, 125.9, 119.3, 115.1 (2 x Ar-C), 106.4, 66.3 (-CONHCH$_2$CH$_2\underline{CH_2}$), 55.9 (-OCH$_3$), 45.9 (-$\underline{CH}$CH$_3$), 36.2, 29.3, 19.3 (-CH$\underline{CH_3}$); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{23}H_{27}N_2O_5S$ = 443.1635, found 443.1637.

2-(±)-(6-Methoxynaphthalen-2-yl)-*N*-(4-(4-sulfamoylphenoxy)butyl)propan amide 2E. Naproxen **2** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxybuthyl)amino)benzenesulfonamide **E** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was triturated with DCM/Petroleum (10 % *v/v*) ether to afford the title compound **2E** as a white solid in 57% yield, silica gel TLC R_f 0.2 (Ethyl acetate/*n*-hexane 66% *v/v*); mp 143.1

ºC; δ_H (400 MHz, DMSO-d_6) 1.44 (3H, d, *J* 7.2, -CH<u>CH$_3$</u>), 1.59-1.52 (2H, m, -CONHCH$_2$<u>CH$_2$</u>CH$_2$CH$_2$), 1.73-1.66 (2H, m, -CONHCH$_2$CH$_2$<u>CH$_2$</u>CH$_2$), 3.16-3.11 (2H, m, -CONH<u>CH$_2$</u>CH$_2$CH$_2$CH$_2$), 3.77-3.72 (1H, q, *J* 6.8, -<u>CH</u>CH$_3$), 3.89 (3H, s, -OCH$_3$), 4.02 (2H, t, *J* 6.4, -CONHCH$_2$CH$_2$CH$_2$<u>CH$_2$</u>), 7.06 (2H, d, *J* 8.8, 2 x naphtalen-H), 7.17 (1H, dd, *J* 2.8/8.8, naphtalen-H), 7.22 (2H, s, SO_2NH_2, exchange with D_2O), 7.30 (1H, d, *J* 2.4, naphtalen-H), 7.48 (1H, dd, *J* 2/8.4, naphtalen-H), 7.78-7.75 (4H, m, naphtalen-H, 3 x Ar-H), 7.80 (1H, d, *J* 8.8, naphtalen-H), 8.05-8.03 (1H, t, *J* 5.6, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 174.2 (C=O), 161.9, 157.9, 138.5, 136.9, 134.0, 130.0, 129.3, 128.5 (2 x Ar-C), 127,5, 127.3, 126.1, 119.4, 115.3 (2 x Ar-C), 106.60, 68.5 (-CONHCH$_2$CH$_2$ CH$_2$<u>CH$_2$</u>), 56.1 (-OCH$_3$), 46.0 (-<u>CH</u>CH$_3$), 39.1, 26.8, 26.5, 19.5 (-CH<u>CH$_3$</u>); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{24}H_{29}N_2O_5S$ = 457.1792, found 457.1796.

2-(±)-(3-Benzoylphenyl)-*N*-(4-sulfamoylphenyl)propanamide 3A. Ketoprofen **3** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), sulfonilamide **A** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in DMF (4.5 ml) were treated according to the **general procedure a** previously reported to yield the title compound **3A** as a white solid in 27% yield, silica gel TLC R_f 0.11 (Ethyl acetate/n-hexane 50% *v/v*); mp 193.0 ºC; δ_H (400 MHz, DMSO-d_6) 1.51 (3H, d, *J* 7.0,-CH<u>CH$_3$</u>), 4.09-4.04 (1H, m ,-<u>CH</u>CH$_3$), 7.28 (2H, s, SO_2NH_2, exchange with D_2O), 7.60-7.57 (3H, m, 3 x Ar-H), 7.66 (1H, d, *J* 7.80, Ar-H), 7.76-7.71 (6H, m, 6 x Ar-H), 7.77 (2H, s, 2 x Ar-H), 7.84 (1H, s, Ar-H), 10.50 (1H, s, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 196.6 (C=O), 173.4 (C=O), 143.0, 143.0 (2 x Ar-C), 139.5, 138.0, 138.0, 133.7, 132.6, 130.6 (2 x Ar-C), 129.8, 129.6 (3 x Ar-C), 129.5, 127.7 (2 x Ar-CH), 119.8 (2 x Ar-C), 46.9 (-<u>CH</u>CH$_3$), 19.6 (-CH<u>CH$_3$</u>); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{22}H_{21}N_2O_4S$ = 409.1216, found 409.1219.

2-(±)-(3-Benzoylphenyl)-*N*-(4-sulfamoylphenethyl)propanamide 3B. Ketoprofen **3** (1.0 eq.), *N,N′*-dicyclohexylcarbodiimide (DCC, 1.1 eq.), *N*-hydroxysuccin imide (NHS, 1.1 eq.) and 4-aminoethylbenzenesulfonamide **B** (2.0 eq.) dissolved in dry DMF (1.5 ml), were treated according to the **general procedure b** previously reported to afford the titled compound **3B** as a white solid in 32% yield, silica gel TLC R_f 0.14 (MeOH/DCM 5 % *v/v*); mp 116.7 °C; δ_H (400 MHz, DMSO-d_6) 1.37 (3H, d, *J* 7.0,-CH$\underline{CH_3}$), 2.77 (2H, m, -CONHCH$_2\underline{CH_2}$), 3.28-3.33 (2H, m, -CONH$\underline{CH_2}$CH$_2$), 3.73-3.67 (1H, m, -$\underline{CH}$CH$_3$), 7.29 (2H, s, SO_2NH_2, exchange with D_2O), 7.31-7.29 (2H, m, 2 x Ar-H), 7.53 (1H, t, *J* 7.6, Ar-H), 7.59 (2H, d, *J* 7.6, 2 x Ar-H), 7.63(2H, d, *J* 8.0, 2 x Ar-H), 7.70 (2H, t, *J* 8.0, 2 x Ar-H), 7.78-7.75 (3H, m, 3 x Ar-H), 8.16 (1H, t, *J* 5.6, CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 196.4 (C=O), 173.5 (C=O), 144.2, 143.3, 142.6, 137.7, 137.5, 133.3 (Ar-C), 132.23, 130.2, 129.7, 129.2 (2 x Ar-C), 129.1 (2 x Ar-CH), 129.1 (2 x Ar-C), 126.2 (2 x Ar-C), 120.7, 45.5 (-$\underline{CH}$CH$_3$), 40.8 (-CONH$\underline{CH_2}$CH$_2$), 35.3 (-CONHCH$_2\underline{CH_2}$), 19.1 (-CH$\underline{CH_3}$); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{24}H_{25}N_2O_4S$ = 437.1529, found 437.1525.

2-(±)-(3-Benzoylphenyl)-*N*-(2-(4-sulfamoylphenoxy)ethyl)propanamide 3C. Ketoprofen **3** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxyethyl)amino)benzenesulfonamide **C** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was purified by silica gel column chromatography using EtOAc/n-hexane 66% *v/v* to afford the title compound **3C** as a white solid in 9 % yield, silica gel TLC R_f 0.33 (EtOAc/n-hexane 66% *v/v*); mp 123.3 ºC; δ_H (400 MHz, DMSO-d_6) 2.11 (3H, d, *J* 7.2, -CH$\underline{CH_3}$), 3.48-3.45 (2H, m., -CONH$\underline{CH_2}$CH$_2$), 3.82-3.77 (1H, m, -$\underline{CH}$CH$_3$), 4.08 (2H, t, *J* 5.6, -CONHCH$_2\underline{CH_2}$), 7.07 (2H, d, *J* 8.8, Ar-H), 7.24 (2H, s, SO_2NH_2, exchange with D_2O), 7.52 (1H, t, *J* 7.6, Ar-H), 7.72-7.58 (5H, m, 5 x Ar-H), 7.77-7.75

(5H, m, 5 x Ar-H), 8.37-8.34 (1H, m, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 196.7 (C=O), 174.3 (C=O), 161.7, 143.6, 138.0, 137.8, 137.3, 133.6, 132.5, 130.5 (2 x Ar-C), 129.5 (2 x Ar-C), 129.5, 129.4, 129.0, 128.6 (2 x Ar-C), 115.4 (2 x Ar-C), 67.6 (-CONHCH$_2$<u>CH$_2$</u>), 45.7 (-<u>CH</u>CH$_3$), 39.1 (-CONH<u>CH$_2$</u>CH$_2$), 19.5 (-CH<u>CH$_3$</u>); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{24}H_{25}N_2O_5S$ = 453.1479, found 453.1471.

2-(±)-(3-Benzoylphenyl)-*N*-(3-(4-sulfamoylphenoxy)propyl)propanamide 3D. Ketoprofen **3** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxypropyl)amino)benzenesulfonamide **D** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product purified by silica gel column chromatography using Ac/DCM 20% *v/v* to yield the title compound **3D** as a white solid in 11% yield, silica gel TLC R_f 0.39 (Ac/DCM 20% *v/v*); mp 139.5 ºC; δ_H (400 MHz, DMSO-d_6) 1.39 (3H, d, *J* 6.8, -CH<u>CH$_3$</u>), 1.86 (2H, pent., *J* 6.8, -CONHCH$_2$<u>CH$_2$</u>CH$_2$), 3.22 (1H, m, -CONH<u>CH$_2$</u>CH$_2$CH$_2$), 3.70-3.80 (1H, m, -<u>CH</u>CH$_3$), 4.01 (2H, t, *J* 6.4, -CONHCH$_2$CH$_2$<u>CH$_2$</u>), 7.03 (2H, d, *J* 8.8, 2 x Ar-H), 7.24 (2H, s, SO_2NH_2, exchange with D_2O), 7.53 (1H, t, *J* 7.6, Ar-H), 7.62-7.58 (4H, m, 4 x Ar-H), 7.73-7.70 (1H, dd, *J* 1.6/7.6, Ar-H), 7.77-7.74 (5H, m, 5 x Ar-H), 8.20 (1H, t, *J* 5.6, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 196.7 (C=O), 173.9 (C=O), 161.8, 143.7, 138.0, 137.8, 137.1, 133.7, 132.5, 130.5, 129.5 (2 x Ar-C), 129.5 (2 x Ar-C), 129.4, 129.0, 128.6 (2 x Ar-C), 115.3 (2 x Ar-C), 66.5 (-CONHCH$_2$CH$_2$<u>CH$_2$</u>), 45.9 (-<u>CH</u>CH$_3$), 36.4, 29.5, 19.4 (-CH<u>CH$_3$</u>); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{25}H_{27}N_2O_5S$ = 467.1635, found 467.1643.

2-(±)-(3-Benzoylphenyl)-*N*-(4-(4-sulfamoylphenoxy)butyl)propanamide 3E. Ketoprofen **3** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-

hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxybuthyl)amino)benzenesulfonamide **E** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was triturated with DCM/Petroleum ether (5 % *v/v*) to afford the title compound **3E** as a white solid in 58% yield, silica gel TLC R_f 0.22 (Ethyl acetate/*n*-hexane 66 % *v/v*); mp 121.6 ºC; δ_H (400 MHz, DMSO-d_6) 1.39 (3H, d, *J* 6.8, -CH<u>CH</u>$_3$), 1.58-1.51 (2H, m, -CONHCH$_2$<u>CH$_2$</u>CH$_2$CH$_2$), 1.73-1.66 (2H, m, -CONHCH$_2$CH$_2$<u>CH$_2$</u>CH$_2$), 3.15-3.10 (2H, m, -CONH<u>CH$_2$</u>CH$_2$CH$_2$CH$_2$), 3.76-3.71 (1H, m, -<u>CH</u>CH$_3$), 4.03 (2H, t, *J* 6.4, -CONHCH$_2$CH$_2$CH$_2$<u>CH$_2$</u>), 7.06 (2H, d, *J* 9.2, 2 x Ar-H), 7.22(2H, s, SO_2NH_2, exchange with D_2O), 7.53 (1H, d, *J* 7.6, Ar-H), 7.75-7.57 (5H, d, *J* 7.6, 5 x Ar-H), 7.77-7.75 (5H, m, 5 x Ar-H), 8.11 (1H, t, *J* 5.6, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 196.7 (C=O), 173.7 (C=O), 161.9, 143.8, 138.0, 137.8, 137.0, 133.6, 132.5, 130.5 (2 x Ar-C), 129.5 (2 x Ar-C), 129.4, 129.4, 129.0, 128.6 (2 x Ar-C), 115.3 (2 x Ar-C), 68.5 (-CONHCH$_2$CH$_2$CH$_2$<u>CH$_2$</u>), 45.9 (-<u>CH</u>CH$_3$), 39.1, 26.8, 26.5, 19.4 (-CH<u>CH$_3$</u>); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{26}H_{29}N_2O_5S$ = 481.1792, found 481.1801.

2-(±)-(2-Fluoro-[1,1'-biphenyl]-4-yl)-*N*-(4-sulfamoylphenyl)propanamide 4A. Flurbiprofen **4** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), sulfonilamide **A** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in DMF (4.5 ml) were treated according to the **general procedure a** previously reported to yield the title compound **4A** as a white solid in 15.1 % yield, silica gel TLC R_f 0.26 (Ethyl acetate/n-hexane 50% *v/v*); mp 220.1 ºC; δ_H (400 MHz, DMSO-d_6) 1.51 (3H, d, *J* 7.0, -CH<u>CH$_3$</u>), 4.00-3.95 (1H, q, *J* 7.0, -<u>CH</u>CH$_3$), 7.30 (2H, s, SO_2NH_2, exchange with D_2O), 7.34 (1H, s, biphenyl-H), 7.37 (1H, dd, *J* 1.5/5.5, biphenyl-H), 7.45-7.41 (1H, m, biphenyl-H), 7.58-7.49 (5H, m, 5 x biphenyl-H), 7.80 (4H, s, 4 x Ar-H), 10.51 (1H, s, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 173.1 (C=O), 159.7 (d, $J_{C\text{-}F}$ = 246.1), 144.2 (d, $J_{C\text{-}F}$

= 7.7), 142.8, 139.4, 135.7, 135.7, 131.6, 131.6, 129.6, 129.6, 129.5 (2 x Ar-C), 128.7, 127.7, 127.6 (2 x Ar-C), 124.7, 124.7, 119.7, 115.8 (d, $J_{C\text{-}F}$ = 23.3), 46.4 (-<u>CH</u>CH_3), 19.3 (-CH<u>CH_3</u>). δ_F (376 MHz, DMSO-*d*$_6$) -118.40 (1F, s).

Experimental in agreement with reported data. [19]

2-(±)-(2-Fluoro-[1,1'-biphenyl]-4-yl)-*N*-(4-sulfamoylphenethyl)propanamide 4B. Flurbiprofen **4** (1.0 eq.), *N,N'*-dicyclohexylcarbodiimide (DCC, 1.1 eq.), *N*-hydroxysuccinimide (NHS, 1.1 eq.) and 4-aminoethylbenzenesulfonamide **B** (2.0 eq.) dissolved in dry DMF (1.5 ml), were treated according to the **general procedure b** previously reported to afford the titled compound **4B** as a white solid in 28% yield, silica gel TLC R_f 0.19 (MeOH/DCM 5 % *v/v*); mp 154.1 °C; δ_H (400 MHz, DMSO-*d*$_6$) 1.37 (3H, d, *J* 7.0, -CH<u>CH_3</u>), 2.82 (2H, t, *J* 6.8-CONHCH$_2$<u>CH_2</u>), 3.31-3.33 (2H, m, -CONH<u>CH_2</u>CH_2), 3.69-3.64 (1H, m, -<u>CH</u>CH_3), 7.27-7.20 (2H, m, biphenyl-H), 7.30 (2H, s, SO_2NH_2, exchange with D_2O), 7.34 (2H, d, *J* 8.3, Ar-H), 7.42 (1H, ddd, *J* 1.2/7.2/14.4 biphenyl-H), 7.53-7.47 (3H, m, biphenyl-H), 7.59-7.57 (2H, m, biphenyl-H), 7.74 (2H, d, *J* 8.3, Ar-H) , 8.17-8.14 (1H, t, *J* 5.8, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-*d*$_6$) 173.7 (C=O), 159.8 (d, $J_{C\text{-}F}$ = 245.7), 145.2 (d, $J_{C\text{-}F}$ = 7.8), 144.7, 143.1, 136.0, 136.0, 131.5, 131.5, 130.2, 129.8, 129.7 (2 x Ar-C), 128.8, 127.5, 127.3, 126.6, 124.8 (2 x Ar-C), 124.8, 115.9 (d, $J_{C\text{-}F}$ = 23.1), 45.6 (-<u>CH</u>CH_3), 34.3 (-CONH<u>CH_2</u>CH_2), 31.6 (-CONHCH$_2$<u>CH_2</u>), 19.3 (-CH<u>CH_3</u>); δ_F (376 MHz, DMSO-*d*$_6$) -118.76 (1F, s); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{23}H_{24}FN_2O_3S$ = 427.1486, found 427.1478.

2-(±)-(2-Fluoro-[1,1'-biphenyl]-4-yl)-*N*-(2-(4-sulfamoylphenoxy)ethyl)propan amide 4C. Flurbiprofen **4** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-

hydroxyethyl)amino)benzenesulfonamide **C** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was triturated with diethyl ether to afford the title compound **4C** as a white solid in 54% yield, silica gel TLC R_f 0.18 (EtOAc/n-hexane 66% *v/v*); mp 152.6 ºC; δ_H (400 MHz, DMSO-d_6) 1.40 (3H, d, *J* 7.2, -CH$\underline{CH_3}$), 3.49 (2H, dd, *J* 5.6/8.4, -CONH$\underline{CH_2}CH_2$), 3.78-3.73 (1H, d, *J* 7.2, -$\underline{CH}CH_3$), 4.11 (2H, t, *J* 5.6, -CONHCH$_2\underline{CH_2}$), 7.11 (2H, d, *J* 8.8, AA'BB'), 7.24 (2H, s, SO_2NH_2, exchange with D_2O), 7.29-7.26 (2H, m, biphenyl-H), 7.56-7.41 (6H, m, 6 x biphenyl-H), 7.77 (2H, d, *J* 8.8, Ar-H) 8.35 (1H, t, *J* 5.6, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 174.1 (C=O), 161.7, 159.7 (d, $J_{C\text{-}F}$ = 244.3), 145.0 (d, $J_{C\text{-}F}$ = 7.6), 137.3, 135.9, 131.4, 131.4, 129.6, 129.6, 129.5, 128.6 (3 x Ar-C), 128.6 (2 x Ar-C), 127.4, 127.2, 124.7, 124.7, 115.8 (d, $J_{C\text{-}F}$=23.0), 115.4 (2 x Ar-C), 67.6 (-CONHCH$_2$CH$_2\underline{CH_2}$), 45.4 (-$\underline{CH}CH_3$), 39.1 (-CONH$\underline{CH_2}CH_2CH_2$), 19.3 (-CH$\underline{CH_3}$); δ_F (376 MHz, DMSO-d_6) -118.75 (1F, s); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{23}H_{24}FN_2O_4S$ = 443.1435, found 443.1443.

2-(±)-(2-Fluoro-[1,1'-biphenyl]-4-yl)-*N*-(3-(4-sulfamoylphenoxy)propyl) propanamide 4D. Flurbiprofen **4** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxypropyl)amino)benzenesulfonamide **D** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product purified by silica gel column chromatography using Ac/DCM 20% *v/v* and crystallized from ethanol to yield the title compound **4D** as a white solid in 66% yield, silica gel TLC R_f 0.37 (Ac/DCM 20% *v/v*); mp 123.3 ºC; δ_H (400 MHz, DMSO-d_6) 1.39 (3H, d, *J* 7.0, -CH$\underline{CH_3}$), 1.90-1.87 (2H, m, -CONHCH$_2\underline{CH_2}$CH$_2$), 3.29-3.21 (2H, m, -CONH$\underline{CH_2}$CH$_2$CH$_2$), 3.69 (1H, q, *J* 3.6, -$\underline{CH}CH_3$), 4.05 (2H, t, *J* 6.6, -CONHCH$_2$CH$_2\underline{CH_2}$), 7.08-7.05 (2H, d, *J* 9.0, Ar-H), 7.24 (2H, s, SO_2NH_2, exchange with D_2O), 7.28-7.27 (2H, m, biphenyl-H), 7.56-7.41 (6H, m,

biphenyl-H), 7.76 (2H, d, *J* 8.9, Ar-H), 8.19 (1H, t, *J* 5.4, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 173.7 (C=O), 161.8, 159.7 (d, J_{C-F} =246.4), 145.2 (d, J_{C-F} = 8.1), 137.1, 135.9, 131.4, 131.4, 129.6 (2 x Ar-C), 129.6 (2 x Ar-C), 129.5, 128.7, 128.6, 127.3, 127.2, 124.7, 124.7, 115.7 (d, J_{C-F} = 23.2), 115.3, 66.5 (-$CONHCH_2CH_2\underline{CH_2}$), 45.6 (-$\underline{CH}CH_3$), 31.6 (-$CONH\underline{CH_2}CH_2CH_2$), 29.5 (-$CONHCH_2\underline{CH_2}CH_2$), 19.3 (-$CH\underline{CH_3}$); δ_F (376 MHz, DMSO-d_6) -118.76 (1F, s); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{24}H_{26}FN_2O_4S$ = 457.1592, found 457.1593.

2-(2-Fluoro-[1,1'-biphenyl]-4-yl)-*N*-(4-(4-sulfamoylphenoxy)butyl)propanamide 4E.

Flurbiprofen **4** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxybuthyl)amino)benzenesulfonamide **E** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was triturated with DCM/Petroleum ether (5 % *v/v*) to afford the title compound **4E** as a white solid in 59.6% yield, silica gel TLC R_f 0.2 (Ethyl acetate/n-hexane 66% *v/v*); mp 126.7 ºC; δ_H (400 MHz, DMSO-d_6) 1.37 (3H, d, *J* 7.2, -$CH\underline{CH_3}$), 1.55 (2H, p, *J* 6.8 ,-$CONHCH_2\underline{CH_2}CH_2CH_2$), 1.70 (2H, p, *J* 6.8 -$CONHCH_2CH_2\underline{CH_2}CH_2$), 3.11 (2H, p, *J* 6.8 -$CONH\underline{CH_2}CH_2CH_2CH_2$), 3.69-3.64 (1H, m, -$\underline{CH}CH_3$), 4.03 (2H, t, *J* 6.4, -$CONHCH_2CH_2CH_2\underline{CH_2}$), 7.06 (2H, d, *J* 8.8, Ar-H), 7.19 (2H, s, SO_2NH_2, exchange with D_2O), 7.23-7.26 (2H, s, biphenyl-H), 7.54-7.37 (6H, m, biphenyl-H), 7.73 (2H, d, *J* 8.8, Ar-H), 8.07 (1H, d, *J* 5.6, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 173.6 (C=O), 161.9, 159.7 (d, J_{C-F} = 244.2), 145.2 (d, J_{C-F} = 7.7), 137.0, 135.9, 135.9, 131.4, 131.3, 129.6, 129.6, 129.5 (3 x Ar-C), 128.6 (2 x Ar-C), 127.3, 127.2, 124.7, 124.7, 115.7 (d, J_{C-F}=23.0), 115.3 (2 x Ar-C), 68.5 (-$CONHCH_2CH_2\underline{CH_2}$), 45.6 (-$\underline{CH}CH_3$), 39.2 (-$CONH\underline{CH_2}CH_2CH_2$), 26.8, 26.5, 19.3 (-$CH\underline{CH_3}$); δ_F

(376 MHz, DMSO-d_6) -118.78 (1F, s); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{25}H_{28}FN_2O_4S$ = 471.1748, found 471.1751.

5-Benzoyl-*N*-(4-sulfamoylphenyl)-2,3-dihydro-1*H*-pyrrolizine-1-carboxamide 5A. Ketorolac **5** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), sulfonilamide **A** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in DMF (4.5 ml) were treated according to the **general procedure a** previously reported to yield the title compound **5A** as a white solid in 10% yield, silica gel TLC R_f 0.19 (Ethyl acetate/n-hexane 50% *v/v*); mp 201.2 ºC; δ_H (400 MHz, DMSO-d_6) 2.91-2.83 (5H, s, 4 x pyrrolizine-CH_2, pyrrolizine-CH), 7.31 (2H, s, SO_2NH_2, exchange with D_2O), 7.84-7.56 (11H, m, 2 x pyrrole-H, 9 x Ar-H), 10.49 (1H, m, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 196.4 (C=O), 173.2 (C=O), 142.8, 142.8, 139.3, 137.8, 133.5, 132.4, 130.4 (2 x Ar-C), 129.6, 129.4 (2 x Ar-C), 129.3, 127.5 (2 x pyrrolizine=CH), 119.6(2 x pyrrolizine=CH), 46.7 (pyrrolizine-CH), 31.5 (pyrrolizine-CH_2), 19.4 (pyrrolizine-CH_2); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{21}H_{20}N_3O_4S$ = 410.1169, found 410.1170.

5-Benzoyl-*N*-(4-sulfamoylphenethyl)-2,3-dihydro-1*H*-pyrrolizine-1-carboxamide 5B. Ketorolac **5** (1.0 eq.), *N,N'*-dicyclohexylcarbodiimide (DCC, 1.1 eq.), *N*-hydroxysuccinimide (NHS, 1.1 eq.) and 4-aminoethylbenzenesulfonamide **B** (2.0 eq.) dissolved in dry DMF (1.5 ml) were treated according to the **general procedure b** previously reported to afford the titled compound **5B** as a white solid in 4.5 % yield, silica gel TLC R_f 0.32 (MeOH/DCM 5 % *v/v*); mp 197.3 ºC; δ_H (400 MHz, DMSO-d_6) 2.73-2.69 (2H, q., *J* 7.2, -CONHCH$_2$<u>CH$_2$</u>), 2.89-2.85 (2H, t, *J* 7.0, -CONH<u>CH$_2$</u>CH$_2$), 3.47-3.39 (1H, m, pyrrolizine-CH_2), 3.96-3.92 (1H, m, pyrrolizine-CH_2), 4.08 (1H, s, pyrrolizine-CH), 4.28-4.35 (1H, m, pyrrolizine-CH_2), 4.40-4.46 (1H, m, pyrrolizine-CH_2), 5.93 (1H, d, *J* 3.8, pyrrole-H), 6.79 (1H, d, *J* 4.0, pyrrole-H), 7.34 (2H, s, SO_2NH_2, exchange with

D_2O), 7.44 (2H, d, *J* 8.4, 2 x Ar-H), 7.57-7.54 (2H, m, 2 x Ar-H), 7.65-7.62 (1H, m, Ar-H), 7.80-7.77 (4H, m, 4 x Ar-H), 8.39-8.37 (1H, t, *J* 5.2, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 183.9 (C=O), 170.8 (C=O), 145.8, 144.0, 142.6, 139.39, 131.8, 129.6 (2 x Ar-C), 128.9 (2 x Ar-C), 128.8 (2 x Ar-C), 126.4, 126.1 (2 x Ar-C), 124.9, 102.8, 48.2, 43.2, 35.1, 31.3, 31.1; ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{23}H_{24}N_3O_4S$ = 438.1482, found 438.1489.

5-Benzoyl-*N*-(3-(4-sulfamoylphenoxy)propyl)-2,3-dihydro-1*H*-pyrrolizine-1-carboxamide 5D. Ketorolac **5** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxypropyl)amino)benzenesulfonamide **D** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product purified by silica gel coloumn chromatography using Ac/DCM 14.3 % *v/v* to yield the title compound **5D** as a white solid in 58% yield, silica gel TLC R_f 0.41 (Ac/DCM 14.29% *v/v*); mp 284.3 ºC; δ_H (400 MHz, DMSO-d_6) 1.27 (2H, s, -CONHCH$_2$<u>CH$_2$</u>CH$_2$), 2.93-2.81 (2H, m, pyrrolizine-CH$_2$), 4.27-4.24 (1H, m, pyrrolizine-CH), 4.52-4.38 (2H, m, pyrrolizine-CH$_2$), 6.17 (1H, d, *J* 4.0, pyrrole-H), 6.81 (1H, d, *J* 4.0, pyrrole-H), 7.30 (2H, s, SO_2NH_2, exchange with D_2O), 7.57-7.53 (2H, m, 2 x Ar-H), 7.65-7.61 (1H, m, Ar-H), 7.80 (2H, m, 2 x Ar-H), 7.82 (4H, s, 4 x Ar-H), 10.75 (1H, s, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 184.6 (C=O), 170.9 (C=O), 145.5, 142.9, 139.9, 139.7, 132.5, 129.5 (2 x Ar-C), 129.4 (2 x Ar-C), 127.7 (2 x pyrrolizine=CH), 127.2 125.5, 120.0 (2 x pyrrolizine=CH), 103.7, 48.8, 44.9, 32.0; ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{24}H_{26}N_3O_5S$ = 468.1588, found 468.1589.

2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)-*N*-(4-sulfamoyl phenyl) acetamide 6A. Sulindac **6** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.),

sulfonilamide **A** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in DMF (4.5 ml) were treated according to the **general procedure a** previously reported to yield the title compound **6A** as a yellow solid in 2% yield, silica gel TLC R_f 0.14 (MeOH/DCM 7.5 % *v/v*); mp 285.4 ºC; δ_H (400 MHz, DMSO-d_6) 2.27 (3H, s, indene- CH_3), 2.86 (3H, s, SO_2CH_3), 3.78 (2H, s, -$\underline{CH_2}$CONH), 6.77 (1H, m, indene-H), 7.24-7.19 (2H, m, 2 x indene-H), 7.30 (2H, s, exchange with D_2O), 7.43 (1H, s, vinyl-H), 7.77 (2H, d, *J* 8.0, 2 x Ar-H), 7.80 (3H, s, 3 x Ar-H), 7.82 (2H, d, *J* 8.0, 2 x Ar-H), 10.61 (1H, s, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 169.4 (C=O), 163.5 (d, $J_{C\text{-}F}$ = 244.4), 148.2, 148.2, 148.1, 147.2, 142.9, 141.3, 139.46, 139.4, 139.3, 133.9, 130.9 (2 x Ar-C), 130.6, 130.4, 127.7 (2 x Ar-C), 124.9 (2 x Ar-C), 124.1 (d, $J_{C\text{-}F}$ =9.1), 119.7 (2 x Ar-C), 111.4 (d, $J_{C\text{-}F}$ =23.2), 107.3, 107.1 (d, $J_{C\text{-}F}$ = 24.2), 44.1, 34.5, 11.4; δ_F (376 MHz, DMSO-d_6) -113.49 (1F, s); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{26}H_{24}FN_2O_4S_2$ = 511.1156, found 511.1147.

2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)-*N*-(4-sulfamoyl phenethyl)acetamide 6B. Sulindac **6** (1.0 eq.), *N,N'*-dicyclohexylcarbodiimide (DCC, 1.1 eq.), *N*-hydroxysuccinimide (NHS, 1.1 eq.) and 4-aminoethylbenzenesulfonamide **B** (2.0 eq.) dissolved in dry DMF (1.5 ml) were treated according to the **general procedure b** previously reported to afford the titled compound **6B** as a yellow solid in 26% yield, silica gel TLC R_f 0.12 (MeOH/DCM 5 % *v/v*); mp 152 ºC; δ_H (400 MHz, DMSO-d_6) 2.19 (3H, s, indene-CH_3), 2.83 (2H, t, *J* 6.8, -CONHCH$_2\underline{CH_2}$), 2.86 (3H, s, SO_2CH_3), 3.34 (2H, m, -CONH$\underline{CH_2}CH_2$), 3.45 (2H, s, -$\underline{CH_2}$CONH), 6.78-6.73 (1H, m, indene-H), 7.15 (1H, dd, *J* 9.3/2.4 Hz, indene-H), 7.22-7.19 (1H, m, indene-H), 7.33 (2H, s, SO_2NH_2, exchange with D_2O), 7.39 (2H, d, *J* 2.0, 2 x Ar-H), 7.41 (1H, s, vinyl-H), 7.78-7.75 (4H, m, 4 x Ar-H), 7.84-7.82 (2H, m, 2 x Ar-H), 8.23-8.21 (1H, t, *J* 5.6, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 169.4 (C=O), 163.1 (d, $J_{C\text{-}F}$ =243.5), 147.9, 147.8, 146.8, 144.3, 142.7, 141.1, 139.2, 138.4, 134.2, 134.2, 130.5 (2 x Ar-C), 130.1, 130.1, 129.8, 129.7 (2 x Ar-C), 126.3 (2 x Ar-C), 124.5 (2 x Ar-C), 123.7 (d, $J_{C\text{-}F}$ =9.8), 110.9 (d, $J_{C\text{-}F}$ =22.8), 106.9 (d,

$J_{C\text{-}F}$ =23.6), 56.6, 43.7, 35.4, 33.3, 11.0; δ_F (376 MHz, DMSO-d_6) -113.52 (1F, s); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{28}H_{28}FN_2O_4S_2$ = 539.1469, found 539.1464.

2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)-*N*-(2-(4-sulfamoylphenoxy)ethyl)acetamide 6C. Sulindac **6** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxyethyl)amino)benzenesulfonamide **C** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was triturated with EtOH to afford the title compound **6C** as a yellow solid in 29 % yield, silica gel TLC R_f 0.28 (Ethyl acetate 100 %); mp 200.7 ºC; δ_H (400 MHz, DMSO-d_6) 2.21 (3H, s, indene-CH_3), 2.86 (3H, s, SO_2CH_3), 3.51 (4H, t, *J* 8.2, -CONH$\underline{CH_2}CH_2$), 4.13 (2H, t, *J* 5.4, -CONHCH$_2\underline{CH_2}$), 6.76-6.71 (1H, m, indene-H), 7.11 (2H, d, *J* 9.2, 2 x indene-H), 7.21-7.14 (2H, m, 2 x Ar-H), 7.24 (2H, s, SO_2NH_2, exchange with D_2O), 7.38 (1H, s, vinyl-H), 7.84-7.74 (6H, m, 2 x Ar-H), 8.44 (1H, t, *J* 5.6, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 170.2 (C=O), 163.5 (d, $J_{C\text{-}F}$ =244.8), 161.7, 148.2, 148.1, 147.1, 141.4, 139.5, 138.8, 137.3, 134.5, 134.5, 130.9 (2 x Ar-C), 130.4, 130.4, 130.2, 128.6 (2 x Ar-C), 124.9, 124.0 (d, $J_{C\text{-}F}$ =9.2), 115.4 (2 x Ar-C), 111.2 (d, $J_{C\text{-}F}$ =22.7), 107.1 (d, $J_{C\text{-}F}$ =24.01), 67.6, 44.0, 39.3, 33.6, 11.3; δ_F (376 MHz, DMSO-d_6) -113.79 (1F, s); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{28}H_{28}FN_2O_5S_2$ = 555.1418, found 555.1426.

2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)-*N*-(3-(4-sulfamoyl phenoxy)propyl)acetamide 6D. Sulindac **6** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxypropyl)amino)benzenesulfonamide **D** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the

general procedure b previously reported. The crude product was purified by silica gel coloumn chromatography using Ac/DCM 20% *v/v* and crystallized from EtOH to afford the title compound **6D** as a yellow solid in 35% yield, silica gel TLC R_f 0.48 (Ac/DCM 20% *v/v*); mp 213.8 ºC; δ_H (400 MHz, DMSO-d_6) 1.92 (2H, t, *J* 6.4, -CONHCH$_2$<u>CH$_2$</u>CH$_2$), 2.22 (3H, s, indene-CH$_3$), 2.86 (3H, s, SO$_2$CH$_3$), 3.27 (2H, q, *J* 6.4, CONH<u>CH$_2$</u>CH$_2$CH$_2$), 3.46 (1H, s, -<u>CH$_2$</u>CONH), 4.10-4.07 (2H, t, *J* 6.2, -CONHCH$_2$CH$_2$<u>CH$_2$</u>), 6.77-6.72 (1H, m, indene-H), 7.07 (2H, d, *J* 9.2, 2 x indene-H), 7.23-7.15 (4H, m, SO$_2$NH$_2$, exchange with D$_2$O, 2 x Ar-H), 7.39 (1H, s, vinyl-H), 7.77-7.74 (4H, m, 4 x Ar-H), 7.84-7.82 (2H, m, 2 x Ar-H), 8.25-8.22 (1H, t, *J* 5.2, -CONH, exchange with D$_2$O); δ_C (100 MHz, DMSO-d_6) 169.8 (C=O), 163.5 (d, $J_{C\text{-}F}$ =243.2), 161.9, 148.3, 148.2, 147.2, 141.5, 139.6, 138.8, 137.2, 134.7, 134.6, 130.9 (2 x Ar-C), 130.5, 130.5, 130.3, 128.7 (2 x Ar-C), 125.0 (2 x Ar-C), 124.1 (d, $J_{C\text{-}F}$ =9.1), 115.4 (2 x Ar-C), 111.3 (d, $J_{C\text{-}F}$ =22.8), 107.2 (d, $J_{C\text{-}F}$ =23.6), 66.6, 44.1, 36.7, 33.9, 29.6, 11.4; δ_F (376 MHz, DMSO-d_6) -113.56 (1F, s); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{29}H_{30}FN_2O_5S_2$ = 569.1575, found 569.1572.

2-(5-Fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)-*N*-(4-(4-sulfamoylphenoxy)butyl)acetamide 6E. Sulindac **6** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxybuthyl)amino)benzenesulfonamide **E** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was triturated with Ac/EtOH to afford the title compound **6E** as a yellow solid in 17% yield, silica gel TLC R_f 0.14 (Ethyl acetate 100%); mp 209.6 ºC; δ_H (400 MHz, DMSO-d_6) 1.62-1.57 (2H, m, -CONHCH$_2$<u>CH$_2$</u>CH$_2$CH$_2$), 1.80-1.73 (2H, m, -CONHCH$_2$CH$_2$<u>CH$_2$</u>CH$_2$), 2.21(3H, s, indene-CH$_3$), 2.85 (3H, s, SO$_2$CH$_3$), 3.17 (2H, q, *J* 6.4, -CONH<u>CH$_2$</u>CH$_2$CH$_2$CH$_2$), 3.47 (2H, s, -<u>CH$_2$</u>CONH), 4.07 (2H, t, *J* 6.6, -CONHCH$_2$CH$_2$CH$_2$<u>CH$_2$</u>), 6.76-6.72 (1H, m, indene-H), 7.09 (2H, d, *J* 8.8, 2 x indene-H), 7.22-7.14

(2H, m, 2 x Ar-H), 7.23 (2H, s, SO_2NH_2, exchange with D_2O), 7.38 (1H, s, vinyl-H), 7.78-7.74 (4H, m, 4 x Ar-H), 7.82 (2H, d, *J* 8.4, Ar-H), 8.19 (1H, t, *J* 5.6, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 169.6 (C=O), 163.4 (d, $J_{C\text{-}F}$ =241.9), 161.9 (2 x Ar-C), 148.3, 148.2, 147.1, 141.4, 139.5, 138.6, 137.0, 134.7, 134.7, 130.8 (2 x Ar-C), 130.4, 130.4, 130.1, 128.6 (2 x Ar-C), 124.9 (2 x Ar-C), 124.0 (d, $J_{C\text{-}F}$ =9.2), 115.3, 111.2 (d, $J_{C\text{-}F}$ =22.5), 107.1 (d, $J_{C\text{-}F}$ =24.0), 68.5, 44.0, 39.3, 33.8, 26.9, 26.6, 11.3; δ_F (376 MHz, DMSO-d_6) -113.78 (1F, s); ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{30}H_{32}FN_2O_5S_2$ = 583.1731, found 583.1731.

2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-*N*-(4-sulfamoyl phenyl)acetamide 7A. Indometacin **7** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), sulfonilamide **A** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in DMF (4.5 ml) were treated according to the **general procedure a** previously reported to yield the title compound **7A** as a white solid in 17% yield, silica gel TLC R_f 0.23 (Ethyl acetate/n-hexane 50% *v/v*); mp 244.4 ºC; δ_H (400 MHz, DMSO-d_6) 2.32 (3H, s, indol-CH_3), 3.78 (3H, s, -OCH_3), 3.83 (2H, s, -$\underline{CH_2}$CONH), 6.77-6.74 (1H, dd, *J* 2.4/4.8, indol-H), 6.96 (1H, d, *J* 9.0, indol-H), 7.21 (1H, d, *J* 2.5, indol-H), 7.27 (2H, s, SO_2NH_2, exchange with D_2O), 7.71 (4H, dd, *J* 8.4, 4 x Ar-H), 7.79 (4H, s, 4 x Ar-H), 10.58 (1H, s, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO- d_6) 170.2 (C=O), 169.0 (C=O), 156.7, 143.0, 139.5, 138.7, 136.6, 135.3, 132.3 (2 x Ar-C), 131.9, 131.4, 130.2 (2 x Ar-C), 127.8 (2 x Ar-C), 119.9 (2 x Ar-C), 115.7, 114.8, 112.3, 103.0, 56.6 (-OCH_3), 33.1, 14.5; ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{25}H_{23}ClN_3O_5S$ = 512.1041, found 512.1049.

2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-*N*-(4-sulfamoyl phenethyl)acetamide 7B. Indometacin **7** (1.0 eq.), *N,N'*-dicyclohexylcarbodiimide (DCC, 1.1 eq.), *N*-hydroxy succin imide (NHS, 1.1 eq.) and 4-aminoethylbenzenesulfonamide **B** (2.0 eq.) dissolved

in dry DMF (1.5 ml) were treated according to the **general procedure b** previously reported to afford the titled compound **7B** as a white solid in 1% yield, silica gel TLC R_f 0.21 (MeOH/DCM 5 % *v/v*); mp 210.3 ºC; δ_H (400 MHz, DMSO-d_6) 2.26 (3H, s, indol-CH_3), 2.82 (2H, s, -CONHCH$_2$<u>CH$_2$</u>), 3.37-3.53 (4H, m, -CONH<u>CH$_2$</u>CH$_2$, -<u>CH$_2$</u>CONH), 3.80 (3H, s,-OCH_3), 6.77 (1H, m, indol-H), 6.98 (1H, d, *J* 8.8, indol-H), 7.16 (1H, s, indol-H), 7.31 (2H, s, SO_2NH_2, exchange with D_2O), 7.39 (2H, d, *J* 7.8, 2 x Ar-H), 7.74-7.70 (6H, m, 6 x Ar-H), 8.16 (1H, s, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 170.4 (C=O), 168.8 (C=O), 156.5, 144.7, 143.0, 138.5, 136.2, 135.2, 132.1 (2 x Ar-C), 131.9, 131.3, 130.1 (2 x Ar-C), 130.0 (2 x Ar-C), 126.6 (2 x Ar-C), 115.5, 115.2, 112.1, 103.0, 56.4 (-OCH_3), 35.9, 32.1, 31.7, 14.3; ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{27}H_{27}ClN_3O_5S$ = 540.1354, found 540.1363.

2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-*N*-(2-(4-sulfamoylphenoxy)ethyl)acetamide 7C. Indometacin **7** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxyethyl)amino)benzenesulfonamide **C** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was triturated with DCM/Petroleum ether (10% *v/v*) to afford the title compound **7C** as a white solid in 13% yield, silica gel TLC R_f 0.22 (EtOAc/n-hexane 66% *v/v*), mp 197.5 ºC; δ_H (400 MHz, DMSO-d_6) 2.26 (3H, s, indol-CH_3), 3.47 (2H, q, *J* 5.6, -CONH<u>CH$_2$</u>CH$_2$), 3.58 (2H, s, -CH_2CONH), 3.74 (3H, s, -OCH_3), 4.13-4.10 (2H, q, *J* 5.6, -CONHCH$_2$<u>CH$_2$</u>), 6.74 (1H, dd, *J* 2.6/9.4, indol-H), 6.98 (1H, d, *J* 9.2, indol-H), 7.09 (2H, d, *J* 7.2, 2 x Ar-H), 7.15-7.14 (1H, m, indol-H), 7.24 (2H, brs, SO_2NH_2, exchange with D_2O), 7.68 (2H, d, *J* 8.8, Ar-H), 7.73 (2H, d, *J* 8.8, 2 x Ar-H), 7.77 (2H, d, *J* 9.2, 2 x Ar-H), 8.35 (1H, t, *J* 5.6, -CONH, exchange with D_2O); δ_C (101 MHz, DMSO-d_6) 170.8 (C=O), 168.8 (C=O), 161.7, 156.5, 138.5, 137.3, 136.1, 135.2, 132.1 (2 x Ar-C), 131.8, 131.2, 130.0 (2 x Ar-C), 128.6 (2 x Ar-C),

115.5, 115.4 (2 x Ar-C), 115.2, 112.1, 102.9, 67.7, 56.3, 39.2, 32.0, 14.3; ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{27}H_{27}ClN_3O_6S$ = 556.1304, found 556.1303.

2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-*N*-(3-(4-sulfamoylphenoxy)propyl)acetamide 7D. Indometacin **7** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxypropyl)amino)benzenesulfonamide **C** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was purified by silica gel column chromatography using Ac/DCM 14.3 % *v/v* to afford the title compound **7D** as a white solid in 3% yield, silica gel TLC R_f 0.24 (Ac/DCM 14.3 % *v/v*); mp 116.7 ºC; δ_H (400 MHz, DMSO-d_6) 1.87-1.94 (2H, m, -CONHCH$_2$<u>CH$_2$</u>CH$_2$), 2.27 (3H, s, indol-CH$_3$), 3.24-3.29 (2H, m, -CONH<u>CH$_2$</u>CH$_2$CH$_2$), 3.54 (2H, s, -<u>CH$_2$</u>CONH), 3.77 (3H, s, -OCH$_3$), 4.06 (2H, t, *J* 6.4, -CONHCH$_2$CH$_2$<u>CH$_2$</u>), 6.76-6.73 (1H, dd, *J* 2.4/9.2, indol-H), 6.98 (1H, d, *J* 9.2, indol-H), 7.03 (2H, d, *J* 8.8, 2 x Ar-H), 7.15 (1H, d, *J* 2.4, indol-H), 7.24 (2H, s, SO_2NH_2, exchange with D_2O),7.67 (2H, d, *J* 8.8, 2 x Ar-H), 7.72 (2H, d, *J* 8.8, 2 x Ar-H), 7.75 (2H, d, *J* 9.2, 2 x Ar-H), 8.17 (1H, t, *J* 5.6, CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 170.4 (C=O), 168.8 (C=O), 161.8, 156.5, 138.5, 137.1, 136.1, 135.2, 132.1 (2 x Ar-C), 131.8, 131.2, 130.0 (2 x Ar-C), 128.6 (2 x Ar-C), 115.5, 115.3 (2 x Ar-C), 115.3, 112.1, 102.8, 66.6, 56.4 (-OCH$_3$), 36.5, 32.2, 29.6, 14.3; ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{28}H_{29}ClN_3O_6S$ = 570.1460, found 570.1449.

2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-*N*-(4-(4-sulfamoyl phenoxy)butyl)acetamide 7E. Indometacin **7** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxybuthyl)amino)benzenesulfonamide **E** (2.0 eq.) and

N,N-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was triturated with DCM/Petroleum ether (10 % *v/v*) to afford the title compound **7E** as a white solid in 30% yield, silica gel TLC R_f 0.31 (Ethyl acetate/*n*-hexane 66 % *v/v*); mp 135.1 ºC; δ_H (400 MHz, DMSO-d_6) 1.56-1.63 (2H, m, -CONHCH$_2$<u>CH$_2$</u>CH$_2$CH$_2$), 1.79-1.72 (2H, m, -CONHCH$_2$CH$_2$<u>CH$_2$</u>CH$_2$), 2.27 (3H, s, indol-CH$_3$), 3.16 (2H, q, *J* 6.4, -CONH<u>CH$_2$</u>CH$_2$CH$_2$CH$_2$), 3.54 (2H, s, -<u>CH$_2$</u>CONH), 3.79 (3H, s, -OCH$_3$), 4.06 (2H, t, *J* 6.4, -CONHCH$_2$CH$_2$CH$_2$<u>CH$_2$</u>), 6.74 (1H, dd, *J* 2.5/9.0, indol-H), 6.99 (1H, d, *J* 9.0, indol-H), 7.08 (2H, d, *J* 9.0, 2 x Ar-H), 7.16 (1H, d, *J* 2.5, indol-H), 7.21 (2H, brs, SO_2NH_2, exchange with D_2O), 7.68 (2H, d, *J* 8.8, 2 x Ar-H), 7.73 (2H, d, *J* 8.8, 2 x Ar-H), 7.76 (2H, d, *J* 9.0, 2 x Ar-H), 8.09 (1H, t, *J* 5.6, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 170.2 (C=O), 168.8 (C=O), 162.0, 156.5, 138.5, 137.0, 136.0, 135.2, 132.0 (2 x Ar-C), 131.8, 131.2, 129.9 (2 x Ar-C), 128.6 (2 x Ar-C), 115.5, 115.3, 115.3 (2 x Ar-C), 112.2, 102.8, 68.5, 56.3, 39.2, 32.1, 26.9, 26.7, 14.3; ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{29}H_{31}ClN_3O_6S$ = 584.1617, found 584.1621.

2-(2-((2,6-Dichlorophenyl)amino)phenyl)-N-(4-sulfamoylphenyl)acetamide 8A. Diclofenac **8** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), sulfonilamide **A** (2.0 eq.) and *N*-methylmorpholine (NMM, 2.0 eq.) in DMF (4.5 ml) were treated according to the to the **general procedure a** previously reported to yield the title compound **8A** as a white solid in 2% yield, silica gel TLC R_f 0.29 (Ethyl acetate/*n*-hexane 50% *v/v*); mp 251.9 ºC; δ_H (400 MHz, DMSO-d_6) 3.88 (2H, s, -<u>CH$_2$</u>CONH), 6.34 (1H, d, *J* 8.4, Ar-H), 6.92 (1H, td, *J* 1.2/6.4, Ar-H), 7.13-7.09 (1H, m, Ar-H), 7.24 (1H, t, *J* 8.2, Ar-H), 7.30 (2H, s, SO_2NH_2, exchange with D_2O), 7.33 (1H, dd, *J* 1.2/7.3, Ar-H), 7.58 (2H, d, *J* 8.0, Ar-H), 7.81 (4H, s, 4 x Ar-H), 7.79 (1H, s, NH), 10.71 (1H, s, -CONH, exchange with D_2O).

Experimental in agreement with reported data.[19]

2-(2-((2,6-Dichlorophenyl)amino)phenyl)-*N*-(4-sulfamoylphenethyl)acetamide 8B. Dichlofenac **8** (1.0 eq.), *N,N'*-dicyclohexylcarbodiimide (DCC, 1.1 eq.), *N*-hydroxysuccinimide (NHS, 1.1 eq.) and 4-aminoethylbenzenesulfonamide **B** (2.0 eq.) dissolved in dry DMF (1.5 ml) were treated according to the **general procedure b** previously reported to afford the titled compound **8B** as a white solid in 37% yield, silica gel TLC R_f 0.24 (MeOH/DCM 5 % *v/v*); mp 186.3 ºC; δ_H (400 MHz, DMSO-d_6) 2.85 (2H, t, *J* 7.0, -CONHCH$_2$CH$_2$), 3.40-3.37 (2H, m, -CONHCH$_2$CH$_2$), 3.60 (2H, s, -CH$_2$CONH), 6.33 (1H, d, *J* 7.8, Ar-H), 6.89 (1H, td, *J* 1.0/7.4, Ar-H), 7.11-7.06 (1H, m, Ar-H), 7.22-7.18 (1H, m, Ar-H), 7.20 (1H, dd, *J* 1.4/7.5, Ar-H), 7.32 (2H, s, SO_2NH_2, , exchange with D_2O), 7.39 (2H, d, *J* 8.3, Ar-H), 7.55 (2H, d, *J* 8.1, 2 x Ar-H), 7.75 (2H, d, *J* 8.4, Ar-H) , 8.37 (1H, s, NH), 8.50-8.47 (1H, t, *J* 6.0, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 172.5 (C=O), 144.4, 143.9, 143.0, 138.1, 131.3, 130.4 (2 x Ar-C), 130.1 (2 x Ar-C), 130.0 (2 x Ar-C), 128.1, 126.6 (2 x Ar-C), 126.3, 126.0, 121.5, 116.8, 35.4, 34.2, 31.6; ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{22}H_{22}Cl_2N_3O_3S$ = 478.0753, found 478.0750.

2-(2-((2,6-Dichlorophenyl)amino)phenyl)-*N*-(2-(4-sulfamoylphenoxy)ethyl) acetamide 8C. Dichlofenac **8** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxyethyl)amino)benzenesulfonamide **C** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was triturated with EtOH to afford the title compound **8C** as a white solid in 27% yield, silica gel TLC R_f 0.64 (Ethyl acetate/n-hexane 50% *v/v*); mp 210.2 ºC; 3.53 (2H, q., *J* 5.6, -CONHCH$_2$CH$_2$), 3.66 (2H, s, -CH$_2$CONH), 4.14 (2H, t, *J* 5.4, -CONHCH$_2$CH$_2$), 6.33 (1H, d, *J* 8.0, Ar-H), 6.87 (1H, td, *J* 7.2/0.8, Ar-H), 7.07 (1H, td, *J* 8.0/1.6, Ar-H), 7.12 (2H, d, *J* 8.8, Ar-H), 7.22-

7.18 (2H, m, 2 x Ar-H), 7.24 (2H, s, SO_2NH_2, exchange with D_2O), 7.55 (2H, d, *J* 8.0, 2 x Ar-H), 7.77 (2H, d, *J* 8.8, Ar-H), 8.33 (1H, s, NH), 8.70-8.68 (1H, t, *J* 5.4, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 172.9 (C=O), 161.7, 143.9, 138.1, 137.3, 131.4, 130.5, 130.1 (2 x Ar-C), 128.6 (2 x Ar-C), 128.2, 126.2 (2 x Ar-C), 126.0, 121.6, 116.9, 115.5 (2 x Ar-C), 67.6, 39.3, 31.6; ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{22}H_{22}Cl_2N_3O_4S$ = 494.0703, found 494.0702.

2-(2-((2,6-Dichlorophenyl)amino)phenyl)-*N*-(3-(4-sulfamoylphenoxy)propyl) acetamide 8D. Dichlofenac **8** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxypropyl)amino)benzenesulfonamide **D** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was purified by silica gel column chromatography using Ethyl acetate/n-hexane 50% *v/v* to afford the title compound **8D** as a white solid in 55% yield, silica gel TLC R_f 0.20 (Ethyl acetate/n-hexane 50% *v/v*); mp 190.2 ºC; 1.93 (2H, q., *J* 6.4, -CONHCH$_2$<u>CH$_2$</u>CH$_2$) 3.31-3.26 (2H, q., *J* 6.4, -CONH<u>CH$_2$</u>CH$_2$CH$_2$), 3.62 (2H, s, -<u>CH$_2$</u>CONH), 4.03 (2H, t, *J* 6.2, -CONHCH$_2$CH$_2$<u>CH$_2$</u>), 6.33 (1H, d, *J* 7.8, Ar-H), 6.88 (1H, dt, *J* 7.2/1.2, Ar-H), 7.09-7.06 (3H, m, 3 x Ar-H), 7.23-7.18 (4H, m, 2 x Ar-H, SO_2NH_2, exchange with D_2O), 7.55(2H, d, *J* 8.0, 2 x Ar-H), 7.77-7.75(2H, m, 2 x Ar-H), 8.38 (1H, s, NH), 8.50 (1H, t, *J* 5.4, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO- d_6) 172.5 (C=O), 161.8, 143.9, 138.1, 137.1, 131.3, 130.4 (2 x Ar-C), 130.1 (2 x Ar-C), 128.5 (2 x Ar-C), 128.1, 126.3, 126.0, 121.6, 116.8, 115.3 (2 x Ar-C), 66.5, 40.5, 36.6, 29.4; ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{23}H_{24}Cl_2N_3O_4S$ = 508.0859, found 508.0850.

2-(2-((2,6-Dichlorophenyl)amino)phenyl)-*N*-(4-(4-sulfamoylphenoxy)butyl) acetamide 8E. Dichlofenac **8** (1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt

(EDCI·HCl, 2.0 eq.) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.0 eq.), 4-((3-hydroxybuthyl)amino)benzenesulfonamide **E** (2.0 eq.) and *N,N*-diisopropylethylamine (DIPEA, 4.0 eq.) in dry DMA (4.5 ml) were treated according to the **general procedure b** previously reported. The crude product was triturated with diethyl ether to afford the title compound **8E** as a white solid in 46% yield, silica gel TLC R_f 0.35 (Ethyl acetate/*n*-hexane 66% *v/v*); mp 170.5 ºC; δ_H (400 MHz, DMSO-d_6) 1.65-1.61 (2H, m, -CONHCH$_2$<u>CH$_2$</u>CH$_2$CH$_2$), 1.81-1.74 (2H, m, -CONHCH$_2$CH$_2$<u>CH$_2$</u>CH$_2$), 3.21-3.16 (2H, m, -CONH<u>CH$_2$</u>CH$_2$CH$_2$CH$_2$), 3.62 (2H, s, -<u>CH$_2$</u>CONH), 4.08 (2H, t, *J* 6.4, -CONHCH$_2$CH$_2$CH$_2$<u>CH$_2$</u>), 6.33 (1H, d, *J* 8.0, Ar-H), 6.90-6.86 (1H, m, Ar-H), 7.07-7.05 (1H, m, Ar-H), 7.09 (2H, d, *J* 8.8, Ar-H), 7.19 (1H, t, *J* 8.2, Ar-H), 7.23-7.21 (3H, m, Ar-H, SO_2NH_2, exchange with D_2O), 7.55(2H, d, *J* 8.0, 2 x Ar-H), 7.77 (2H, d, *J* 8.8, Ar-H), 8.44-8.41 (2H, m, NH, -CONH, exchange with D_2O); δ_C (100 MHz, DMSO- d_6) 172.4 (C=O), 161.9, 143.9, 138.1, 137.0, 131.3, 130.3, 130.1 (2 x Ar-C), 128.6 (2 x Ar-C), 128.1, 126.5 (2 x Ar-C), 125.9, 121.6, 116.9, 115.3 (2 x Ar-C), 68.5, 40.5, 39.3, 26.9, 26.4; ESI-HRMS (*m/z*) calculated for $[M+H]^+$ ion species $C_{24}H_{26}Cl_2N3O_4S$ = 522.1016, found 522.1014.

Carbonic Anhydrase Inhibition. The CA-catalyzed CO_2 hydration activity was performed on an Applied Photophysics stopped-flow instrument using phenol red (at a concentration of 0.2 mM) as a pH indicator with 20 mM Hepes (pH 7.5) as the buffer, 20 mM Na_2SO_4, and following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10−100 s and working at the maximum absorbance of 557 nm.[17] The CO_2 concentrations ranged from 1.7 to 17 mM. For each inhibitor six traces of the initial 5−10 % of the reaction have been used in order to determin the initial velocity. The uncatalyzed reaction rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled water, and dilutions up to 0.01 nM were prepared. Solutions containing inhibitor and enzyme were preincubated for 15 min at room temperature prior to assay in order to allow the formation of the

E−I complex. The inhibition constants were obtained as nonlinear least squares protocols using PRISM 3, [21-23] and are the mean from at three different measurements. All hCAs were recombinant ones and were obtained in house. [21-23]

Animals. Sprague Dawley rats (Harlan, Italy,Varese) of the weight of 200-250 g were used. Animals were housed in the Centro Stabulazione Animali da Laboratorio (University of 65 Florence) and used 1 week after arrival. Four rats were housed per cage (size 26 cm × 41 cm); animals were fed a standard laboratory diet and tap water ad libitum and kept at 23 ± 1 °C with a 12 h light/dark cycle (light at 7 a.m.). All animal manipulations were done according to the European Community guidelines for animal care [DL 116/92, application of the European Communities Council Directive of 24 November 1986 (86/609/EEC)]. The ethical policy of the University of Florence complies with the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health (NIH Publication No. 85-23, revised 1996; University of Florence assurance number A5278-01). Formal approval to conduct the experiments described was obtained from the Animal Subjects Review Board of the University of Florence. Experiments involving animals have been reported according to ARRIVE - Animal Research: Reporting of In Vivo Experiments – guidelines. [24] Efforts were made in order to minimize suffering and to reduce the number of animals used.

Complete Freund's adjuvant-induced arthritis. Articular damage was induced by injection into the tibiotarsal joint of the Complete Freund's adjuvant (CFA; Sigma-Aldrich St Louis, MO, USA), which contained 1 mg/ml heat-killed and dried *Mycobacterium tuberculosis* in paraffin oil and mannide monooleate. [25-27] The rats were anesthetized with 2% isoflurane, the left leg skin was sterilized with 75% alcohol and the lateral malleolus located by palpation. A 28-gauge needle was inserted to penetrate the skin and turned distally into the articular cavity at the gap between the

tibiofibular and tarsal bone. A volume of 50 µl of CFA was injected (day 0). Control rats received 50 µl of saline solution within the tibiotarsal joint.

Administration of compounds. Compounds **2B**, **3B**, **6B** and **8B** were tested at the doses of 0.1, 1.0 and 10 mg kg^{-1}. The relative NSAIDs, naproxen, ketoprofen, sulindac and diclofenac were administered equimolarly to 10 mg/kg of the corresponding hydrid. All compounds were suspended in a 1% CMC solution and administered per os (p.o.) on day 14 after CFA i.a. injection.

Paw pressure test. The nociceptive threshold of rats was assessed with an analgesimeter (Ugo Basile, Varese, Italy). [28] A constantly increasing pressure was applied to a small area of the dorsal surface of the hind paw using a blunt conical probe as a mechanical device. Mechanical pressure was increased until vocalization or a withdrawal reflex occurred while the animals were restrained. Vocalization or withdrawal reflex thresholds were expressed in grams. Rats scoring below 40 g or over 75 g during the test before drug administration were rejected (25%). For analgesia measures the mechanical pressure application limit was set at 120 g.

Incapacitance test. Weight-bearing changes were measured using an incapacitance apparatus (Linton Instrumentation, Norfolk, UK) to detect modifications in postural equilibrium after a hind limb injury occurred. [29] Rats were trained to stand on their hind paws in a box with an 65° inclined plane. This box was placed above the incapacitance apparatus. The value reported for each animal is the mean of five and consecutive measurements. In the absence of hind limb injury the animals applied an equal weight distribution on both hind limbs (postural equilibrium), whereas an unequal distribution indicated a monolateral decreased pain threshold. Data are obtained as the difference

between the weight applied to the limb contralateral to the injury and the weight applied to the ipsilateral limb (Δ Weight).

Statistical analysis. Behavioural measurements were performed on 8 rats used for each treatment carried out in two different experiments. The results were expressed as mean (S.E.M.) with one-way analysis of variance. The Bonferroni's significant difference procedure was used as a post hoc comparison. P-values <0.05 or <0.01 were considered significant. Data were analysed on the Origin 9 software (OriginLab, Northampton, MA, USA).

Supporting Information. Supporting information is available free of charge on the ACS Publications website: SMILES representation for compounds (CSV), Enzymatic activity curves of compounds **5A, 2B, 3C, 3D, 3E** and **AAZ** on hCAs I, II, IV, IX and XII.

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Corresponding Authors. FC, Phone: +39-055-4573666; fax: +39-055-4573385; E-mail: fabrizio.carta@unifi.it; CTS, Phone: +39-055-4573729; fax: +39-055-4573385; E-mail: claudiu.supuran@unifi.it

Abbreviations Used. Nonsteroidal-Anti-Inflammatory-Drugs (NSAIDs); Carbonic-Anhydrase-Inhibitors (CAIs); Rheumatoid Arthritis (RA); Complete Freund's Adjuvant (CFA);

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