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Curcumin Conjugates of Non-steroidal Anti-Inflammatory Drugs: Synthesis, Structures, Anti-proliferative Assays, Computational Docking, and Inflammatory Response

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In an effort to combine the anti-proliferative effect of CUR-BF_2 and CUR compounds with anti-inflammatory benefits of non-steroidal anti-inflammatory drugs (NSAIDs), a library of the *bis*- and *mono*-NSAID/CUR-BF_2 and NSAID/CUR conjugates were synthesized by coupling flufenamic acid, flurbiprofen, naproxen, indomethacin, and ibuprofen to diversely substituted hydroxybenzaldehydes via an ester linkage, and by subsequent reaction with acetylacetone-BF_2 to form the *bis*- and the *mono*-NSAID/CUR-BF_2 adducts. Since conversion to NSAID/CUR by the previously developed decomplexation protocol showed limited success, a set of NSAID/CUR conjugates were independently prepared by directly coupling the NSAIDs with parent curcumin. The *bis*-NSAID/CUR-BF_2 and *bis*-NSAID-CUR hybrids exhibited low cytotoxicity in NCI-60 assay, and in independent cell viability assay on colorectal cancer (CRC) cells (HCT116, HT29, DLD-1, RKO, SW837, CaCo2) and in normal CR cells (CCD841CoN). By contrast, the mono-naproxin and mono-flurbiprofen CUR-BF_2 adducts exhibited remarkable anti-proliferative and apoptopic activity in NCI-60 assay most notably against HCT-116 (colon), OVCAR-3 (ovarian), and ACHN (renal) cells. Computational molecular docking calculations showed favorable binding energies to HER2, VEGFR2, BRAF, and Bcl-2 as well as to COX-1 and COX-2, which in several cases exceeded known inhibitors. The main interactions between the ligands and the proteins were hydrophobic, although several hydrogen bonds were also observed. A sub-set of six compounds that had exhibited little or no cytotoxicity were tested for their anti-inflammatory response with THP-1 human macrophages in comparison to parent NSAIDs or parent curcumin.

1. Introduction

Whereas potential health benefits of parent curcumin and its anticancer, anti-inflammatory, antioxidant, and anti-mutagenic effects have been extensively studied,[1–5] its complex signaling pathways and biological profile, coupled to poor pharmacokinetic properties have been major obstacles in developing a CUR-based anti-cancer drug, despite much effort to devise delivery methods by nanotechnology formulation or encapsulation into liposomes, or by inclusion into water soluble host molecules such as β-cyclodextrin.[6–8]

Much work has been devoted to improving solubility, metabolic stability, lipophilicity, and other properties through synthesis of analogs, and these developments have been summarized in recent reviews.[9,10] Work from this laboratory has focused on structural modifications by tuning steric and electronic effects through introduction of activating and deactivating substitutents, introducing fluorinated moieties in an effort to improve lipophilicity and metabolic stability, synthesis of pyazole and isoxazole derivatives, and synthesis of libraries of CUR-inspired heterocyclic analogs.[11–14] Libraries of deuterated-CUR-BF_2 and CUR analogs were also synthesized and studied.[15] These strategies produced diverse libraries of "CUR-inspired" compounds, and *in-vitro* bioassay studies identified several potential "hit compounds" with high anti-proliferative and apoptotic efficacy, most notably in multiple myeloma and colorectal cancer (CRC) that warrant further studies. The CUR-BF_2 adducts of some of these compounds proved especially effective. Other laboratories have found new applications for some CUR-BF_2 analogs as fluorescence imaging probes for detecting amyloids, and half-CUR-BF_2 compounds as PET imaging probes.[16] Taking into account the connection between inflammatory response and development of cancer,[17] synthesis of hybrid compounds that could combine the anti-cancer properties of a pro-drug with the anti-inflammatory response of an NSAID is a desirable goal. We know of only two published studies on CUR/NSAIDs.[18,19] with one study reporting inhibitory

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effect on proliferation of RAW 264.5 cell line.[18] The reported IC50 values showed low cytotoxicity for most, except for a *mono*-NSAID/CUR adduct employing flufenamic acid and a *bis*-NSAID/CUR adduct with salicylic acid. A salicylic acid *mono*-adduct, and a salalate *bis*-adduct showed notable anti-inflammatory effect against RAW 264.4 cell line. The other study reported spectroscopic and computational study on the *mono*-ibuprofen/CUR compound.[19]

CUR has recently been classified as both a PAINS (pan-assay interference compounds) and an IMPS (invalid/improbable metabolic panaceas) candidate.[20,21] In view of the ongoing active debate in the medicinal chemistry community concerning the therapeutic efficacy of parent curcumin,[20–22] a continuing search for CUR-inspired compounds that could overcome these drug-discovery challenges appears worthy.

The present study reports on (1) synthesis of a diverse set of *bis*-NSAID/CUR-BF_2, *bis*-NSAID/CUR, and *mono*-NSAID/CUR compounds, (2) computational docking studies to determine binding energies to HER2, VEGFR2, BRAF, Bcl-2, COX-1, and COX-2, (3) anti-proliferative activity as compared to CUR-BF_2 and CUR compounds in i*n-vitro* bioassay against a panel of 60 cancer cell lines, and more specifically in human CRC cells (HCT116, HT29, DLD-1, RKO, SW837, and Caco2), and (4) comparative anti-inflammatory assay for a subset of flufenamic acid, indomethacin, and ibuprofen conjugates with THP-1 human macrophages in comparison to the parent NSAIDs and parent curcumin. Several factors were considered in the selection of the NSAIDS in this study: flufenamic acid and flurbiprofen were chosen because of the presence of fluorines, well known to increase lipophilicity and metabolic stability, with the added benefit of ^{19}F NMR as a diagnostic tool to monitor reaction progress and to confirm structural integrity of the synthesized hybrid compounds. Selection of naproxen and ibuprofen stemmed from their wide use as over the counter drugs, and indomethacin because of its efficacy in pancreatic cancer by down-regulating of COX-2.[23]

2. Results and Discussion

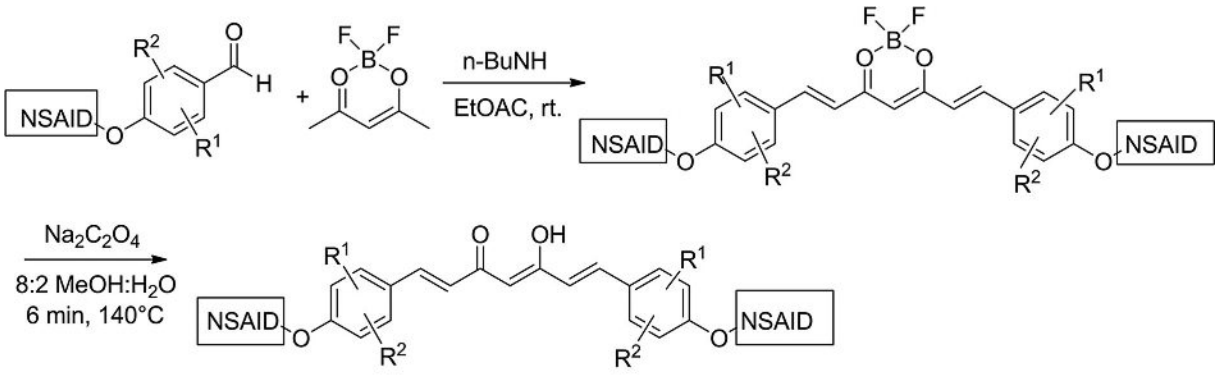
Variously substituted hydroxybenzaldehydes were coupled to flufenamic acid, flurbiprofen, indomethacin, naproxen, and ibuprofen using classical Steglich esterification protocol, employing DCC/DMAP, and the coupling adducts were obtained in good to excellent isolated yields. These were then reacted with acetylacetone-BF_2 complex (Scheme 1) in 2:1 ratio, following previously established procedures,[11–15] to obtain the corresponding *bis*-NSAID/CUR-BF_2 adducts. Thermal decomplexation of the BF_2 adducts in the Monowave reactor[24] were successful in some cases depending on the NSAID, and from this simple route the corresponding *bis*-NSAID/CUR compounds were directly obtained (Scheme 1).

By using a different strategy (Scheme 2) a library of *bis*-NSAID/CUR and *mono*-NSAID/CUR conjugates were synthesized, from which the corresponding CUR-BF_2 adducts were obtained by reaction with BF_3.[25]

Collectively these efforts led to the synthesis and isolation of libraries of hybrid compounds shown in Figures 1–2. Octanol/water partition coefficients (LogP) are displayed below each structure. LogP is a measure of lipophilicity, useful in estimating the distribution of drugs within the body. Hydrophobic compounds with high logP values are mainly distributed into hydrophobic regions such as lipid bilayers, while hydrophilic molecules (low logP values) are primarily found in blood serum.

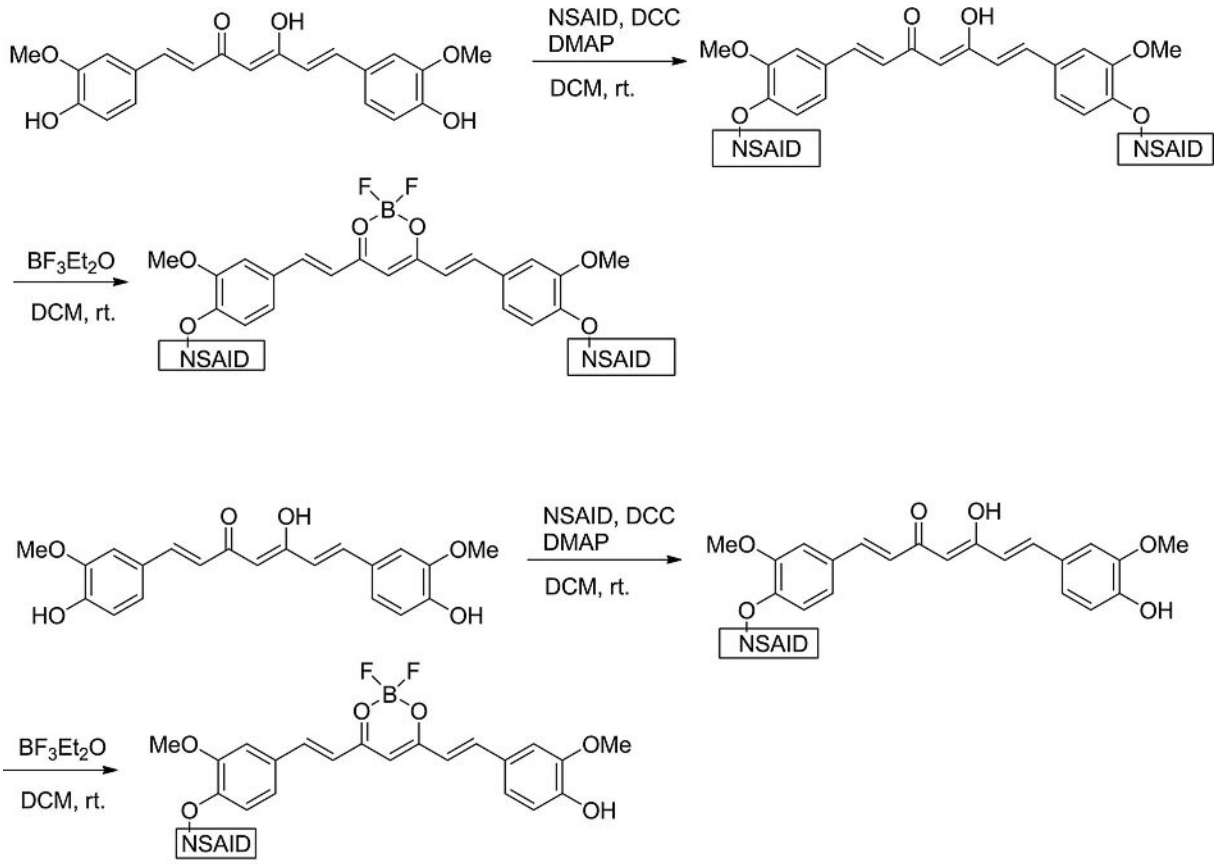
2.1. Structural Studies

Since efforts to grow crystals suitable for X-ray analysis were unsuccessful, the geometries of the compounds were optimized by density functional theory (DFT) computations at the B3LYP/6-31G* level. Some representative analogs are shown in Figure S1. The planar bis-α,β-unsaturated-β-diketone backbone



R^1/R^2 = 3,5-dimethoxy and 2,6-dimethoxy; NSAID = flufenamic acid; flurbiprofen; indomethacin; ibuprofen; R^1 = 5-F/R^2 = H; NSAID = flufenamic acid

Scheme 1. General procedure for the synthesis of symmetrical *bis*-NSAID/CUR-BF_2 -> *bis*-NSAID/CUR. R^1/R^2 = 3,5-dimethoxy and 2,6-dimethoxy; NSAID = flufenamic acid; flurbiprofen; indomethacin; ibuprofen; R^1 = 5-F/R^2 = H.



Scheme 2. Synthesis of symmetrical *bis*-NSAID/CUR -> *bis*-NSAID/CUR-BF_2 and mono-NSAID/CUR-> mono-NSAID/CUR-BF_2. NSAID = flufenamic acid; naproxen, flurbiprofen; ibuprofen.

converged to the enolic tautomer, and the CUR-BF_2 adducts presented a symmetrically BF_2-coordinated structure.

2.1.1. In-vitro *Bioassay*

The *bis*-NSAID/CUR-BF_2 and their corresponding CUR adducts proved to have little or no cytotoxicity by the NCI-60 assay (SI file and experimental section). Similar results were also observed in independent cell viability assay on colorectal cancer (CRC) cells (HCT116, HT29, DLD-1, RKO, SW837, CaCo2) and in normal CR cells (CCD841CoN) (Figure S2). By contrast the *mono*-NSAID/CUR-BF_2 compounds proved to be highly active, with *mono*-flurbiprofen/CUR-BF_2 **8** and *mono*-naproxin/CUR-BF_2 **10** exhibiting remarkable inhibitory effect on proliferation and apoptosis (mean values 20.3 and 36.6 respectively), in particular for colon, ovarian, and renal cancer cells (Tables S14 and S11), while the *mono*-flufenamic acid/CUR-BF_2 adduct **5** was less potent (mean value 81). Removal of the BF_2 results in significant loss of activity with the mean values for the *mono*-naproxin/CUR **20** and *mono*-ibuprofen/CUR **21** dropping to 89.8 and 87.9 respectively, and with mono-flufenamtic acid/CUR adduct **16** displaying essentially no cytotoxicity. Compounds **8** and **10** were then subjected to the five-dose screening assay by the NCI at concentrations 10^{-5} to 10^{-8} molar. Whereas compound **10** retained anti-proliferative activity at 10^{-6} molar, notably toward leukemia (RPMI 8226), colon (HCT-116), CNS (U-251), and ovarian (OVAR-8) cancer cells (Table S14b), compound **8** lost significant anti-proliferative activity at 10^{-6} molar concentration.

2.1.2. Stability in plasma and in solvent

Since one of the important functions of the proteins in human plasma is to transport drugs, in a test experiment the bis-NSAID/CUR-BF_2 compound **1** was allowed to incubate with citrated human plasma in water/DMSO (see experimental) at 37 °C with 5% CO_2 for 8 hours. The ^{19}F and ^{1}H NMR spectra (and relative integrals) of the recovered material showed that circa 90% of the compound had remained structurally intact, implying that the release of NSAID is not a major contributor to the observed bioactivity. Furthermore, the NMR samples of NSAID/CUR-BF_2 and NSAID/CUR compounds remained unchanged when stored at r.t. for days.

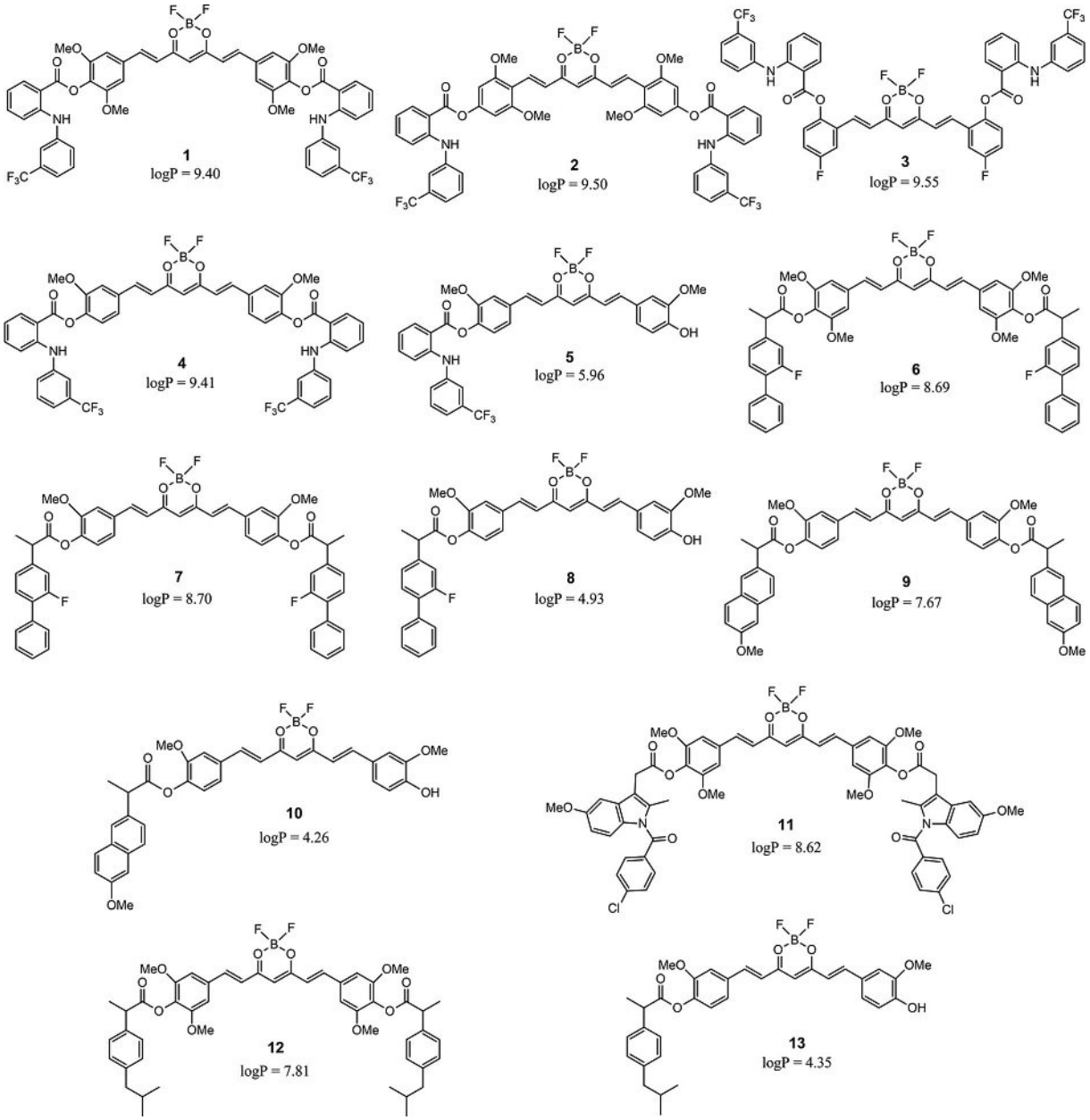


Figure 1. The NSAID/CUR-BF_2 library with calculated logP values (octanol/water partition coefficient, see Computational Methods section).

2.2. Computational/Docking Studies

Molecular docking calculations for model compounds were performed with the aim to elucidate the factors determining the bioactivity of the hybrid compounds. Binding energies in the active site of various proteins involved in carcinogenic processes were determined and compared with the binding affinities of their corresponding known inhibitors applied in anticancer therapies. The proteins selected for these docking studies are involved in diverse oncogenic pathways, which were described in previous works.[11–14] In addition, the potential anti-inflammatory activity of the CUR-NSAID conjugates was evaluated by docking calculations in the active site of cyclo-oxygenase enzymes COX-1 and COX-2. These enzymes are responsible for inflammatory processes, and their pharmacological inhibition can relieve the symptoms of inflammation and pain.[26,27] Moreover, the expression of COX-2 was found to be increased in a variety of malignancies including pancreatic cancer, and COX-2-mediated synthesis of prostaglandins favors the growth of tumor cells by stimulating proliferation and angiogenesis.[28]

In general, the studied curcuminoids yielded very good binding energies that were similar to, and in several cases more favorable than, the usually employed chemotherapeutic inhibitors (Tables S1 and S2). The main interactions between the ligands and the proteins were hydrophobic, although some hydrogen bonds were also observed (Figures 3 and 4). It should be noted that in COX-1 the binding mode of the conjugates

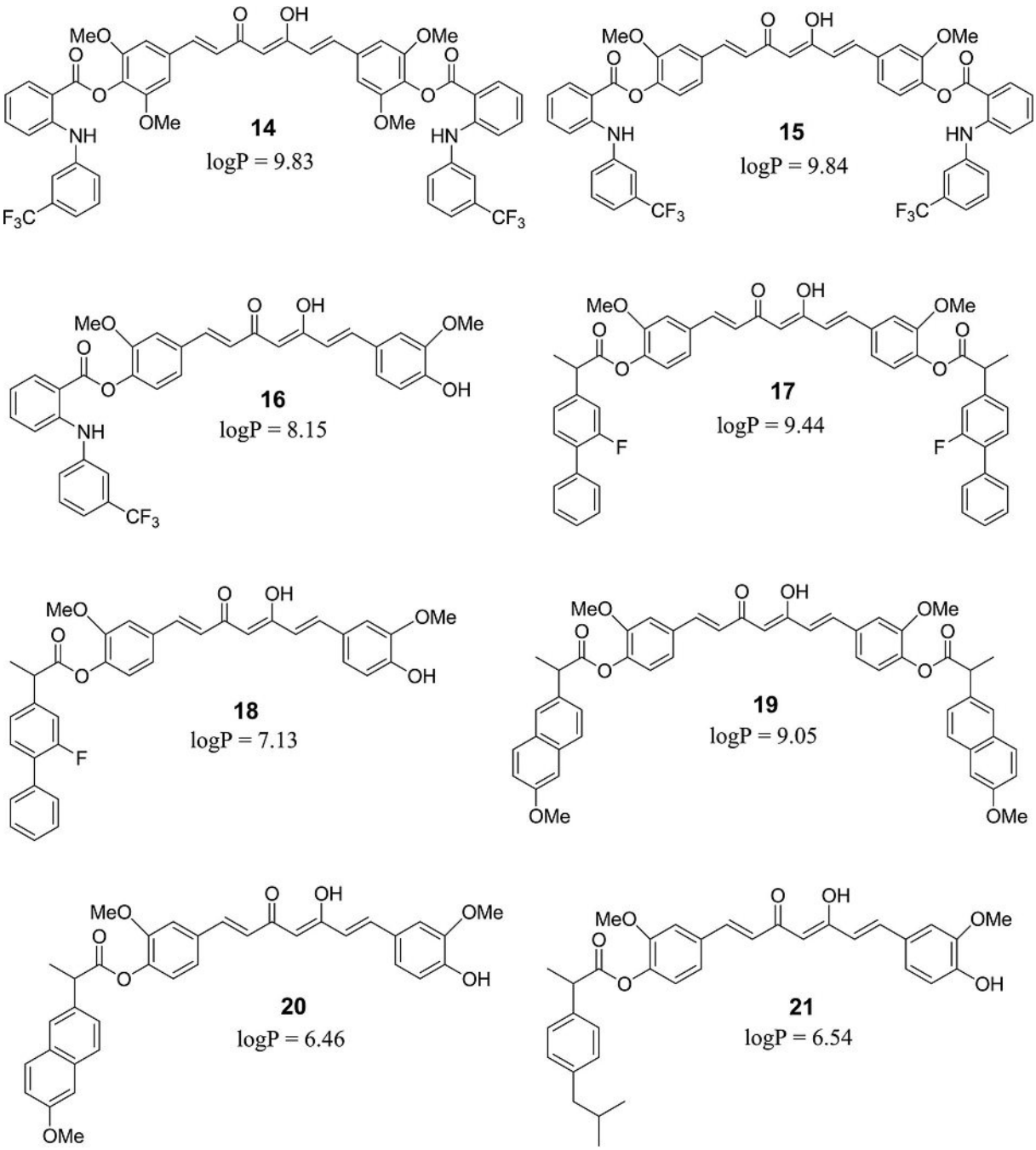


Figure 2. The NSAID/CUR library with calculated logP values (octanol/water partition coefficient, see Computational Methods section).

were different from that of the NSAIDs. In COX-2, the studied compounds docked into the same site as meloxicam and indomethacin.

Among the NSAID/CUR-BF_2 and NSAID-CUR conjugates that showed significant binding affinities with cyclooxygenases, most presented greater inhibitory action against the inducible isoform COX-2 (Table S16) which is implicated in the inflammatory response, than against the constitutive form of this enzyme (COX-1), inhibition of which is associated with gastric, renal and other adverse effects, such as inhibition of platelet aggregation.[29] These observations appear promising for the development of new anti-inflammatory agents with an improved tolerability profile. Nevertheless, although favorable docking is a necessary requirement for bioactivity, processes that occur prior to ligand-protein interaction, such as solubility, absorption, transport, metabolism, and membrane permeability, can affect the observed *in vitro* and *in vivo* activities.

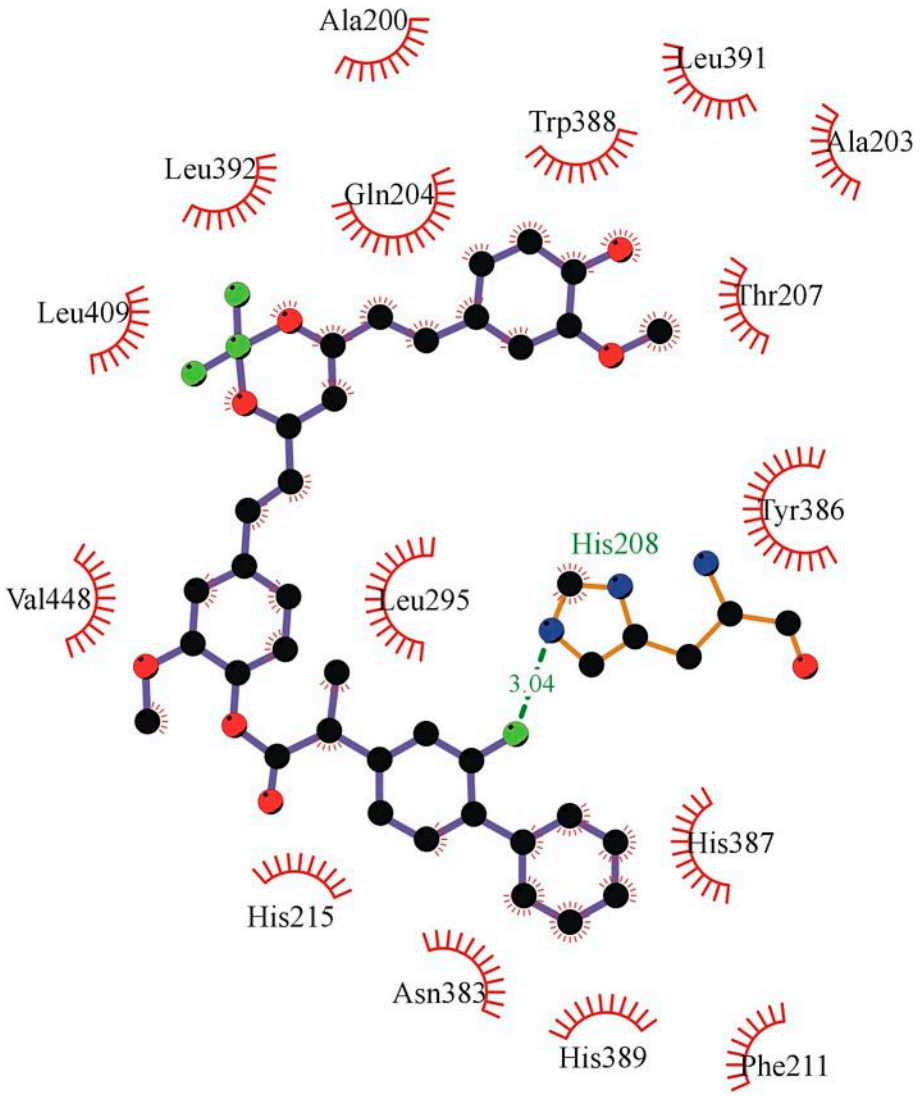


Figure 3. 2D representation of the most favorable binding pose of the *mono*-NSAID-CUR **8** in the active site of COX-2. The hydrophobic interactions between the ligand atoms and the protein residues are illustrated as red radial lines, and hydrogen bonds as green dotted lines.

2.3. Anti-inflammatory Assay

Lack of cytotoxicity in the bis-NSAID-CUR-BF_2 adducts prompted a preliminary investigation into their anti-inflammatory effect. A comparative anti-inflammatory assay using IL-1β, a pre-inflammatory cytokine, with THP-1 human macrophage cell line on a sub-set of six NSAIDS-CUR compounds showed better anti-inflammatory response (suppression of LPS-induced IL-1β expression) compared to parent curcumin (Figure 3). The flufenamic acid/CUR conjugate induced better suppression of LPS-induced inflammatory response than the parent flufenamate acid (Figure 5), while the anti-inflammatory response by ibuprofen/CUR and flurbiprofen/CUR conjugates was not significantly improved compared to the respective parent NSAIDS. It has been reported that fenamate NSAIDs (e.g., flufenamic acid, meclofenamic acid, and metenamic acid) suppressed the release and production of IL-1β in mouse bone marrow-derived macrophages (BMDMs) via inhibition of **NLRP3** inflammasome which is one of the most well-characterized inflammasome pathways.[30] By contrast, ibuprofen hardly inhibited the release and production of IL-1β and **NLRP3** inflammasome pathway in BMDMs.[30] Interestingly, parent curcumin also suppressed the release and production of IL-1β through the inhibition of **NLRP3** inflammasome in BMDMs.[31] Therefore, we surmise that flufenamic acid/CUR conjugates may act as selective and synergistic **NLRP3** inflammasome inhibitors.

3. Summary

A series of the *bis*- and the *mono*-NSAID/CUR-BF_2 and NSAID-CUR hybrids were synthesized and characterized. Whereas the *bis*-adducts exhibited little or no cytotoxicity in *in-vitro* bioassay, *the mono*-NSAID-CUR-BF_2 compounds, in particular the naproxen and flurbiprofen conjugates **8** and **10**, proved to be highly potent. Removal of the BF_2 group, as in the mono-naproxin/

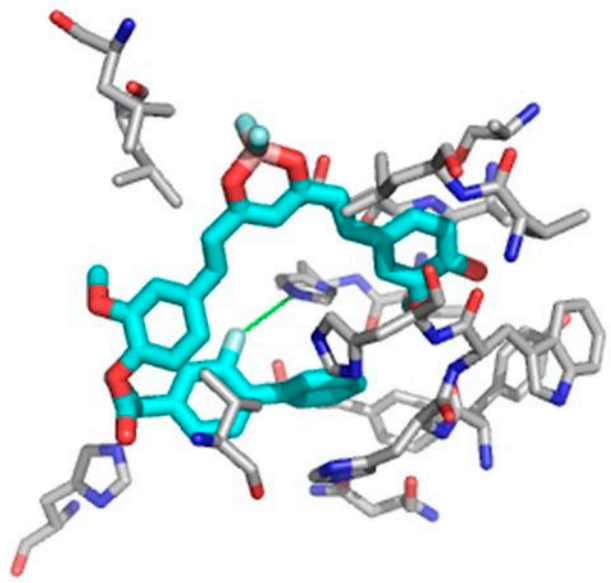


Figure 4. 3D representation of the most favorable binding mode of compound **8** in the active site of COX-2. Hydrogen-bond interactions are depicted as green lines.

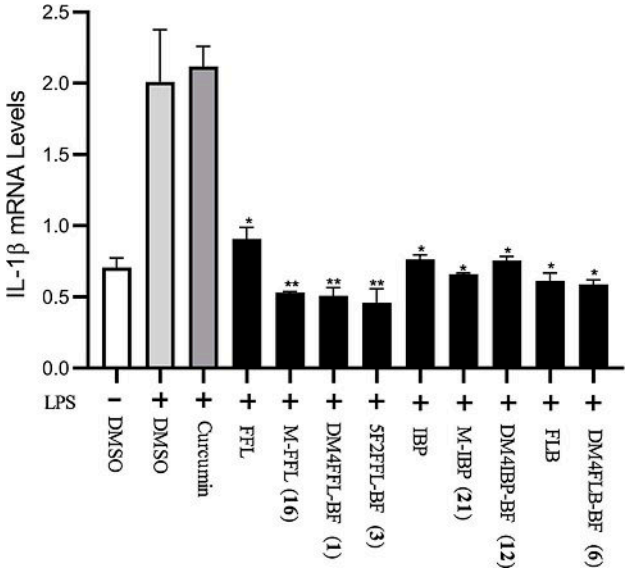


Figure 5. Inflammation Assay. THP-1 cells were treated with LPS (100 ng/ml) for two hours. After the induction of inflammatory response by LPS treatment, cells were treated with DMSO or CUR compounds (10 μM) for 24 hours. The IL-1β expression levels were normalized using the β-actin gene expression. Error bars represent SEM. Each groups contained more than two samples. *significantly different relative to LPS-treated controls ($p < 0.05$), ** significantly different relative to LPS/flufenamic acid-treated samples. Abbreviations: FFL: flufenamic acid; IBP: ibuprofen; FLB: flurbiprofen; M-FFL: compound **16**; M-IBP: compound **21**; DM4IBP-BF: compound **12**; DM4FFL-BF: compound **1**; DM4FLB-BF: compound **6**; 5F2FFL-BF: compound **3**.

CUR **20** and mono-ibuprofen/CUR **21** resulted in significant loss of activity. Computational molecular docking calculations showed favorable binding energies to HER2, VEGFR2, BRAF, and Bcl-2 as well as to COX-2. Finally, we found that the CUR-conjugated flufenamic acid compounds showed better anti-inflammatory response relative to parent flufenamaic acid, parent curcumin, and other NSAIDs (ibuprofen, flurbiprofen). Present studies suggest that fenamate NSAIDs/CUR conjugates could become effective and selective drug candidates for several inflammatory diseases implicated in IL-1β and **NLRP3** inflammasome.[32]

It is worth noting that some of the new molecules show promising activity toward multiple targets. The multitarget approach of drug discovery has several advantages (lower dose requirement, less side effects, reduced pharmaceutical pollution, etc.), especially in treating complex diseases.

Experimental Section

General – The NSAIDs, substituted hydroxyl-benzaldehydes, DCC and DMAP were all high purity commercially available samples and were used without further purification. NMR spectra were recorded on a 500 MHz instrument using $CDCl_3$, DMSO-d_6 or acetone-d_6 as solvent. ^{19}F NMR were referenced relative to external $CFCl_3$. HRMS analyses were performed on a Finnigan Quantum ultra-AM in electrospray mode using methanol as solvent. Decomplexation of CUR-BF_2 adducts to CUR was effected by using a benchtop Monowave reactor (Anton Paar). FT-IR spectra were recorded in ATR mode (as thin films formed via DCM evaporation). Melting points were measured in open capillaries and are not corrected.

Synthesis of NSAID-Aldehyde Adducts- The selected hydroxybenzaldehyde was added to a round bottom flask and dissolved in a minimal amount of chloroform. The NSAID (1.5 mmol), DCC (1.6 mmol), and DMAP (1.6 mmol) were subsequently added and the reaction mixture was flushed with nitrogen and allowed to stir at room temperature overnight. Upon completion (monitored by TLC) it was transferred to a freezer to allow the dicyclohexylurea (DCU) side product to fall out of solution. The reaction mixture was then quickly filtered through a Buchner funnel and the filtrate was transferred to a small beaker, washed with a 5% HCl solution (3× 10 mL) and extracted with chloroform. The organic layer was dried (sodium sulfate), filtered, and the solvent was evaporated under vacuum to give the crude product as an oil, which was purified by crystallization from isopropanol followed by vacuum drying.

Synthesis of bis-NSAID-CUR-BF_2 Adducts (Method 1) – To the NSAID-aldehyde adduct (0.55 mmol) charged into a multi-neck round bottom flask, was added acetylacetone-BF_2 adduct (0.25 mmol) and the mixture was dissolved in a minimal amount of ethyl acetate and flushed with nitrogen. Then n-butylamine (0.055 mmol) was introduced dropwise under stirring and the reaction was allowed to continue overnight, whereupon a solid precipitate was formed. When the spot due to NSAID-aldehyde adduct was no longer detectable by TLC, the reaction mixture was cooled to 0 °C and the solid product was filtered through a Buchner funnel, washed with cold ethyl acetate and dried under vacuum.

Synthesis of bis-NSAID-CUR Adducts (Method 1) – The *bis*-NSAID-CUR-BF_2 adduct synthesized via method 1 (0.1 mmol) was mixed with sodium oxalate (0.2 mmol) and charged into a Monowave reactor[21] vial equipped with a stir bar. Then 6 mL of 8:2 methanol/water was added and the tube was sealed and heated to 140 °C for 6 minutes in the Monowave reactor. After cooling, the product was filtered, washed with water, and dried under vacuum.

Synthesis of bis- and mono-NSAID-CUR Adducts (Method 2) – synthetic curcumin (0.5 mmol), DCC (0.55 mmol), DMAP (0.05 mmol) and the NSAID (1.05 mmol for *bis*-adduct and

0.45 mmol for the *mono*-adduct) were added to a round bottom flask and dissolved in DCM (15 mL). The mixture was stirred and the reaction was allowed to continue for 24 hours. TLC analysis of the crude reaction mixture showed the formation of both *mono*-, and *bis*-adducts (irrespective of molar equivalence of NSAID) along with unreacted curcumin. The crude mixture was washed with saturated $NaHCO_3$ solution, and the DCM layer was separated. The products were separated by column chromatography using hexane/ethyl acetate (40%). Fractions containing the respective products were combined and the solvent was removed leaving behind an oil, which was crystallized by dissolving in DCM and adding hexane, followed by filtration and vacuum drying.

Synthesis of bis- and mono-NSAID-CUR-BF_2 Adducts (Method 2) – To a multi-neck round bottom flask, 0.05 mmol of either the *bis*- or the *mono*-NSAID-CUR conjugate synthesized via method 2 was added and was dissolved in dry DCM. The solution was flushed with nitrogen with efficient stirring, then 0.075 mmol of BF_3 (as a solution of 48% BF_3-etherate) diluted in 0.5 mL of dry DCM was added dropwise into the flask via a syringe through a septum, and the reaction was allowed to stir at r.t. for circa 2 hours (completion was checked by TLC). The solvent was evaporated and the product was washed with diethyl ether, filtered, and dried under vacuum.

Stability Tests in Human Plasma and in Solvent

Compound **1** (20 mg) was dissolved in DMSO and allowed to mix with citrated human plasma (sigma-Aldrich) (1 mL) in a 24-well plate and incubated at 37 °C with 5% CO_2 for 8 hours. The resulting mixture was freeze-dried, extracted with acetone-d_6 and directly checked by ^{19}F and ^{1}H NMR.

NMR samples of the bis- and the mono-NSAID/CUR-BF_2 adducts in acetone-d_6 or DMSO-d_6 and those of the bis- and mono-NSAID/CUR adducts dissolved in $CDCl_3$ showed minimal decomposition (< 5%) by NMR when kept at r.t. overnight or stored in refrigerator for several days.

***bis*-Flufenamic acid/CUR-BF_2 Adduct (1)**: Yield 55%, off-white solid, mp 240–242 °C, Rf 0.70 (40% EtOAc in hexane). ^{1}H NMR (acetone-d_6, 500 MHz): δ 9.43 (s, 2H), 8.28 (dd, J = 1.5 and 8.0 Hz, 2H), 8.08 (d, 16.0 Hz, 2H), 7.65–7.57 (unresolved m, 8H), 7.45–7.41 (unresolved m, 4H), 7.36 (s, 4H), 7.23 (d, J = 15.5 Hz, 2H), 7.01 (pseudo-dt, J = 1.0 and 8.5 Hz, 2H), 6.54 (s, 1H), 3.94 (s, 12H). ^{19}F NMR (acetone-d_6, 470 MHz): δ −63.30 (6F, CF_3), −140.25 (s, ^{11}B–F), −140.18 (s, ^{10}B–F). ^{13}C NMR (acetone-d_6, 125 MHz): δ 180.6, 165.4, 153.1, 147.0, 146.9, 146.5, 141.7, 141.6, 135.3, 133.0, 132.4, 131.4, 131.2, 130.4, 124.8, 121.8, 119.6 (q, J_{CF} = 3.9), 118.8, 117.7 (q, J_{CF} = 3.8), 114.7, 112.0, 106.2, 102.1, 55.9. IR (cm^{-1}): 2941, 1705, 1620, 1593, 1557, 1501, 1456, 1423, 1331, 1256, 1207, 1130, 1047.

***bis*-Flufenamic acid/CUR Adduct (14)**: Yield 96%, Yellow solid, mp 209 °C, Rf 0.62 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.50 (s, 2H), 8.32 (d, J = 7.7 and 2.0 Hz, 2H), 7.65 (d, J = 16.5 Hz, 2H), 7.50–7.30 (unresolved m, 14 H!), 6.93–6.90 (unresolved m, 2H), 6.88 (s, 4H), 6.61 (d, J = 15.5 Hz, 2H), 5.93 (s, 1H), 3.89 (s, 12H). ^{19}F NMR ($CDCl_3$, 470 MHz): δ −62.87. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 183.1, 166.3, 152.8, 147.3, 141.4, 140.4, 135.0, 133.6, 132.8, 131.8 (q, J = 32.4 Hz), 130.1, 129.9, 124.5, 124.4, 123.9 (q, J = 271.8 Hz), 119.7 (q, J = 3.8), 118.5, 118.0 (q, J = 3.9), 114.2, 111.7, 104.8, 101.8, 56.3. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{51}H_{41}F_6N_2O_{10}$: 955.26654; found: 955.2612. IR (cm^{-1}): 3331, 2961–2843, 1701, 1595, 1506, 1456, 1418, 1333, 1254, 1206, 1130, 1040.

***bis*-Flufenamic acid/CUR-BF_2 Adduct (2)**: Yield 67%, red solid, mp 273 °C, Rf 0.61 (40% EtOAc in hexane). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.24 (br s, NH), 8.28 (d, J = 16.0 Hz, 2H), 8.17 (dd, J = 8.2, 2.0 Hz, 2H), 7.61–7.54 (m, 9H), 7.38–7.34 (m, 6H), 7.04 (pseudo-dt, J = 7.0, 1.0 Hz, 2H), 6.81 (s, 4H), 6.64 (s, 1H), 3.94 (s, 12H). ^{19}F NMR (DMSO-d_6, 470 MHz): δ −61.31 (s, 6F), −137.74 (s, ^{11}B–F). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 180.3, 165.7, 161.7, 155.9, 146.3, 142.5, 136.6, 135.9, 132.6, 131.1, 130.7 (q, J = 31.4 Hz), 124.0 (q, J = 271.8 Hz), 124.1, 122.8, 120.0, 119.2 (q, J = 4.0 Hz), 116.9 (q, J = 2.9 Hz), 116.7, 113.8, 109.7, 103.7, 99.8, 57.1. IR (cm^{-1}): 3331, 2947–2845, 1695, 1599, 1530, 1454, 1335, 1304, 1254, 1223, 1123, 1057.

***bis*-Flufenamic acid/CUR-BF_2 Adduct (3)**: Yield 32%, orange solid, mp 224–226 °C, Rf 0.62 (40% EtOAc in hexane). ^{1}H NMR (acetone-d_6, 500 MHz): δ 9.36 (s, NH), 8.35 (dd, J = 8.2 and 2.0 Hz, 2H), 8.12 (dd, J = 1.5 and 16.0 Hz, 2H), 7.84 (dd, J = 3.0 and 9.5 Hz, 2H), 7.63–7.53 (m, 8H), 7.55 (d, J = 5 Hz, 1H), 7.52 (d, J = 5 Hz, 1H), 7.47–7.41 (m, 6H), 7.29 (d, J = 16.0 Hz, 2H), 7.04 −7.01 (m, 2H), 6.54 (s, 1H). ^{19}F NMR (acetone-d_6, 470 MHz): δ −63.31 (s, 6F), −116.92 (m, 2F), −139.53 (s, ^{11}B–F), −139.46 (s, ^{10}B–F). ^{13}C NMR (acetone-d_6, 125 MHz): δ 180.8, 166.3, 160.4 (d, J_{CF} = 244 Hz), 147.5, 146.5 (d, J_{CF} = 2.8 Hz), 141.4, 138.5 (d, J_{CF} = 1.9 Hz), 135.7, 132.2, 131.3 (q, J_{CF} = 31.5 Hz), 130.4, 129.1 (d, J_{CF} = 8.7 Hz), 125.3 (q, J_{CF} = 271.8 Hz), 125.9 (d, J_{CF} = 8.5 Hz), 125.2, 124.7, 119.8 (q, J_{CF} = 3.9 Hz), 119.4 (d, J = 23.8 Hz), 118.9, 118.1 (J_{CF} = 3.8 Hz), 114.7, 114.1 (d, J_{CF} = 24.9 Hz), 111.4, 103.5. IR (cm^{-1}): 2359, 1715, 1620, 1585, 1541, 1541, 1520, 1491, 1456, 1418, 1337, 1258, 1221, 1163, 1123, 1042.

***bis*-Flufenamic acid/CUR-BF_2 Adduct (4)**: Yield 96.8%, orange solid, mp 199 °C, Rf 0.86 (40% EtOAc in hexane). ^{1}H NMR (acetone-d_6, 500 MHz): δ 9.42 (s, 2H), 8.27 (dd, J = 8.3 Hz and 2.0 Hz, 2H), 8.12 (d, J = 16.0 Hz, 2H), 7.73 (d, J = 2.0 Hz, 2H), 7.64–7.57 (m, 10H), 7.46–7.42 (m, 6H), 7.23 (d, J = 15.5 Hz, 2H), 7.02 (t, J = 7.5 Hz, 2H), 6.59 (s, 1H), 3.97 (s, 6H). ^{19}F NMR (acetone-d_6, 470 MHz): δ −63.32 (6F), −140.23 (s, ^{10}B–F), −140.29 (s, ^{11}B–F). ^{13}C NMR (acetone-d_6, 125 MHz): δ 180.6, 165.7 152.1, 147.1, 146.1, 142.7, 141.7, 135.3, 133.5, 132.2, 131.3 (q, J_{CF} = 32.4 Hz), 130.4, 124.5 (q, J_{CF} = 271.8 Hz), 124.0, 122.5, 121.7, 119.9 (distorted-q, J_{CF} = 3 Hz), 118.8, 114.7, 112.9, 112.1, 102.1, 55.7.

***bis*-Flufenamic acid/CUR Adduct (15)**: Yield 57.5%, yellow solid, mp 107 °C, Rf (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): 9.50 (s, 2H), 8.28 (dd, J = 8.3 Hz and 1.5 Hz, 2H), 7.69 (d, J = 15.5 Hz, 2H), 7.51–7.32 (unresolved m, 12H), 7.26–7.21 (m, 6H), 6.92 (dt, J = 8.0 Hz and 1.0 Hz, 2H), 6.63 (d, J = 16.0 Hz, 2H), 5.91 (s, 1H), 3.91 (s, 6H). ^{19}F NMR ($CDCl_3$, 470 MHz): δ −62.85 (CF_3).^{13}C NMR ($CDCl_3$, 125 MHz): δ 183.1, 166.5, 151.7, 147.5, 141.3, 141.2, 140.0, 135.2, 134.2, 132.5, 131.9 (q, J_{CF} = 32.4 Hz), 130.0, 124.7, 124.4, 123.6, 123.9 (q, J_{CF} = 272.7 Hz), 121.2, 119.9 (q, J = 3.8 Hz), 118.4, 118.2 (q, 3.9 Hz), 114.2, 111.6, 101.9, 56.1. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{49}H_{37}F_6N_2O_8$: 895.245411; found: 895.1868. IR (cm^{-1}): 3331, 3074–2841, 1699, 1632, 1582, 1506, 1454, 1416, 1333, 1252, 1227, 1200, 1161, 1119, 1045.

***mono*-Flufenamic acid/CUR-BF_2 Adduct (5)**: Yield 67.8%, red solid, mp 170 °C, Rf 0.20 (40% EtOAc in hexane). ^{1}H NMR (acetone-d_6, 500 MHz): δ 9.42 (s, NH), 8.64 (s, 1H), 8.27 (dd, J = 8.5 Hz and 1.5 Hz, 1H), 8.05 (d, J = 15.0, 1H), 8.03 (d, J = 16.0, 1H), 7.69 (d, J = 2.0, 1H), 7.64–7.53 (m, 6H), 7.46–7.40 (m, 4H), 7.16 (d, J = 15.5, 1H), 7.03–6.97 (m, 3H), 6.47 (s, 1H), 3.97 (s, 3H), 3.96 (s, 3H). ^{19}F NMR (acetone-d_6, 470 MHz): δ −63.32 (CF_3), −140.70 (s, ^{10}B–F), −140.76 (s, ^{11}B–F). ^{13}C NMR (acetone-d_6, 125 MHz): δ 181.2, 178.9, 165.7, 152.1, 151.4, 148.1, 147.0, 144.7, 142.4, 141.7, 135.2, 133.7, 132.3, 131.3 (q, J_{CF} = 32 Hz), 130.4, 126.6., 125.3, 124.8, 124.7, 123.9 (q, J_{CF} = 271.0 Hz), 122.2, 121.9, 119.5 (q, J_{CF} = 3.7 Hz), 118.8, 118.0, 117.7 (q, J_{CF} = 4.7 Hz), 115.7, 114.7, 112.7, 112.1, 111.9, 101.6, 55.7, 55.5.

***mono*-Flufenamic acid/CUR Adduct (16)**: Yield 29.5%, yellow solid, mp 133 °C, Rf 0.53 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.48 (s, 1H), 8.27 (dd, J = 8.0 Hz and 1.0 Hz, 1H), 7.64 (d, J = 15.5 Hz, 1H), 7.62 (d, J = 15.5 Hz, 1H), 7.49–7.30 (m, 6H), 7.24–7.19 (m, 3H), 7.13 (dd, J = 8.0 Hz and 1.5 Hz, 1H), 7.06 (s, 1H), 6.94 (d,

J = 8.0 Hz, 1H), 6.91 (t, J = 6.5 Hz, 1H), 6.59 (d, J = 16.0 Hz, 1H), 6.50 (d, J = 16.0 Hz, 1H), 5.89 (br s, 1H), 5.85 (s, 1H), 5.30 (s, 1H), 3.95 (s, 3H), 3.89 (s, 3H).^{19}F NMR ($CDCl_3$, 470 MHz): δ −62.85 (CF_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 184.6, 181.8, 166.5, 151.7, 148.0, 147.5, 146.8, 141.3, 141.2, 141.0,139.4, 135.2, 134.3, 132.5, 131.9 (q, J_{CF} = 32.4 Hz), 130.0, 127.6, 124.7, 124.4, 123.9 (q, J_{CF} = 272.7 Hz), 123.5, 123.1, 121.8, 121.0, 119.9 (q, J_{CF} = 3.8 Hz), 118.4, 118.2 (q, J = 3.8 Hz), 114.9, 114.2, 111.6, 111.5, 109.7, 101.6, 56.0, 55.9. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{35}H_{29}O_7F_3N$: 632.1896; found: 632.2006. IR (cm^{-1}): 3331, 3069–2841, 1699, 1628, 1582, 1506, 1454, 1429, 1416, 1333, 1254, 1202, 1161, 1121, 1034.

***bis*-Flurbiprofen/CUR-BF$_2$ Adduct (6)**: Yield 63%, Dark orange solid, mp: 230–232 °C, Rf 0.36 (40% EtOAc in hexane). ^{1}H NMR (acetone-d_6, 500 MHz): δ 8.02 (d, J = 15.5 Hz, 2H), 7.63–7.37 (complex region, 16H), 7.26 (s, 4H), 7.17 (d, J = 15.5 Hz, 2H), 6.50 (s, 1H), 4.22 (q, J = 7.0 Hz, 2H), 3.88 (s, 12H), 1.65 (d, J = 7.5 Hz, 6H). ^{19}F NMR (acetone-d_6, 470 MHz): δ −119.5 (t, J = 11.8 Hz, 2F), −140.2 (s, ^{10}B–F), −140.3 (s, ^{11}B–F). ^{13}C NMR (acetone-d_6, 125 MHz): δ 180.6, 170.9, 159.5 (d, J_{CF} = 246.0 Hz), 152.8, 146.5, 142.3 (J_{CF} = 8.5 Hz), 135.5, 132.7, 131.8, 130.7 (d, J = 4.0 Hz), 128.8, 128.5, 127.7, 127.6 (J_{CF} = 13.0 Hz), 124.3 d, J = 3.7 Hz), 121.7, 115.4 (J = 24.0 Hz), 106.2, 102.0, 55.8, 44.4, 18.3. IR (cm^{-1}): 2359, 1759, 1622, 1593, 1558, 1501, 1456, 1420, 1342, 1254, 1132, 1065, 1011.

***bis*-Flurbiprofen/CUR-BF$_2$ Adduct (7)**: Yield 73.6%, orange solid, mp 198 °C, Rf 0.39 (40% EtOAc in hexane). ^{1}H NMR (acetone-d_6, 500 MHz): δ 8.05 (d, J = 15.5 Hz, 2H), 7.62–7.37 (complex region, 20H), 7.20 (d, J = 8.5 Hz, 2H), 7.15 (d, J = 16.0 Hz, 2H), 6.53 (s, 1H), 4.22 (q, J = 7.0 Hz, 2H), 3.89 (s, 6H), 1.66 (d, J = 7.0 Hz, 6H). ^{19}F NMR (acetone-d_6, 470 MHz): δ −119.33 (2F), −140.22 (s, ^{10}B–F), −140.28 (s, ^{11}B–F).^{13}C NMR (acetone-d_6, 125 MHz): δ 180.6, 171.3, 160.5, 158.6, 151.9, 146.1, 143.0, 142.2 (d, J_{CF} = 8.6 Hz), 135.5, 133.4, 130.8 (d, J_{CF} = 3.9 Hz), 128.9 (d, J_{CF} = 3.8 Hz), 128.5, 127.7, 127.6, 124.2 (d, J_{CF} = 2.9 Hz), 123.4, 123.1 (q, J_{CF} = 245.0 Hz), 122.5, 121.6, 115.4 (d, J_{CF} = 23.9 Hz), 112.9, 102.0, 55.5, 44.5, 18.2.

***bis*-Flurbiprofen/CUR Adduct (17)**: Yield 24%, yellow solid, mp 131 °C, Rf 0.84 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.69 (d, J = 16.0 Hz, 2H), 7.59–7.57 (m, 4H), 7.48–7.46 (m, 6H), 7.46–7.37 (m, 2H), 7.29 (unresolved d, 4H), 7.15 (dd, J = 8.5 Hz and 1.5 Hz, 2H), 7.10 (d, J = 2.0 Hz, 2H), 7.03 (d, J = 8.0 Hz, 2H), 6.56 (d, J = 15.5 Hz, 2H), 5.86 (s, 1H), 4.07 (q, J = 7.5 Hz, 2H), 3.81 (s, 6H), 1.68 (d, J = 7.5 Hz, 6H). ^{19}F NMR ($CDCl_3$, 470 MHz): δ −117.6 (pseudo-t). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 183.1, 171.8, 160.7, 158.7, 151.4, 141.4, 141.3 (d, J_{CF} = 7.7 Hz), 139.9, 135.5, 134.0, 130.8 (d, J_{CF} = 3.9 Hz), 129.0 (d, J_{CF} = 2.9 Hz), 128.0 (d, J_{CF} = 7.5 Hz), 127.7, 124.3, 123.8 (d, J_{CF} = 2.9 Hz), 123.1, 121.1, 115.5 (d, J_{CF} = 24.0 Hz), 111.4, 101.8, 55.8, 44.9, 18.6. HRMS (ESI): m/z $[M-H]^-$ calcd for $C_{51}H_{41}F_2O_8$: 819.276951; found: 819.2860. IR (cm^{-1}): 3059–2938, 1759, 1628, 1599, 1506, 1483, 1418, 1300, 1254, 1121, 1072, 1034.

***mono*-Flurbiprofen/CUR-BF$_2$ Adduct (8)**: Yield 62.9%, dark-red solid, mp 227 °C, Rf 0.15 (40% EtOAc in hexane). ^{1}H NMR (acetone-d_6, 500 MHz): δ 8.63 (s, 1H), 8.04 (d, J = 15.5 Hz, 1H), 7.97 (d, J = 15.5 Hz, 1H), 7.63–7.57 (m, 4H)), 7.51–7.49 (m, 3H), 7.54–7.36 (m, 5H), 7.18 (d, J = 7.5 Hz, 1H), 7.11 (d, J = 16.0 Hz, 1H), 6.98 (d, J = 15.5 Hz, 1H), 6.97 (d, J = 8.5 Hz, 1H), 6.45 (s, 1H), 4.22 (q, J = 7.5 Hz, 1H), 3.96 (s, 3H), 3.89 (s, 3H), 1.66 (d, J = 6.5 Hz, 3H). ^{19}F NMR (acetone-d_6, 470 MHz): δ −119.34 (pseudo-t, 1F), −140.68 (s, ^{10}B–F), −140.74 (s, ^{11}B–F). ^{13}C NMR (acetone-d_6, 125 MHz): δ 181.2, 179.0, 171.3, 160.7, 158.7, 151.9, 151.4, 151.2, 148.0 (d, J_{CF} = 6.6 Hz), 144.7, 142.7, 142.2 (d, J_{CF} = 8.5 Hz), 135.5, 133.6, 130.8 (d, J_{CF} = 4.7 Hz), 128.9 (d, J_{CF} = 2.7 Hz), 128.5, 127.7 (d, J_{CF} = 3.0 Hz), 126.6, 125.3, 124.2 (d, J_{CF} = 2.8 Hz), 123.3, 122.2, 121.8, 117.9, 115.6, 115.4 (d, J_{CF} = 24.7 Hz), 112.7, 111.8, 101.6, 55.5, 44.5, 18.2.

***mono*-Flurbiprofen/CUR Adduct (18)**: Yield 17.2%, orange solid, mp 92 °C, Rf 0.58 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): 7.62 (d, J = 16.0 Hz, 1H), 7.60 (d, J = 15.0 Hz, 1H), 7.58 (d, J = 8.5 Hz, 2H), 7.48–7.45 (m, 3H), 7.39 (pseudo-tt, 1H), 7.37 (d and s overlapping, 3H), 7.16–7.14 (m, 2H), 7.08 (dd, J = 15.7 Hz and 1.5 Hz, 2H), 7.03 (d, J = 8.5 Hz, 1H), 6.95 (d, J = 8.0 Hz, 1H), 6.55 (d, J = 16.0 Hz, 1H), 6.50 (d, J = 15.5 Hz, 1H), 5.84 (s, 1H), 4.06 (q, J = 7.5 Hz, 1H), 3.96 (s, 3H), 3.81 (s, 3H), 1.68 (d, J = 7.5 Hz, 3H). ^{19}F NMR ($CDCl_3$, 470 MHz): δ −117.7 (pseudo t). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 184.5, 181.8, 171.9, 160.7, 158.7, 151.4, 148.0, 146.8, 141.3 (d, J_{CF} = 7.7 Hz), 141.3, 141.1, 139.4, 135.5, 134.2, 130.8 (d, J_{CF} = 3.8 Hz), 129.0 (d, J_{CF} = 2.9 Hz), 128.5, 128.0 (d, J_{CF} = 13.3 Hz), 127.7, 127.6, 124.3, 123.8 (d, J_{CF} = 2.9 Hz), 123.0 (d, J_{CF} = 2.8 Hz), 121.8, 120.9, 115.5 (d, J_{CF} = 23.7 Hz), 111.4, 109.7, 101.5, 56.0, 55.8, 44.9, 18.6. HRMS (ESI): m/z $[M-H]^-$ calcd for $C_{36}H_{30}FO_7$: 593.19756; found: 593.1715. IR (cm^{-1}): 3524, 3416, 3061–2841, 1757, 1626, 1587, 1506, 1450, 1418, 1296, 1267, 1204, 1121, 1072, 1032.

***bis*-Naproxen/CUR-BF$_2$ Adduct (9)**: Yield 75.5%, dark red solid, mp 167 °C, Rf 0.30 (40% EtOAc in hexane). ^{1}H NMR (acetone-d_6, 500 MHz): δ 8.03 (d, J = 16.0 Hz, 2H), 7.88 (d, J = 1.5 Hz, 2H), 7.86 (s, 2H), 7.84 (d, J = 1.5 Hz, 2H), 7.58–7.55 (partially overlapping doublets, J = 2.0 Hz, 4H), 7.44 (dd, J = 8.5 Hz and 2.0 Hz, 2H), 7.33 (d, J = 2.5 Hz, 2H), 7.18 (dd, J = 9.0 Hz and 3.0 Hz, 2H), 7.13 (d, J = 15.5 Hz, 2H), 7.12 (d, J = 7.5 Hz, 2H), 6.52 (s, 1H), 4.24 (q, J = 7.0 Hz, 2H), 3.94 (s, 6H), 3.83 (s, 6H), 1.67 (d, J = 6.5 Hz, 6H). ^{19}F NMR (acetone-d_6, 470 MHz): δ −140.28 (s, ^{10}B–F), −140.34 (s, ^{11}B–F).^{13}C NMR (acetone-d_6, 125 MHz): δ 188.4, 171.6, 157.7, 151.8, 145.8, 142.9, 135.5, 133.8, 133.0, 129.0, 128.8, 126.9, 126.2, 126.0, 123.2, 122.3, 121.3, 118.7, 112.6, 105.4, 101.8, 65.0, 55.3, 54.5, 18.1, 14.5.

***bis*-Naproxen/CUR Adduct (19)**: Yield 20%, yellow solid, mp 179 °C, Rf 0.49 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.80 (s, 2H), 7.56 (pseudo-t, J = 7.5 Hz, 4H), 7.59 (d, J = 16.0 Hz, 2H), 7.53 (dd, J = 8.7 Hz and 2.0 Hz, 2H), 7.18–7.15 (m, 4H), 7.11 (dd, J = 8.25 Hz and 2.0 Hz, 2H), 7.06 (d J = 1.5 Hz, 2H), 6.96 (d, J = 8.5 Hz, 2H), 6.53 (d, J = 7.5 Hz, 2H), 5.83 (s, 1H), 4.16 (q, J = 7.5 Hz, 2H), 3.94 (s, 6H), 3.72 (s, 6H), 1.71 (d, J = 7.0 Hz, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 183.1, 172.5, 157.7, 151.5, 141.6, 140.0, 135.1, 133.9, 129.3, 129.0, 127.1, 126.4, 126.3, 124.2, 123.1, 121.0, 119.0, 111.5, 105.6, 101.7, 55.8, 55.3, 45.3, 18.7. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{49}H_{45}O_{10}$: 793.301275; found: 793.2805. IR (cm^{-1}): 2934, 1755, 1630, 1605, 1506, 1452, 1416, 1300, 1265, 1121, 1070, 1032.

***mono*-Naproxen/CUR-BF$_2$ Adduct (10)**: Yield 83.0%, maroon solid, mp 151 °C, Rf 0.12 (40% EtOAc in hexane). ^{1}H NMR (acetone-d_6, 500 MHz): δ 8.64 (s, 1H), 8.03 (d, J = 16.0 Hz, 1H), 7.95 (d, J = 15.5 Hz, 1H), 7.88 (d, J = 1.5 Hz, 1H), 7.86 (s, 1H), 7.85 (d, J = 1.5 Hz, 1H), 7.57 (dd, J = 8.5 Hz and 2.0 Hz, 1H), 7.55 (d, J = 2.0 Hz, 1H), 7.51 (d, J = 2.0 Hz, 1H), 7.42–7.39 (m, 2H), 7.33 (d, J = 2.5 Hz, 1H), 7.18 (dd, J = 9.0 Hz and 2.5 Hz, 1H), 7.11 (d, J = 8.0 Hz, 1H), 7.08 (d, J = 15.5 Hz, 1H), 6.98 (d, J = 15.0 Hz, 1H), 6.96 (d, J = 8.5 Hz, 1H), 6.44 (s, 1H), 4.23 (q, J = 6.5 Hz, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 3.82 (s, 3H), 1.67 (d, J = 7.5 Hz, 3H). ^{19}F NMR (acetone-d_6, 470 MHz): δ −140.67 (s, ^{10}B–F), −140.74 (s, ^{11}B–F). ^{13}C NMR (acetone-d_6, 125 MHz): δ 181.2, 179.0, 171.9, 158.0, 151.9, 148.1, 148.0, 144.7, 142.9, 135.5, 134.0, 133.4, 129.2, 129.0, 127.1, 126.6, 126.4, 126.1, 125.2, 123.3, 123.3, 122.1, 121.7, 118.9, 118.9, 115.7, 112.7, 111.9, 105.6, 101.5, 55.5, 55.4, 45.0, 18.3.

***mono*-Naproxen/CUR Adduct (20)**: Yield 30%, yellow solid, mp 148 °C, Rf 0.34 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.80 (s, 1H), 7.75 (pseudo-t, 8.5 Hz, 2H), 7.60 (d, J = 16.0, 1H), 7.57 (d, J = 14.5, 1H), 7.53 (dd, J = 8.5 Hz and 2.0 Hz, 1H), 7.18–7.19 (overlapping set of doublets, 4H), 7.06–7.01 (overlapping doublets, 2H), 6.96 (d, J = 9.0 Hz, 1H), 6.94 (d, J = 8.0 Hz, 1H), 6.52 (d, J = 16.0 Hz, 1H), 6.49 (d, J = 15.5 Hz, 1H), 5.87 (s, 1H), 5.82 (s, 1H), 4.15 (q, J = 7.5, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 1.71 (d, J = 6.5 Hz, 3H). ^{13}C NMR ($CDCl_3$,

125 MHz): δ 184.4, 181.9, 172.5, 157.7, 151.5, 148.0, 146.8, 141.5, 141.1, 139.5, 135.2, 134.0, 133.8, 129.3, 129.0, 127.6, 127.1, 126.4, 126.3, 124.2, 123.1, 123.0, 121.8, 120.9, 119.0, 114.8, 111.5, 109.6, 105.6, 101.5, 55.9, 55.8, 55.3, 45.3, 18.7. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{35}H_{33}O_8$: 581.2175; found: 581.2328. IR (cm^{-1}): 3524, 3408, 3003–2839, 1755, 1628, 1601, 1508, 1462, 1267, 1123, 1032.

***bis*-Indomethacin/CUR-BF_2 Adduct (11)** : Yield 54%, dark-red solid, mp 168–170 °C, Rf 0.27 (40% EtOAc in hexane). ^{1}H NMR (acetone-d_6, 500 MHz): δ 8.01 (d, J = 16.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 4H), 7.65 (d, J = 8.5 Hz, 4H), 7.26 (s, 4H), 7.21 (d, J = 2.5 Hz, 2H), 7.17 (d, J = 15.5 Hz, 2H), 7.01 (d, J = 9.0 Hz, 2H), 6.75 (dd, J = 3.0 Hz and 9.2 Hz, 2H), 6.51 (s, 1H), 4.07 (s, 4H), 3.87 (s, 12H), 3.86 (s, 6H), 2.41 (s, 6H). ^{19}F NMR (acetone-d_6, 470 MHz): δ −140.26 (s, ^{10}B–F), −140.32 (s, ^{11}B–F). ^{13}C NMR (acetone-d_6, 125 MHz): δ 180.6, 168.1, 156.2, 152.8, 146.5, 138.4, 136.0, 134.6, 132.7, 132.0, 131.3, 130.9, 129.1, 121.7, 114.7, 112.5, 111.3, 106.3, 101.9, 55.9, 55.1, 12.7. IR (cm^{-1}): 2916, 2848, 1763, 1684, 1616, 1593, 1541, 1503, 1458, 1342, 1260, 1130.

***bis*-Ibuprofen/CUR-BF_2 Adduct (12)**Yield 68%, orange solid, mp: 239–240 °C, Rf 0.64 (40% EtOAc in hexane). ^{1}H NMR (acetone-d_6, 500 MHz): δ 7.97 (d, J = 15.5 Hz, 2H), 7.38 (d, J = 8.0 Hz, 4H), 7.21 (d, J = 7.0 Hz, 4H), 7.20 (s, 4H), 7.12 (d, J = 15.5 Hz, 2H), 6.47 (s, 1H), 4.07 (q, J = 7.5 Hz, 2H), 3.82 (s, 12H), 2.50 (d, J = 7.5 Hz, 4H), 1.90 (septet, J = 6.5 Hz, 2H), 1.58 (d, J = 7.5 Hz, 6H), 0.92 (d, J = 6.0 Hz, 12H). ^{19}F NMR (acetone-d_6, 470 MHz): δ −140.08 (s, ^{10}B–F), −140.74 (s, ^{11}B–F). ^{13}C NMR (acetone-d_6, 125 MHz): δ 180.5, 171.5, 152.9, 146.6, 140.3, 137.8, 132.6, 132.1, 129.1, 127.5, 121.5, 106.3, 102.0, 55.7, 44.7, 44.6, 30.1, 21.7, 18.6. IR (cm^{-1}): 2953,1749, 1618, 1595, 1531, 1504, 1456, 1425, 1383, 1341, 1308, 1258, 1130, 1063, 1015.

***mono*-Ibuprofen/CUR-BF_2 Adduct (13)**: Yield 81%, red solid, mp: 120 °C, Rf 0.33 (40% EtOAc in hexane). ^{1}H NMR (acetone-d_6, 500 MHz): δ 8.96 (s, 1H), 8.04 (d, J = 16.0 Hz, 1H), 7.95 (d, J = 16.0 Hz, 1H), 7.55 (d, J = 2.0 Hz, 1H), 7.51 (d, J = 2.0 Hz, 1H), 7.41 (pseudo-dt, J = 5.0 Hz and 2.0 Hz, 2H), 7.37 (distorted d, J = 8.0 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 7.10 (d, J = 8.0 Hz, 1H), 7.08 (d, J = 15.5 Hz, 1H), 6.97 (d, J = 15.0 Hz, 1H), 6.96 (d, J = 8.5 Hz, 1H), 6.44 (s, 1H), 4.06 (q, J = 6.5 Hz,1H), 3.96 (s, 3H), 3.84 (s, 3H), 2.50 (d, J = 7.5 Hz, 2H), 1.89 (sept, J = 6.5 Hz, 1H), 1.58 (d, 7.5 Hz, 3H), 0.92 (d, J = 6.5 Hz, 6H). ^{19}F NMR (acetone-d_6, 470 MHz): δ −140.66 (s, ^{10}B–F), −140.71 (s, ^{11}B–F). ^{13}C NMR (acetone-d_6, 125 MHz): δ 181.1, 179.0, 172.0, 152.0, 148.1, 148.0, 144.7, 142.8, 140.4, 137.7, 133.4, 129.2, 127.4, 126.6, 125.3, 123.3, 122.2, 121.7, 121.5, 118.0, 115.7, 112.7, 111.8, 101.5, 55.5, 55.4, 44.6, 30.0, 21.73, 21.71, 18.4.

***mono*-Ibuprofen/CUR Adduct (21)**: Yield 29%, yellow solid, mp 100 °C, Rf 0.62 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.61 (d, J = 16.0 Hz, 1H), 7.58 (d, J = 16 Hz, 1H), 7.33 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 2.0 Hz, 1H), 7.16–7.11 (overlapping doublets, 4H), 7.07 (d, J = 6.5 Hz, 2H), 6.97 (d, J = 8.0 Hz, 1H), 6.94 (d, J = 8.0 Hz, 1H), 6.53 (d, J = 15.5 Hz, 1H), 6.49 (d, J = 16.0 Hz, 1H), 5.87 (s, 1H), 5.82 (s, 1H), 3.99 (q, J = 6.5 Hz, 1H), 3.95 (s, 3H), 3.76 (s, 3H), 2.49 (d, J = 7.5 Hz, 2H), 1.88 (sept, J = 6.5 Hz, 1H), 1.63 (d, J = 7.5 Hz, 3H), 0.92 (d, J = 6.5 Hz, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 184.4, 181.9, 172.6, 151.5, 147.9, 146.8, 141.5, 141.0, 140.7, 139.5, 137.2, 134.0, 129.3, 127.6, 127.4, 124.1, 123.1, 123.0, 121.8, 121.0, 114.8, 111.5, 109.6, 101.5, 55.9, 55.8, 45.1, 30.2, 22.39, 22.38, 18.6. HRMS (ESI): m/z $[M-H]^-$ calcd for $C_{34}H_{35}O_7$: 555.2382; found: 555.2431

3,5-Dimethoxybenzaldehyde/4-Flufenamic Adduct: Yield 72%, white solid, mp 118 °C, Rf 0.65 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.97 (s, 1H), 9.44 (s, 1H), 8.31 (dd, J = 8.3 Hz and 1.0 Hz, 1H), 7.51–7.40 (m, 4H), 7.36 (d, J = 8.0 Hz, 1H), 7.32 (d, J = 7.5 Hz, 1H), 7.23 (s, 2H), 6.92 (t, J = 8.0 Hz, 1H), 3.93 (s, 6H). ^{19}F NMR ($CDCl_3$, 470 MHz): δ −62.85. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 191.0, 165.8, 153.2, 147.5, 141.2, 135.2, 134.6, 133.6, 131.9 (q, J_{CF} = 32.4 Hz), 130.0, 124.7, 123.9 (q, J_{CF} = 272.7 Hz), 119.8 (q, J_{CF} = 3.8 Hz), 118.5, 118.2 (q, J_{CF} = 7.7 Hz), 114.2, 111.4, 106.1, 56.5.. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{23}H_{19}F_3NO_5$: 446.1214; found: 446.1326.

2,6-Dimethoxybenzaldehyde/4-Flufenamic Adduct: Yield 37%, white solid, mp 105 °C, Rf 0.57 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 10.48 (s, 1H), 8.21 (dd, J = 8.2 Hz and 1.5 Hz, 1H), 7.51–7.42 (m, 4H), 7.37–7.32 (m, 2H), 3.92 (s, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 188.2, 166.3, 163.2, 156.8, 148.0, 141.0, 135.6, 132.2, 132.0 (q, J_{CF} = 32.4 Hz), 130.1, 125.1, 123.9 (q, J_{CF} = 272.8 Hz), 120.3 (q, J_{CF} = 4.6 Hz), 118.6 (q, J_{CF} = 3.8 Hz), 118.4, 114.4, 112.4, 111.0, 98.4, 56.3. ^{19}F NMR ($CDCl_3$, 470 MHz): δ −62.84 (3F). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{23}H_{19}F_3NO_5$: 446.12153; found: 446.1058.

5-Fluorobenzaldehyde/2-Flufenamic Adduct: Yield 47%, light-yellow solid, mp 96 °C, Rf 0.82 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 10.19 (d, J = 2.5 Hz, 1H), 9.34 (s, 1H), 8.27 (dd, J = 8.2 Hz and 2.0 Hz, 1H), 7.66 (dd, J = 8.2 Hz and 3.0 Hz, 1H), 7.51–7.46 (m, 3H), 7.43–7.39 (m, 2H), 7.36 (d, J = 7.5 Hz, 1H), 7.35–7.31 (m, 2H), 6.93 (td, J = 7.0 Hz and 1.0 Hz, 1H). ^{19}F NMR ($CDCl_3$, 470 MHz): δ −62.84 (3F), −114.13 (q, J = 9.9 Hz, 1F). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 187.0, 166.8, 160.4 (d, J_{CF} = 248.0 Hz), 148.2, 148.1 (J_{CF} = 2.8 Hz), 140.8, 135.8, 132.2, 132.0 (q, J_{CF} = 32.4 Hz), 130.1, 129.8 (J_{CF} = 5.7 Hz), 123.8 (q, J_{CF} = 273.0 Hz), 125.5 (J_{CF} = 7.7 Hz), 125.3, 122.3 (J_{CF} = 23.9 Hz), 120.4 (q, J_{CF} = 4.8 Hz), 118.8 (q, J_{CF} = 3.8 Hz), 118.5, 115.7 (J_{CF} = 24.0 Hz), 114.3, 110.4. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{21}H_{14}F_4NO_3$: 404.09098; found: 404.0966.

3,5-Dimethoxybenzaldehyde/4-Flurbiprofen Adduct: Yield 37.3% (141 mg), white solid, mp 115–116 °C, Rf 0.82 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.91 (s, 1H), 7.58-7.56 (m, 2H), 7.48 – 7.44 (m, 3H), 7.39 (tt, J = 7.5 Hz and 1.0 Hz, 1H), 7.32–7.28 (complex m, 2H), 4.13 (q, J = 7.5 Hz, 1H), 3.85 (s, 6H), 1.69 (d, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 191.0, 171.2, 160.7, 158.7, 152.9, 141.4 (J_{CF} = 7.7 Hz), 135.5, 134.4, 133.8, 130.7 (J_{CF} = 3.8 Hz), 129.0 (J_{CF} = 2.8 Hz), 128.5, 128.0, 127.9, 127.7, 56.2, 44.8, 18.7. ^{19}F NMR ($CDCl_3$, 470 MHz): δ −117.88 (pseudo-t, J = 8.5 Hz). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{24}H_{22}FO_5$: 409.14513; found: 409.1482.

3,5-Dimethoxybenzaldehyde/4-Naproxen Adduct: Yield 46%, white solid, mp 69–71 °C, Rf 0.61 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.87 (s, 1H), 7.83 (d, J = 1 Hz, 1H), 7.75 (t, J = 8.5 Hz, 2H), 7.54 (dd, J = 8.2 and 2.0 Hz, 1H), 7.18–7.16 (m, 2H), 7.09 (s, 2H), 4.22 (q, J = 7.5 Hz, 1H), 3.93 (s, 3H), 3.75 (br s, 6H), 1.73 (d, J = 7.5 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 191.1, 171.9, 157.7, 153.0, 135.2, 134.3, 134.1, 133.8, 129.3, 129.0, 126.9, 126.6, 126.3, 119.0, 106.1, 105.6, 56.2, 55.3, 45.2, 18.8. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{23}H_{23}O_6$: 395.149465; found: 395.1459.

2,6-Dimethoxybenzaldehyde/4-Naproxen Adduct: Yield 47%, white solid, mp 140–141 °C, Rf 0.41 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 10.39 (s, 1H), 7.78–7.73 (m, 3H), 7.49 (dd, J = 7.0 Hz and 1.5 Hz, 1H), 7.18 (dd, J = 6.0 Hz and 2.5 Hz, 1H), 7.15 (d, J = 2.5 Hz, 1H), 6.26 (s, 2H), 4.09 (q, J = 6.5 Hz, 1H), 3.93 (s, 3H), 3.82 (s, 6H), 1.71 (d, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 188.2, 172.3, 163.1, 157.9, 157.0, 134.5, 133.9, 129.3, 129.0, 127.5, 126.2, 126.0, 119.3, 112.1, 105.6, 97.8, 56.2, 55.3, 45.7, 18.5. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{23}H_{23}O_6$: 395.149465; found: 395.1400.

3,5-Dimethoxybenzaldehyde/4-Ibuprofen Adduct: Yield 60%, white solid, mp 83 °C, Rf 0.63 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.88 (s, 1H), 7.35 (d, J = 8.0 Hz, 2H), 7.15 (d, J = 8.5 Hz, 2H), 7.10 (s, 2H), 4.05 (q, J = 7.0 Hz, 1H), 3.78 (s, 6H), 2.48 (d, J = 6.5 Hz, 2H), 1.88 (septet, J = 7.0 Hz, 1H), 1.64 (d, J = 7.5 Hz, 3H), 1.22 (d, J = 6.0 Hz), 0.92 (dd, J = 6.5 Hz and 1.0 Hz, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 191.1, 171.9, 153.0, 140.6, 137.2, 134.2, 129.2, 127.5, 106.1, 56.2, 45.1, 44.9, 30.2, 25.4, 22.4, 18.7. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{22}H_{27}O_5$: 371.18585; found: 371.1897.

5-Fluorobenzaldehyde/2-Ibuprofen Adduct: Yield 13%, white solid, mp 85 °C, Rf 0.86 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.68 (d, J = 2.5 Hz, 1H), 7.53 (dd, J = 8.2. and 3.5 Hz, 1H), 7.31–7.28 (m, 3H), 7.18 (d, J = 8.0 Hz, 2H), 7.11 (dd, J = 9.2 and 4.5 Hz, 1H), 4.04 (q, J = 6.5 Hz, 1H), 2.49 (d, J = 7.0 Hz, 2H), 1.88 (sept, J = 6.5 Hz, 1H), 1.66 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 6.5 Hz, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 187.0, 172.9, 160.2 (J = 247.9 Hz), 148.4 (J = 2.8 Hz), 141.4, 136.5, 129.8, 129.4 (J = 6.7 Hz), 127.2, 125.0 (J = 7.7 Hz), 122.1 (J = 23.9 Hz), 115.0 (J = 23.9 Hz), 45.2, 45.0, 30.2, 22.4, 18.0. ^{19}F NMR ($CDCl_3$, 470 MHz): δ −114.63 (m).

3,5-Dimethoxybenzaldehyde/4-Indomethacin Adduct: Yield 96%, white solid, mp 149 °C, Rf 0.56 (40% EtOAc in hexane). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.67 (dt, J = 6.5 Hz and 2.0 Hz, 2H), 7.46 (dt, J = 8.5 Hz and 2.5 Hz, 2H), 7.12 (s, 2H), 7.09 (d, J = 2.5 Hz, 1H), 6.90 (d, J = 9.0 Hz, 1H), 6.69 (dd, J = 9.2 Hz and 2.5 Hz, 1H), 3.98 (s, 2H), 3.84 (s, 3H), 3.81 (s, 6H), 2.44 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 191.0, 168.3, 168.1, 156.0, 152.9, 139.3, 136.3, 134.4, 133.9, 131.2, 130.9, 130.7, 129.1, 114.8, 112.2, 111.7, 111.4, 106.1, 101.9, 101.3, 56.3, 55.7, 29.7, 13.4. HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{28}H_{25}ClNO_7$: 522.13195; found: 522.14.

4. Computational Methods

B3LYP/6-31G*[33] geometry optimizations were carried out with the Gaussian 09 program.[34] Molecular docking calculations were performed with the software AutoDock Vina (version 1.1.2)[35] for modeling the binding modes and assessing the interaction energies of the studied compounds as ligands for several enzymes. The three-dimensional coordinates of the proteins were obtained from the RCSB Protein Data Bank (PDB IDs: 3PP0[36] (HER2), 3SDK[37] (20S proteasome), 4AG8[38] (VEGFR2), 4XV2[39] (BRAF), 4LVT[40] (Bcl-2), 4O1Z[41] (COX-1), and 4PH9[42] (COX-2)). Chain A of HER2, VEGFR2, BRAF, Bcl-2, COX-1 and COX-2, and chains K (β5 subunit) and L (β6 subunit) of 20S proteasome were selected as target templates for the docking computations. Co-crystallized ligands and crystallographic water molecules were removed. Addition of hydrogens, merger of nonpolar hydrogens to the atom to which they were linked, and assignment of partial charges were computed with AutoDockTools. Docking areas were constrained to a 30x30x30 Å box centered at the active site of the proteins, providing proper space for rotational and translational movement of the ligands. Octanol/water partition coefficients (LogP) were evaluated by free Molinspiration molecular property calculation service.[43]

5. Bioassay Methods

NCI-60 assay: samples were submitted to the National Cancer Institute (NCI of NIH) Developmental Therapeutics anticancer screening program (DTP) for human tumor cell line assay by NCI-60 screening against leukemia, lung, colon, and CNS cancers, as well as melanoma, ovarian, renal, prostate, and breast cancers. Compounds are initially tested at a single dose of 10^{-5} molar. Data are reported as mean graph of percent growth (GP). Selected data output are shown in SI file. Growth inhibition is represented by values between 0 and 100 and lethality by values less than zero. Detailed procedures for the one-dose and five-dose screening assays are reported here: https://dtp.cancer.gov/discovery_development/nci-60/methodology.htm

6. Cell viability, Cytotoxicity and Apoptosis Assay for Colorectal Cells

Colorectal cancer (HCT116, HT29, DLD-1, RKO, SW837 and Caco2) and normal colon cell lines (CCD841CoN) were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cells were maintained in DMEM medium (Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (Life Technologies), 1% non-essential amino acids (Life Technologies), 1% penicillin-streptomycin (Life Technologies), and 1% glutamine (Life Technologies) at 37 °C and 5% CO_2. For the assessment of cell viability, cells were seeded in 96-well plates with approximately 5.0×10^3 cells / well and incubated in RPMI1640 medium (supplemented with 10% fetal bovine serum and 1% glutamine) for 24 hours. Cells were then treated with DMEM medium containing CUR compounds (10 μM) or vehicle (DMSO) for 72 hours and the number of viable cells was determined using CellTiter-Glo® cell viability assay system (Promega, Madison WI). After addition of the reagent (CytoTox-Glo™ or Caspase-Glo® 3/7 Assay System) to the cell culture medium, luminescence was measured by infinite M200 Pro microplate reader (TECAN).

7. Inflammation response (Figure S3)

Human macrophage cells (THP-1) was obtained from the ATCC (Manassas, VA). Cells were maintained in DMEM medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids, 1% penicillin-streptomycin, and 1% glutamine at 37 °C and 5% CO_2. For the inflammation assay, cells were seeded in T75 flask with approximately 9.0×10^5 cells / flask and incubated in DMEM medium for 24 hours. Cells were treated for 2 hours with Lipopolysaccharide (LPS, Millipore-Sigma, St Louis, MO) at a concentration of 100 ng/ml or vehicle control (Phosphate-Buffered Saline (PBS, Life Technologies) to induce an inflammatory state. After the incubation, culture media containing LPS or PBS was removed and replaced for 24 hours with DMEM media containing curcumin derivatives, the parent compound at a concentration of 10 μM or vehicle control (DMSO). Twenty-four hours after the treatment, cells were rinsed by PBS, and total RNAs were isolated using Qiagen's RNeasy Micro Kit (Qiagen, Hilden, Germany) per the manufacturer's instructions. Complimentary DNA (cDNA) was generated using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The mRNA levels of Interleukin 1β (IL-1β) and β-actin (the structural housekeeping gene) were measured using TaqMan Gene Expression Assay (Applied Biosystems) and Quant-Studio 6 Quantitative Real-Time PCR (qRT-PCR) system (Thermo Fisher Scientific, Waltham, MA). Raw

cycle threshold (Ct) values were used via the ΔΔCt method to calculate fold change in gene expression.

8. Statistical analysis

All statistical analyses were performed using GraphPad Prism 7 software. A statistically significant difference was determined using unpaired t-test with Welch's correction. Data were presented as mean ± standard error of mean and was considered statistically significant when p-value was < 0.05.

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Conflict of Interest

The authors declare no conflict of interest.

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[1] a) G. Radhakrishna Pillai, A. S. Srivastava, T. I. Hassanein, D. P. Chauhan, E. Carrier, *Cancer Lett.* **2004**, *208*,163–170; b) A. L. Lopresti, S. D. Hood, P. D. Drummond, *J. Psychopharmacol.* **2012**, *26*, 1512–1524; c) D. Perrone, F. Ardito, G. Giannatempo, M. Dioguardi, G. Troiano, L. Lo Russo, A. De Lillo, L. Laino, L. Lo Muzio, *Exp. Ther. Med.* **2015**, *10*, 1615–1623.

[2] S. C. Gupta, S. Prasad, J. H. Kim, S. Patchva, L. J. Webb, I. K. Priyadarsini, B. B. Aggarwal, *Nat. Prod. Rep.* **2011**, *28*, 1937–1955.

[3] D. Perrone, F. Ardito, G. Giannatempo, M. Dioguardi, G. Troiano, L. Lo Russo, A. De Lillo, L. Laino, L. Lo Muzio, *Exp. Ther. Med.* **2015**, *10*, 1615–1623.

[4] A. Minassi, G. Sánchez-Duffhues, J. A. Collado, E. Muñoz, G. Appendino, *J. Nat. Prod.* **2013**, *76*, 1105–1112.

[5] A. Rajasekhar Reddy, P. Dinesh, A. S. Prabhakar, K. Umasankar, B. Shireesha, M. Bhagavan Raju, *Mini-Rev. Med. Chem.* **2013**, *13*, 1769–1777.

[6] J. Liu, S. Chen, L. Lv, L. Song, S. Guo, S. Hunag, *Curr. Pharm. Des.* **2013**, *19*, 1974–1993; M. Mimeault, S. K. Batra, *Chin. Med.* **2011**, *6*, 1–19.

[7] C. Cheng, S. Peng, Z. Li, L. Zou, W. Liu, C. Liu *RSC Adv.* **2017**, *7*, 25978–25986.

[8] a) L. Zhang, S. Man, H. Qiu, Z. Liu, M. Zhang, L. Ma, *Environ. Toxicol. Pharmacol.* **2016**, *48*, 31–38; b) M. M. Yallapu, M. Jaggi, S. C. Chauhan, *Colloids Surf. B* **2010**, *79*, 113–125; c) B. Tang, L. Ma, H.-Y. Wang, G.-Y. Zhang, *J. Agric. Food Chem.* **2002**, *50*, 1355–1361.

[9] K. Bairwa, J. Grover, M. Kania, S. M. Jachak, *RSC Adv.* **2014**, *4*, 13946–13978.

[10] A. Vyas, P. Dandawate, P. S. Padhye, A. Ahmad, A. F. Sarkar, *Curr. Pharm. Des.* **2013**, *19*, 2047–2069.

[11] K. K. Laali, B. M. Rathman, S. D. Bunge, X. Qi, G. L. Borosky, *J. Fluorine Chem.* **2016**, 191, 29–41.

[12] K. K. Laali, W. J. Greves, S. J. Correa Smits, A. T. Zwarycz, S. D. Bunge, G. L. Borosky, A. Manna, A. Paulus, A. Chanan-Khand, *J. Fluorine Chem.* **2018**, 206, 82–98.

[13] K. K. Laali, W. J. Greves, A. T. Zwarycz, S. J. Correa Smits, F. J. Troendle, G. L. Borosky, S. Akhtar, A. Manna, A. Paulus, A. Chanan-Khan, M. Nukaya, G. D. Kennedy *ChemMedChem* **2018**, *13*, 1895–1908.

[14] K. K. Laali, A. T. Zwarycz, S. D. Bunge, G. L. Borosky, M. Nukaya, G. D. Kennedy, *ChemMedChem* **2019**, *14*, 1173–1184.

[15] K. K. Laali, A. T. Zwarycz, S. D. Bunge, G. L. Borosky, M. Nukaya, G. D. Kennedy, *ChemMedChem* **2019**, *14*, 1173–1184.

[16] a) X. Zhang, Y. Tian, Z. Li, X. Tian, H. Sun, H. Liu, A. Moore, C. Ran J Am, *Chem. Soc.* **2013**, 135, 16397–16409; b) X. Zhang, Y. Tian, P. Yuan, Y. Li, M. A. Yaseen, J. Grutzendler, A. Moore, C. Ran, *Chem. Commun.* **2014**, *50*, 11550–11553; c) J. Yang, R. Cheng, H. Fu, J. Yang, M. Kumar, J. Lu, Y. Xu, S. H. Liang, M. Cui, C. Ran, *Chem. Commun.* 2019, 55, 3630–3633.

[17] a) A. Mullard, *Nat. Rev. Drug Discovery* **2016**, *15*, 219–221; b) E. Elinav, R. Nowarski, C. A. Thaiss, B. Hu, C. Jin, R. A. Flavell, *Nat. Rev. Cancer* **2013**, *13*, 759–771; c) S. Rakoff-Nahoum, *Yale J. Biol. Med.* **2006**, 79, 123–130.

[18] W. Liu, Y. Li, Y. Yue, K. Zhang, Q. Chen, H. Wang, Y. Lu, M.-T. Hunag, X. Zheng, Z. Du, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 3044–3051.

[19] S. Srivastava, P. Gupta, A. Sethi, R. P. Singh *J. Mol. Struct.* **2016**, *1117*, 173–180.

[20] K. M. Nelson, J. L. Dahlin, J. Bisson, J. Graham, G. F. Pauli, M. A. Walters, *J. Med. Chem.* **2017**, *60*, 1620–1637.

[21] K. M. Nelson, J. L. Dahlin, J. Bisson, G. F. Pauli, M. A. Walters *ACS Med. Chem. Lett.* **2017**, *8*, 467–470 and references cited therein.

[22] F. Bahadori, M. Demiray *ACS Med. Chem. Lett.* **2017**, *8*, 893–896 and references cited therein.

[23] L. Sun, K. Chen, Z. Jiang, X. Chen, J. Ma, Q. Ma, W. Duan, *Oncol. Rep.* **2018**, *39*, 2243–2251.

[24] D. Obermayer, D. Znidar, G. Glotz, A. Stadler, D. Dallinger, C. O. Kappe, *J. Org. Chem.* **2016**, *81*, 11788–1180.

[25] R. Abonia, K. K. Laali, D. Raja-Somu, S. D. Bunge, E. C. Wang, *ChemMedChem* **2020**, *15*, 354–362.

[26] G. Banuppriya, R. Sribalan, V. Padmini, V. Shanmugaiah, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 1655–1659.

[27] C. Selvam, S. M. Jachak, R. Thilagavathib, A. K. Chakraborti, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1793–1797.

[28] S. Padhye, S. Banerjee, D. Chavan, S. Pandye, K. V. Swamy, S. Ali, J. Li, Q. P. Dou, F. H. Sarkar, *Pharm. Res.* **2009**, *26*, 2438–2445.

[29] J. R. Vane, R. M. Botting, *Inflammation Res.* **1995**, *44*,1–10.

[30] M. J. Daniels J Rivers-Auty, T. Schilling N G Spencer, W. Watremez, V. Fasolino, S. J. Booth, C. S. White, A. G. Baldwin, S. Freeman, R. Wong R, C. Latta, S. Yu, J. Jackson J, N. Fischer, V. Koziel, T. Pillot, J. Bagnall, S. M. Allan, P. Paszek, J. Galea, M. K. Harte, C. Eder, C. B. Lawrence, D. Brough, *Nat. Commun.* **2016**, *7*, 12504.

[31] M. T. Palizgir, M. Akhtari, M. Mahmoudi, S. Mostafaei, A. Rezaiemanesh, F. Shahram, *Immunopharmacol. Immunotoxicol.* **2018**, *40*, 297–302.

[32] C. A. Dinarello, *Blood* **2011**, *117*, 3720–32.

[33] a) A. D. Becke, *J. Chem. Phys.* **199**3, 98, 5648–5652; b) C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1988**, *37*, 785–789; c) B. Miehlich, A. Savin, H. Stoll, H. Preuss, *Chem. Phys. Lett.* **1989**, *157*, 200–206.

[34] Gaussian 09, Revision E.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian, Inc., Wallingford, CT, **2009**.

[35] O. Trott, A. J. Olson, *J. Comput. Chem.* **2010**, *31*, 455–461.

[36] K. Aertgeerts, R. Skene, J. Yano, B.-C. Sang, H. Zou, G. Snell, A. Jennings, K. Iwamoto, N. Habuka, A. Hirokawa, T. Ishikawa, T. Tanaka, H. Miki, Y. Ohta, S. J. Sogabe, *J. Biol. Chem.* **2011**, *286*, 18756–18765.

[37] C. Blackburn, C. Barrett, J. L. Blank, F. J. Bruzzese, N. Bump, L. R. Dick, P. Fleming, K. Garcia, P. Hales, Z. Hu, M. Jones, J. X. Liu, D. S. Sappal, M. D.

Sintchak, C. Tsu, K. M. Gigstad, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6581–6586.
[38] M. McTigue, B. W. Murray, J. H. Chen, Y.-L. Deng, J. Solowiej, R. S. Kania, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 18281–18289.
[39] C. Zhang, W. Spevak, Y. Zhang, E. A. Burton, Y. Ma, G. Habets, J. Zhang, J. Lin, T. Ewing, B. Matusow, G. Tsang, A. Marimuthu, H. Cho, G. Wu, W. Wang, D. Fong, H. Nguyen, S. Shi, P. Womack, M. Nespi, R. Shellooe, H. Carias, B. Powell, E. Light, L. Sanftner, J. Walters, J. Tsai, B. L. West, G. Visor, H. Rezaei, P. S. Lin, K. Nolop, P. N. Ibrahim, P. Hirth, G. Bollag, *Nature* **2015**, *526*, 583–586.
[40] A. J. Souers, J. D. Leverson, E. R. Boghaert, S. L. Ackler, N. D. Catron, J. Chen, B. D. Dayton, H. Ding, S. H. Enschede, W. J. Fairbrother, D. C. S. Huang, S. G. Hymowitz, S. Jin, S. L. Khaw, P. J. Kovar, L. T. Lam, J. Lee, H. L. Maecker, K. C. Marsh, K. D. Mason, M. J. Mitten, P. M. Nimmer, A. Oleksijew, C. H. Park, C.-M. Park, D. C. Phillips, A. W. Roberts, D. Sampath, J. F. Seymour, M. L. Smith, G. M. Sullivan, S. K. Tahir, C. Tse, M. D. Wendt, Y. Xiao, J. C. Xue, H. Zhang, R. A. Humerickhouse, S. H. Rosenberg, S. W. Elmore, *Nat. Med.* **2013**, *19*, 202–208.
[41] S. Xu, D. J. Hermanson, S. Banerjee, K. Ghebreselasie, G. M. Clayton, R. M. Garavito, L. J. Marnett, *J. Biol. Chem.* **2014**, *289*, 6799–6808.
[42] B. J. Orlando, M. J. Lucido, M. G. Malkowski, *J. Struct. Biol.* **2015**, *189*, 62–66.
[43] Molinspiration Cheminformatics free web services, https://www.molinspiration.com.

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