This is an open access article published under a Creative Commons Attribution (CC-BY) License, which permits unrestricted use, distribution and reproduction in any medium, provided the author and source are cited.

ACS OMEGA

Article

Cite This: ACS Omega 2019, 4, 8824−8833

http://pubs.acs.org/journal/acsodf

Carboranyl Analogues of Ketoprofen with Cytostatic Activity against Human Melanoma and Colon Cancer Cell Lines

Antonio Buzharevski,[†] Svetlana Paskas,[‡] Markus Laube,[§] Peter Lönnecke,[†] Wilma Neumann,[†] Blagoje Murganic,[‡] Sanja Mijatovic,[‡] Danijela Maksimovic-Ivanic,[‡] Jens Pietzsch,[§,∥] and Evamarie Hey-Hawkins*[,†]

[†]Institut für Anorganische Chemie, Universität Leipzig, Johannisallee 29, D-04103 Leipzig, Germany

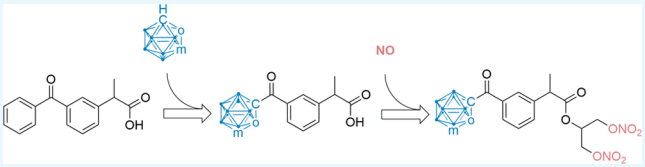
[‡]Department of Immunology, Institute for Biological Research "Sinisa Stankovic", Belgrade University, Belgrade 11060, Serbia

[§]Institut für Radiopharmazeutische Krebsforschung, Helmholtz-Zentrum Dresden-Rossendorf, Bautzner Landstrasse 400, D-01328 Dresden, Germany

[∥]Faculty of Chemistry and Food Chemistry, Technische Universität Dresden, Mommsenstrasse 4, D-01062 Dresden, Germany

Supporting Information

ABSTRACT: Ketoprofen is a widely used nonsteroidal anti-inflammatory drug (NSAID) that also exhibits cytotoxic activity against various cancers. This makes ketoprofen an attractive structural lead for the development of new NSAIDs and cytotoxic agents. Recently, the incorporation of carboranes as phenyl mimetics in structures of established drugs has emerged as an attractive strategy in drug design. Herein, we report the synthesis and evaluation of four novel carborane-containing derivatives of ketoprofen, two of which are prodrug esters with an nitric oxide-releasing moiety. One of these prodrug esters exhibited high cytostatic activity against melanoma and colon cancer cell lines. The most pronounced activity was found in cell lines that are sensitive to oxidative stress, which was apparently induced by the ketoprofen analogue.



1. INTRODUCTION

Ketoprofen (Figure 1) is a nonsteroidal anti-inflammatory drug (NSAID) belonging to the group of substituted propionic acids. It is used to treat rheumatoid arthritis, osteoarthritis, dysmenorrhea, and to alleviate moderate pain. Ketoprofen has pharmacologic activity similar to that of other NSAIDs, and this is associated with the inhibition of prostaglandin synthesis catalyzed by both cyclooxygenase (COX) isoforms, COX-1 and COX-2. Its anti-inflammatory effects result from the inhibition of both COX-1 and COX-2, the latter being responsible for the production of prostaglandins under inflammatory conditions.[1−3] In addition to its effects on COX, ketoprofen inhibits the lipoxygenase pathway of the arachidonic acid cascade. It is known that lipoxygenase inhibitors have also the potential to attenuate inflammation, and this inhibition is complementary to the COX inhibitory action.[4,5] Furthermore, it is a powerful inhibitor of bradykinin, an important peptidic mediator of pain and inflammation.[2]

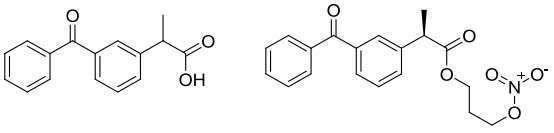


Figure 1. Ketoprofen and the nitric oxide (NO)-releasing analogue of ketoprofen HCT-2037.[6]

Generally, the side effects of ketoprofen are similar to those of other classical NSAIDs.[7] Serious side effects result mostly from gastrointestinal (GI) damage. In fact, ketoprofen is known to be one of the most ulcerogenic NSAIDs with a risk factor for serious GI complications close to that of ibuprofen.[8] These side effects are mainly caused by the fact that ketoprofen is a nonselective COX inhibitor. In contrast to COX-2, COX-1 is constitutively expressed within the body and is responsible for the production of prostaglandins that have important physiological functions, and thus inhibition of this isoform can result in side effects.[9]

The search for therapeutics with fewer side effects has led to the development of nitric oxide (NO)-releasing prodrugs of established drugs.[10,11] Given the gastrotoxicity of ketoprofen, the design of an NO-releasing prodrug is beneficial as NO exhibits cytoprotective properties in the digestive mucosa.[12] There are known examples of NO-releasing analogues of ketoprofen that have an aliphatic-linked NO-releasing moiety, i.e., a nitrate. The antinociceptive effect and efficacy of these compounds were tested on rats, and one of the compounds,

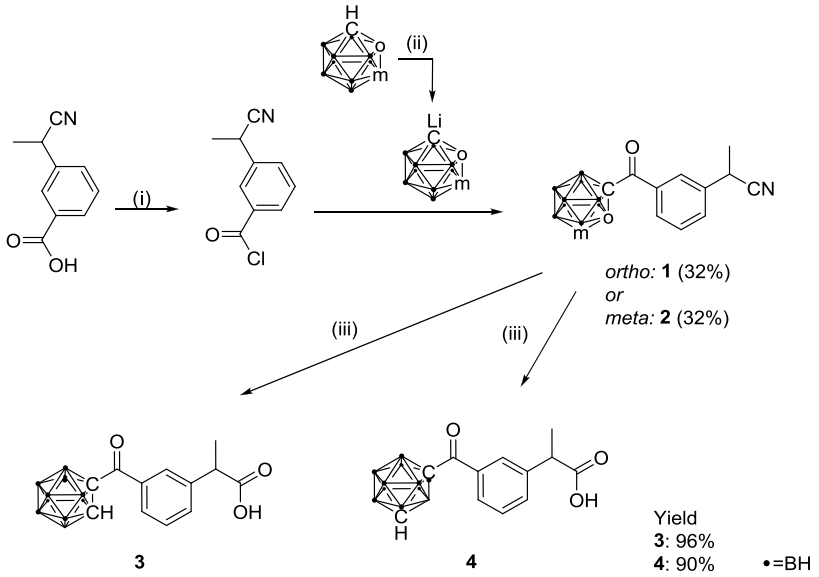
Received: February 13, 2019
Accepted: April 17, 2019
Published: May 23, 2019

Downloaded by NATL LBRY OF SERBIA at 04:20:40:208 on June 21, 2019
from https://pubs.acs.org/doi/10.1021/acsomega.9b00412.

© 2019 American Chemical Society

DOI: 10.1021/acsomega.9b00412
ACS Omega 2019, 4, 8824−8833

Scheme 1. Synthesis of Ketoprofen Analogues 3 (from *ortho*-Carborane) and 4 (from *meta*-Carborane)[a]



[a](i) $SOCl_2$, reflux; (ii) *n*-BuLi, anhydrous Et_2O, and toluene (1:1) at −40 °C; (iii) with either **1** (for **3**) or **2** (for **4**) as starting material; mixture of acetic acid and 6 M HCl (2:1), refluxed overnight.

HCT-2037 (Figure 1), was found to be a potent and effective analgesic agent.[6]

The versatile biological role of NSAIDs is further fortified by the findings that besides their anti-inflammatory action they exhibit cytotoxic properties.[13−15] Ketoprofen was found to exhibit cytostatic activity against various cancer types, including cervical cancer,[16] osteosarcoma,[17] and colon cancer,[16,18] based on diverse effects ranging from inhibition of metastasis[17] to affecting angiogenesis.[19]

Due to their importance for the treatment of inflammation and their cytotoxic potential, new NSAIDs are constantly developed, mostly by derivatizing established COX inhibitors. An attractive drug design strategy is the incorporation of dicarba-*closo*-dodecaboranes, or carboranes,[20,21] in place of hydrophobic moieties.[22,23] Carboranes are icosahedral clusters composed of 2 CH and 10 BH vertices. Based on the arrangement of the carbon atoms within the boron cluster, ortho, meta, and para isomers exist. Due to their size, shape, and hydrophobicity, they are considered as phenyl mimetics,[23] and several examples of carboranyl analogues of NSAIDs have been studied already.[24−28] In the case of indomethacin, substitution of one phenyl ring with a carborane cluster resulted in a shift from nonselective to COX-2-selective inhibitory activity.[24] Furthermore, due to their high boron content, boron clusters are used in boron neutron capture therapy.[20,21,29,30] Incorporation of a carborane moiety in the scaffolds of bioactive molecules is expected to increase their metabolic stability given that carborane clusters are highly resilient toward metabolic transformations.[23,31]

Herein, we report the synthesis of carborane-containing derivatives of ketoprofen. The carborane moiety was intended to induce selectivity toward COX-2 and increase the metabolic stability of the resulting ketoprofen analogue. Furthermore, we introduced NO-releasing moieties to obtain derivatives that incorporate the beneficial cytoprotective activity of NO.[32] The synthesized compounds were tested to determine their potential as COX inhibitors as well as for their potential cytotoxic activity.

2. RESULTS AND DISCUSSION

2.1. Molecular Design and Synthesis of Ketoprofen Analogues. Ketoprofen's molecular structure features two phenyl rings, which, in principle, allow for introduction of the carborane moiety. In this work, the nonsubstituted ring was chosen to be replaced with a carborane cluster (Scheme 1, compounds **3** and **4**) as metabolic transformations of ketoprofen occur at this phenyl ring.[33] This is also a good surrogate for comparing the effect of a carborane substitution of a keto-bound benzene ring to the biological activity of the native drug.

As the most viable option for the synthesis of the carboranyl derivative of ketoprofen, the synthetic methodology involving a reaction between a lithio carborane and an acyl halide was chosen. This synthetic approach was performed with both an *ortho*-carborane and a *meta*-carborane to examine the effect of the position of the cluster carbon atoms on the chemical and biological properties of the compound. The meta isomer may be preferred as it is more stable toward deboronation compared to its ortho counterpart.[23] On the other hand, the ortho cluster can be easily transformed into a *nido*-carborane,[34] which may also be beneficial for the properties of the ketoprofen analogue.

The reaction conditions were chosen based on those previously reported by Zakharkin et al. for the synthesis of a methyl ketone derivative of *ortho*-carborane.[35] To obtain a high yield of the monosubstituted derivative, a mixture of toluene and diethyl ether was used as the solvent for the lithiation step, and an equimolar amount of *n*-BuLi was reacted with *ortho*- or *meta*-carborane. The lithiated carborane was then added to freshly prepared 3-(1-cyanoethyl)benzoyl chloride dissolved in diethyl ether (Scheme 1). The addition step should be performed by adding the nucleophile to the electrophile to minimize the possibility of reduction of the newly formed keto group to a tertiary alcohol. By this approach, compounds **1** and **2** were obtained. Besides the characterization of the intermediates, the molecular structure of the ortho derivative **1** could be verified by X-ray

DOI: 10.1021/acsomega.9b00412
ACS Omega 2019, 4, 8824−8833

crystallography (Supporting Information (SI), Table S1, Figure S1).

The synthesis of the propionitrile derivatives **1** and **2** created intermediates that could be further transformed into the desired propionic acid derivatives **3** and **4** (Scheme 1). This is easily achievable by hydrolysis of the cyano group to a carboxylic group under acidic or basic conditions. To minimize the possibility of a deboronation side reaction of *ortho*-carborane, which is even more likely to occur due to the electron-withdrawing carbonyl group attached at the carborane cluster,[36] the nitrile hydrolysis was done under acidic conditions. The hydrolysis was performed under reflux employing a mixture of acetic acid as the solvent and concentrated hydrochloric acid. This afforded the ketoprofen analogues **3** and **4** in quantitative yields. **3** and **4** were fully characterized and their structures were confirmed by X-ray crystallography (Figure 2; SI, Tables S2, S3 and Figures S2, S3).

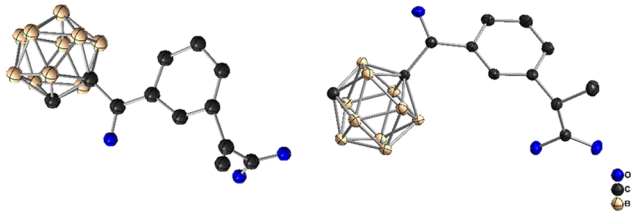


Figure 2. Crystal structures of the *ortho*-carboranyl derivative of ketoprofen **3** (left) and the *meta*-carboranyl derivative **4** (right). (Oak Ridge thermal ellipsoid plot: displacement thermal ellipsoids are drawn at 50% probability level. Hydrogen atoms are omitted for clarity.) Detailed crystallographic parameters can be found in the SI (Tables S2 and S3).

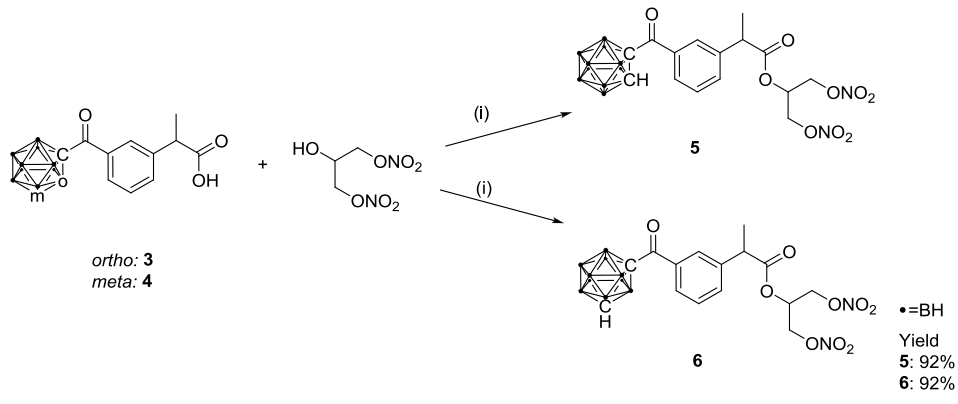
To expand the functionality of the synthesized carboranyl derivatives of ketoprofen, a nitrate group was introduced to generate a prodrug with an NO-releasing moiety. Within the carborane-based ketoprofen analogues, the carboxylic group is the most accessible functionality to insert a linker with one or more nitrate groups attached. For this work, an aliphatic derivative of dinitroglycerol was chosen because aromatic linkers are known to be associated with increased carcinogenicity.[37] Furthermore, this allows the release of two NO molecules per inhibitor molecule. Activation of the carboxylic group as acyl halide and reaction with 1,3-dinitroglycerol or the corresponding alcoholate did not yield the desired product, even after prolonged reaction times and at elevated temperature. This may be attributed to the poor nucleophilicity of 1,3-dinitroglycerol. Finally, the dinitrate esters **5** and **6** could successfully be generated by employing Steglich esterification conditions with dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) as coupling reagents (Scheme 2). Due to the presence of a chiral center in the nitrate esters **5** and **6**, the methylene protons of the isopropyl group are diastereotopic and can be observed as pairs of doublets via coupling to the methine proton in the ^{1}H NMR spectra.

2.2. Evaluation of COX Inhibitory and Cytotoxic Activities. All compounds were tested for their COX inhibitory activity employing a commercial enzymatic COX assay ("COX Fluorescent Inhibitor Screening Assay Kit", Item no. 700100, Cayman Chemical, Ann Arbor, MI) as described elsewhere.[38] However, only compound **5**, the ketoprofen analogue bearing an *ortho*-carborane and a nitrate moiety, exhibited weak COX inhibition without selectivity for one of the isoforms (SI, Table S4). The other compounds did not exhibit any COX inhibitory activity.

To investigate the antitumor activity of the carboranyl analogues of ketoprofen, three melanoma as well as three colon cancer cell lines were used. The selection of the cell lines was based on their ability to express COX-2; A375,[39] B16F10,[40] B16,[41] CT26CL25[42] are expressing COX-2, whereas HCT116 and SW480 do not.[43] The four synthesized compounds were applied at 0.8−50 μM, and the percentage of viable cells was determined by measuring mitochondrial respiration [MTT assay employing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] as well as DNA/RNA content of attached cells (crystal violet (CV) assay employing crystal violet). After 48 h incubation, the colorimetric assays revealed that only compound **6** affected cell viability of the tested cells in a dose-dependent manner with an inhibitory activity varying among the cell lines (Table 1).

Interestingly, the highest sensitivity was found for the COX-2-expressing melanoma cell line A375 and the COX-2-negative colon cancer cell line HCT116, indicating that the cytotoxic activity of **6** is probably COX-2 independent. This is consistent with the observation that **6** does not exhibit COX inhibitory activity. Comparing the sensitivities among the different cell lines showed that the redox-sensitive cell lines[44] are most susceptible to **6**. The IC_{50} values determined with both assays

Scheme 2. Steglich Esterification To Generate the NO-Releasing Prodrugs 5 and 6[a]



[a](i) With either **3** (for **5**) or **4** (for **6**) as starting material; DCC, DMAP, CH_2Cl_2.

DOI: 10.1021/acsomega.9b00412
ACS Omega 2019, 4, 8824−8833

Table 1. Cytotoxic Activities of Compounds 3−6[a]

	compounds	3		4		5		6	
IC_{50} (μM)	assay	MTT	CV	MTT	CV	MTT	CV	MTT	CV
cell line[b]	A375	>50	>50	>50	>50	>50	>50	17.15 ± 1.55	19.7 ± 0.0[c]
	B16	>50	>50	>50	>50	>50	>50	23.1 ± 0.0[c]	24.5 ± 0.0[c]
	B16F10	>50	>50	>50	>50	>50	>50	47.25 ± 2.75	46.15 ± 3.86
	HCT116	>50	>50	>50	>50	>50	>50	11 ± 1.2	18.95 ± 0.95
	CT26CL25	>50	>50	>50	>50	>50	>50	32.55 ± 3.17	50 ± 0.0[c]
	SW480	>50	>50	>50	>50	>50	>50	50 ± 0.0[c]	49.4 ± 0.6

[a]IC_{50} values determined by MTT and CV assays (three independent experiments each). [b]A375: human melanoma, B16: mouse solid melanoma, B16F10: mouse metastatic melanoma, HCT116: human colon carcinoma, CT25CL26: mouse colon carcinoma, SW480: human colon carcinoma. [c]Standard deviation varies in the third decimal place.

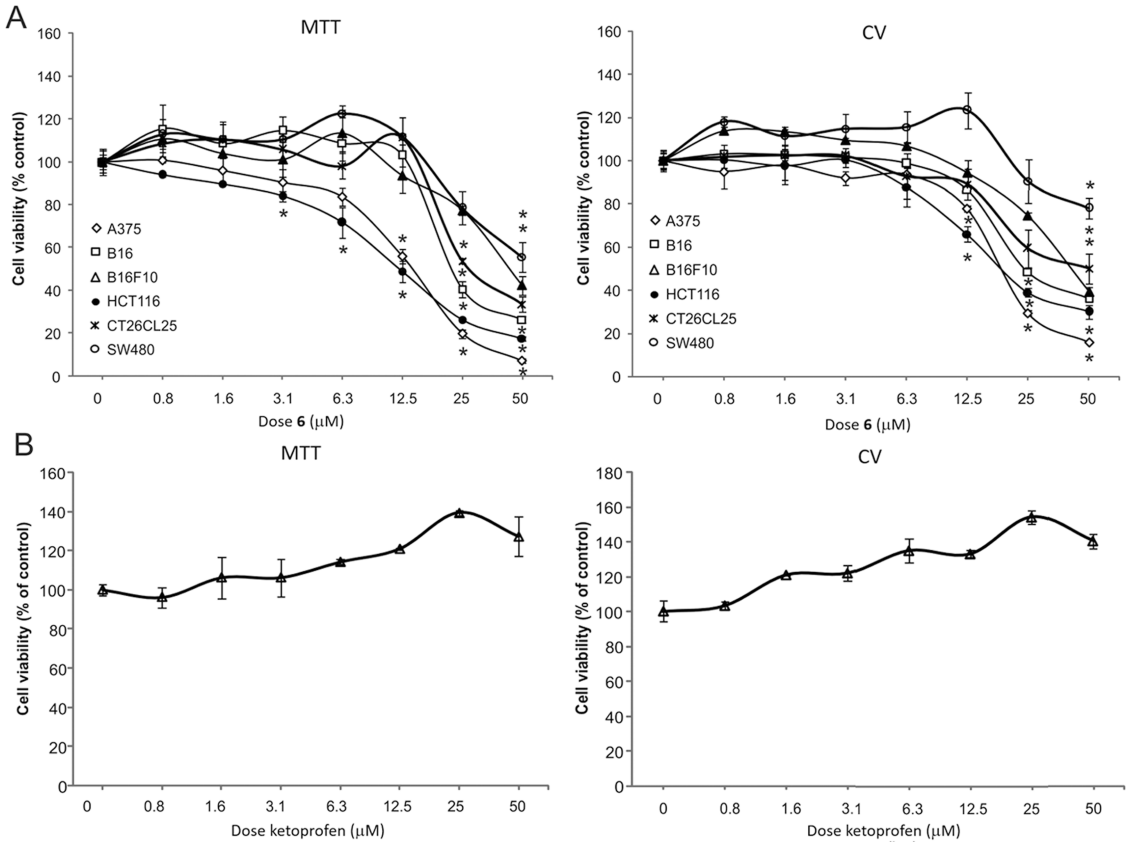


Figure 3. Effect of ketoprofen and **6** on the cell viability of different cell lines. Cells were exposed to **6** (A) or ketoprofen (B) for 48 h, and cell viability was analyzed using MTT (left panel) and CV assays (right panel). *$p < 0.05$ compared to untreated controls.

(MTT, CV) were similar and showed that **6** affects the cell viability in a micromolar range; in contrast, ketoprofen did not affect cell viability in the same dose range (Figure 3). This result is in agreement with the previously reported low effectiveness of ketoprofen against different cell lines.[45−47] However, a significant cytostatic effect was observed upon exposure of human cervical carcinoma (HeLa) and human colon cancer cells (Caco-2) to ketoprofen, which may be attributed to the NF-κB inhibition that was detected.[16]

Comparison of sensitivities toward **6** of malignant cells (Table 1) versus primary macrophages (IC_{50} = 46.55 ± 3.36 μM) showed that the compound is relatively selective for cancer cells; a selectivity index toward the transformed phenotype is given in Table S5, SI. Given that **6** showed high activity against the A375 melanoma cells, this cell line was used to determine the mechanism of the cytotoxic effect of ketoprofen analogue **6**. To determine the main cause of decreased cell viability, the influence of **6** on cell division and cell death was investigated. As presented in Figure 4A, cell division was inhibited upon 48 h treatment of A375 cells with **6**. Within the same period, only 5% of double-positive, late-apoptotic cells were determined suggesting only a symbolic contribution of that type of cell death to decreased viability in response to compound **6** (Figure 4B). Accordingly, only a small amplification of total caspase activity was observed after the treatment (Figure 4C). Given that minor apoptosis usually correlates with autophagic processes, the cells were examined for the presence of autophagosomes. Indeed, enhanced autophagy was detected by flow cytometry after acridine orange (AO) staining in response to the treatment with **6** (Figure 4D). In fact, it is well documented that apoptosis and autophagy confront each other, but under some circumstances, autophagy becomes a regular way of cell death, so it can contribute to the cytotoxicity of compound **6**.[44]

DOI: 10.1021/acsomega.9b00412
ACS Omega 2019, 4, 8824−8833

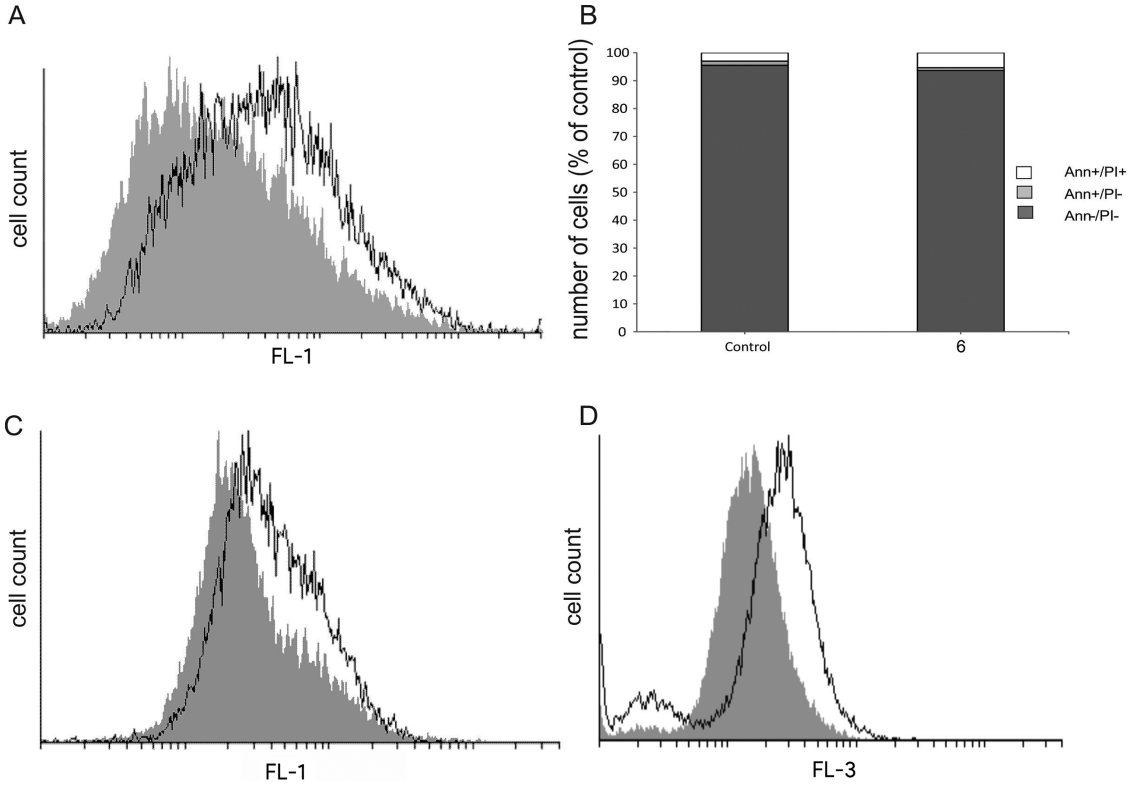


Figure 4. Mechanism of the cytotoxic action of **6** against A375 cells. Cells were exposed to **6** for 48 h, and cell proliferation (A), presence of apoptosis by Ann/propidium iodide (PI) (Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide) double staining (B), caspase activation by apostat (C), and autophagy by AO staining (D) were assessed by flow cytometry. Treatments with **6** are presented as black-lined histograms and controls as gray histograms.

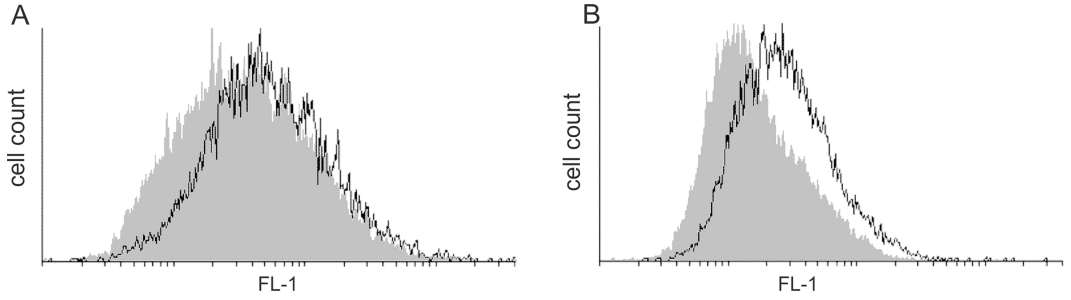


Figure 5. Mechanism of cytotoxic action of **6** against A375 cells. Intracellular NO (A) and ROS/RNS production (B) were measured after incubation with **6** for 48 h by flow cytometry. Treatments with **6** are presented as black-lined histograms and controls as gray histograms.

It has been reported that ketoprofen induced oxidative damage of gastrointestinal mucosa,[48] and it was attempted to circumvent this damage by incorporating an NO-releasing moiety based on the cytoprotective, prostaglandin-like properties of NO.[6] Since **6** is designed to be an NO-releasing ketoprofen analogue, the level of intracellular as well as extracellular NO in response to the treatment was determined. The release of NO from a nitrate moiety is a three-electron reduction that is normally catalyzed by multiple possible enzymes.[49] However, compound **6** was found not to affect the intracellular amount of NO in A375 cells (Figure 5A). Having in mind that A375 cells are able to produce NO, it can be concluded that compound **6** neither liberated NO nor changed endogenous production of this molecule in the melanoma cell line. Given that NO donors also release NO extracellularly and that the absence of intracellular NO release is not a proof that a compound is not able to liberate NO at all, spontaneous as well as the extracellular release of NO from **6** was measured. Release of NO in the extracellular compartment is typically determined by the Griess reaction that measures the resultant nitrite accumulation.[50,51] Nitrite accumulation was measured in culture medium, conditioned culture medium (medium taken from cell cultures upon incubation for 72 h) that contains soluble cellular products or cell debris, as well as in the supernatants from A375 cells that were exposed to **6** (10–40 μM) for 48 h. However, the determined nitrite accumulation was at the detection limit of the Griess reaction (1.5 μM), even at the highest applied dose, suggesting that **6** did not release NO in the extracellular compartment. Moreover, the production of reactive oxygen species/reactive nitrogen species (ROS/RNS) was measured employing dihydrorhodamine-123 (DHR). Indeed, ROS/RNS production was elevated after the exposure to **6** (Figure 5B), indicating that the compound promoted oxidative stress, which

DOI: 10.1021/acsomega.9b00412
ACS Omega 2019, 4, 8824–8833

correlates with the fact that cell lines highly susceptible to oxidative stress were affected most by **6**.

3. CONCLUSIONS

We report the synthesis of two carboranyl analogues of ketoprofen bearing an *ortho*- or *meta*-carborane (**3** and **4**), as well as derivatives additionally bearing nitrate moieties (**5** and **6**). The *ortho*-carborane nitrate ester **5** showed weak COX inhibitory activity. On the other hand, compound **6**, the *meta*-carborane analogue of **5**, exhibited cytotoxic activity toward colon and melanoma cancer cell lines in the micromolar range with a special effect on the A375 cell line. It was determined that the cytotoxic effect is due to its potential to promote autophagy and oxidative stress in cells. Further research should provide better insights into the mechanism of action and the molecular targets of the ketoprofen analogue **6**.

4. EXPERIMENTAL SECTION

4.1. Syntheses. *4.1.1. Materials and Methods.* All commercial reagents and solvents were used without further purification. Reactions including carboranes were carried out under a nitrogen atmosphere using the standard Schlenk technique. Compounds **2**, **4**, and **6** were synthesized with ^{10}B-enriched *m*-carborane. For column chromatography, silica gel (60 Å) from the company ACROS was used. The particle size was in the range of 0.035−0.070 mm. Thin-layer chromatography was used to monitor the reaction process of the syntheses. For this purpose, glass plates coated with silica gel plates 60 F254 from the company MERCK were used. Carborane-containing substances were stained with a 5% solution of palladium chloride in methanol (MeOH). All received ^{1}H, ^{13}C, and ^{11}B NMR spectra were recorded with an ADVANCE DRX 400 spectrometer from BRUKER. The chemical shifts are reported in parts per million (ppm). Quaternary carbons (e.g. substituted cluster carbon atoms) were not always observed because of their long relaxation time. The melting points were determined in glass capillaries using a GALLENKAMP apparatus and were uncorrected. IR spectra were obtained using an FTIR spectrometer (GENESIS ATI, Mattson/Unicam) in the range of 400−4000 cm^{-1} in KBr. High-resolution mass spectra in the positive or negative mode were recorded with a BRUKER Daltonics APEX II FT-ICR spectrometer. For these measurements, dichloromethane, acetonitrile (ACN), methanol (MeOH), formic acid (FA), or a mixture of these solvents was used.

Compounds **1**−**6** were obtained and employed as racemic mixtures.

*4.1.2. Synthesis of 2-[3-(1,2-Dicarba-closo-dodecaboranoyl)phenyl]propionitrile (**1**).* *o*-Carborane (0.0144 mol, 1.64 g) was dissolved in a mixture of dry Et_2O and toluene (1:1). To this solution, 7.6 mL of *n*-BuLi (1 equiv) was added dropwise at −40 °C. The reaction mixture was stirred at −40 °C for 1 h. Separately, 3-(1-cyanoethyl)benzoyl chloride was prepared by refluxing 2 g (0.0144 mol) of 3-(1-cyanoethyl)benzoic acid in $SOCl_2$ (10 mL) for 1 h. Excess $SOCl_2$ was removed under reduced pressure, and the acid halide was dissolved in 10 mL of dry diethyl ether. The solution of lithiated *m*-carborane was then added dropwise at −40 °C to the solution of 3-(1-cyanoethyl)benzoyl chloride, and the reaction was left to slowly warm to room temperature overnight. The reaction mixture was washed with 2 M HCl. The organic phase was collected, dried over $MgSO_4$, and the solvent was removed under reduced pressure to yield a yellow viscous oil. Purification was carried out by column chromatography (*n*-hexane/ethyl acetate 4:1). This gave compound **1** as a colorless solid. Yield: 32% (1.4 g, 4.6 mmol); mp: 69−70 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.04 (d, 1H, CH_{aromat}), 7.97 (s, 1H, CH_{aromat}), 7.7 (d, 1H, CH_{aromat}), 7.56 (t, 1H, CH_{aromat}), 4.56 (s, 1H, $CH_{cluster}$), 4.00 (q, $^{3}J_{HH}$ = 8 Hz, 1H, CH), 1.71 (d, $^{3}J_{HH}$ = 8 Hz, 3H, CH_3), 3.2−1.7 (m, 10H, $BH_{cluster}$); ^{11}B{^{1}H} NMR ($CDCl_3$, 128 MHz): δ = −1.3 (s, 1B), −2.7 (s, 2B), −8.0 (s, 3B), −11.7 (s, 2B), −13.4 (s, 2B); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): δ = 219.7 (CO), 185.2 (CN), 137.9 (qC_{aromat}), 134.0 (qC_{aromat}), 132.5 (CH_{aromat}), 130.1 (CH_{aromat}), 129.5 (CH_{aromat}), 128.6 (CH_{aromat}), 59.2 ($CH_{cluster}$), 32.1 (CH), 21.2 (CH_3); IR (KBr, cm^{-1}): $\tilde{\nu}$ = 3044 (m, ν(C−$H_{cluster}$)), 2992−2877 (w, ν(C−$H_{aliphat}$)), 2615 (s, ν(B−H)), 2240 (w, ν(C≡N)) 1687 (s, ν(C═O)); high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) (negative mode, ACN) *m*/*z* [M − H]$^-$: calcd for $C_{12}H_{18}B_{10}NO$: 300.2391, found: 300.2401; the observed isotopic pattern was in agreement with the calculated one. Elemental analysis: calcd for C: 47.82, H: 6.47, N: 4.65, found for C: 47.79, H: 6.35, N: 4.49.

*4.1.3. Synthesis of 2-[3-(1,7-Dicarba-closo-dodecaboranoyl)phenyl]propionitrile (**2**).* This compound was synthesized in a manner similar as described for the ortho isomer, starting from 1.5 g (0.011 mol) of *m*-carborane. Compound **2** was obtained as a colorless solid. Yield: 32% (1.1 g, 3.65 mmol); mp: 80−81 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.64−7.54 (m, 3H, CH_{aromat}), 7.46 (t, 1H, CH_{aromat}), 3.95 (q, $^{3}J_{HH}$ = 8 Hz, 1H, CH), 3.09 (s, 1H, $CH_{cluster}$), 1.67 (d, $^{3}J_{HH}$ = 8 Hz, 3H, CH_3), 3.35−1.4 (m, 10H, $BH_{cluster}$); ^{11}B{^{1}H} NMR ($CDCl_3$, 128 MHz): δ = −5.5 (br, 3B), −10.8 (br, 3B), −12.9 (s, 2B), −15.2 (s, 2B); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): δ = 188.4 (CO), 137.4 (qC_{aromat}), 137.1 (qC_{aromat}), 130.6 (CH_{aromat}), 129.0 (CH_{aromat}), 128.3 (CH_{aromat}), 126.9 (CH_{aromat}), 120.8 (CN), 55.0 ($CH_{cluster}$), 31.0 (CH), 21.2 (CH_3); IR (KBr, cm^{-1}): $\tilde{\nu}$ = 3072 (m, ν(C−$H_{cluster}$)), 2998−2870 (w, ν(C−$H_{aliphat}$)), 2615 (s, ν(B−H)), 2247 (s, ν(C≡N)), 1683 (s, ν(C═O)); HR-ESI-MS (positive, ACN) *m*/*z* [M + Na]$^+$: calcd for $C_{12}H_{19}{}^{10}B_{10}NNaO$: 316.2658, found: 316.2672; the observed isotopic pattern was in agreement with the calculated one. Elemental analysis: calcd for C: 49.12, H: 6.52, N: 4.77, found for C: 48.26, H: 6.47, N: 4.77.

*4.1.4. Synthesis of 2-[3-(1,2-Dicarba-closo-dodecaboranoyl)phenyl]propanoic Acid (**3**).* Compound **1** (1 g, 3.32 mmol) was dissolved in 20 mL of acetic acid and 10 mL of 6 M HCl. The solution was left to reflux overnight and then it was neutralized with saturated $NaHCO_3$. The resulting slurry was extracted with ethyl acetate (2 × 30 mL). The organic phase was collected and dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. Purification was carried out by column chromatography (*n*-hexane/ethyl acetate 2:1). Yield: 96% (1.02 g, 3.18 mmol); mp: 91−92 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.99 (s, 1H, CH_{aromat}), 7.96 (d, 1H, CH_{aromat}), 7.62 (d, 1H, CH_{aromat}), 7.46 (t, 1H, CH_{aromat}), 4.55 (s, 1H, $CH_{cluster}$), 3.83 (q, $^{3}J_{HH}$ = 7.1 Hz, 1H, CH), 1.57 (d, $^{3}J_{HH}$ = 7.1 Hz, 3H, CH_3), 3.45−1.6 (m, 10H, $BH_{cluster}$); ^{11}B{^{1}H} NMR ($CDCl_3$, 128 MHz): δ = −1.4 (s, 1B), −2.5 (s, 2B), −8.1 (s, 2B), −11.5 (s, 3B), −13.4 (s, 2B); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): δ = 185.2 (CO), 178.4 (COOH), 140.4 (qC_{aromat}), 133.7 (CH_{aromat}), 133.4 (qC_{aromat}), 129.7 (CH_{aromat}), 128.9 (CH_{aromat}), 59.2 ($CH_{cluster}$), 44.9 (CH), 18.1 (CH_3); IR (KBr, cm^{-1}): $\tilde{\nu}$ = 3088 (m, ν(C−

DOI: 10.1021/acsomega.9b00412
ACS Omega 2019, 4, 8824−8833

$H_{cluster}$)), 3000−2726 (w, ν(C−$H_{aliphat}$)), 2592 (s, ν(B−H)), 1713 (s, ν(C═O)), 1671 (s, ν(C═O)), 1263 (s, ν(C−O)), 1233 (s, ν(C−O)); HR-ESI-MS (negative mode, ACN) m/z [M − CO_2 − H]$^-$: calcd for $C_{11}H_{19}B_{10}O$ 275.2439: found: 275.2483; m/z [M − H]$^-$: calcd for $C_{12}H_{19}B_{10}O_3$: 319.2337, found: 319.2370; the observed isotopic pattern was in agreement with the calculated one. Elemental analysis: calcd for C: 44.99, H: 6.29, found for C: 44.33, H: 6.22.

*4.1.5. Synthesis of 2-[3-(1,7-Dicarba-closo-dodecaboranoyl)phenyl]propanoic Acid (**4**).* This compound was synthesized in a manner similar as described for the ortho isomer starting from 0.95 g (3.15 mmol) of **2**. Compound **4** was obtained as a colorless solid. Yield: 90% (0.91 g, 2.84 mmol); mp: 85−86 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.61 (s, 1H, CH_{aromat}), 7.56 (d, 1H, CH_{aromat}), 7.50 (d, 1H, CH_{aromat}), 7.38 (t, CH_{aromat}), 3.79 (q, $^3J_{HH}$ = 4 Hz, 1H, CH), 3.06 (s, 1H, $CH_{cluster}$), 1.54 (d, $^3J_{HH}$ = 4 Hz, 3H, CH_3), 3.36−1.49 (m, 10H, $BH_{cluster}$); ^{11}B{^{1}H} NMR ($CDCl_3$, 128 MHz): δ = −10.8 (br, 4B), −12.9 (s, 3B), −15.2 (s, 3B); ^{13}C{^{1}H} NMR ($CDCl_3$, ppm): δ = 188.5 (CO), 177.6 (COOH), 139.9 (qC_{aromat}), 136.4 (qC_{aromat}), 131.7 (CH_{aromat}), 128.5 (CH_{aromat}), 127.9 (CH_{aromat}), 127.8 (CH_{aromat}), 54.8 ($CH_{cluster}$), 44.8 (CH), 18.2 (CH_3); IR (KBr, cm^{-1}): $\tilde{\nu}$ = 2108−3000 (w, ν(C−H_{aromat})), 3000−2766 (w, ν($CH_{aliphat}$)), 2615 (s, ν(B−H)), 1717 (s, ν(C═O)), 1687 (s, ν(C═O)), 1279 (m, ν(C−O)), 1259 (m, ν(C−O)). HR-ESI-MS (negative mode, ACN) m/z [M − CO_2 − H]$^-$: calcd for $C_{11}H_{19}{}^{10}B_{10}O$: 267.2730, found: 267.2792; m/z [M − H]$^-$: calcd for $C_{12}H_{19}{}^{10}B_{10}O_3$: 311.2628, found: 311.2672; the observed isotopic pattern was in agreement with the calculated one. Elemental analysis: calcd for C: 46.13, H: 6.45, found for C: 45.40, H: 6.45.

*4.1.6. Synthesis of 1,3-Bis(nitrooxy)propan-2-yl-2-[3-(1,2-dicarba-closo-dodecaboranoyl)phenyl]propanoate (**5**).* 2-[3-(1,2-Dicarba-*closo*-dodecaboranoyl)phenyl]propanoic acid (2.03 g, 8.0 mmol), 3.80 mL (40 mmol) of *tert*-butanol, 147 mg (1.2 mmol) of DMAP, and anhydrous CH_2Cl_2 (20 mL) were added to a 100 mL round-bottom flask. DCC (2.23 g, 10.8 mmol) was added in one portion to this stirred slurry. The formation of a colorless precipitate was observed immediately. After stirring at room temperature overnight, the precipitate was removed via filtration, and the filtrate was concentrated under vacuum. The residue was purified with silica gel flash chromatography using *n*-hexane/ethyl acetate 20:1 to 10:1 as an eluent. The desired product was obtained as a viscous clear and colorless oil. Yield: 92% (2.28 g, 4.7 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.97 (d, ^{1}H, CH_{aromat}), 7.91 (s, 1H, $CH_{aromatic}$), 7.55 (d, 1H, CH_{aromat}), 7.46 (t, 1H, CH_{aromat}), 5.37 (m, 1H, CH), 4.74 (dd, $^2J_{HH}$ = 6 Hz, $^4J_{HH}$ = 12.8 Hz, 1H, CH), 4.64 (dd, $^2J_{HH}$ = 6 Hz, $^4J_{HH}$ = 12.8 Hz, 1H, CH), 4.55 (dd, $^2J_{HH}$ = 6 Hz, $^4J_{HH}$ = 12.8 Hz, 2H, CH, and $CH_{cluster}$), 4.74 (dd, $^2J_{HH}$ = 6 Hz, $^4J_{HH}$ = 12.8 Hz, 1H, CH), 3.83 (q, $^3J_{HH}$ = 7.1 Hz, 1H, CH), 1.56 (d, 3H, $^3J_{HH}$ = 7.1 Hz, CH_3), 3.29−1.5 (m, 10H, $BH_{cluster}$); ^{11}B{^{1}H} NMR ($CDCl_3$, 128 MHz): δ = −1.4 (s, 1B), −2.8 (s, 2B), −8.1 (s, 2B), −11.5 (s, 3B), −13.5 (s, 2B); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): δ = 184.9 (CO), 172.8 (COOR), 140.0 (qC_{aromat}), 133.6 (qC_{aromat}), 133.4 (CH_{aromat}), 129.7 (CH_{aromat}), 129.4 (CH_{aromat}), 129.1 (CH_{aromat}), 69.3 (CH_2), 69.2 (CH_2), 67.1 (CH), 59.2 ($CH_{cluster}$), 45.0 (CH), 18.1 (CH_3); IR (KBr, cm^{-1}): $\tilde{\nu}$ = 3094 (m, ν(C−$H_{cluster}$)), 2634−2556 (s, ν(B−$H_{cluster}$)), 1741 (m, v(C═O)), 1678 (s, ν(C═O) or ν(NO)), 1636 (s, ν(C═O) or ν(NO)), 1258 (s, ν(C−O) or ν(NO)), 851 (s, ν(N−O)). HR-ESI-MS (negative mode, ACN, FA) m/z [M + FA − H]$^-$: calculated for the formic acid (FA) adduct $C_{16}H_{25}B_{10}N_2O_{11}$: 529.2413, found: 529.2452; the observed isotopic pattern was in agreement with the calculated one. Elemental analysis: calcd for C: 37.19, H: 4.99, N: 5.78, found for C: 37.37, H: 4.96, N: 5.80.

*4.1.7. Synthesis of 1,3-Bis(nitrooxy)propan-2-yl-2-[3-(1,7-dicarba-closo-dodecaboranoyl)phenyl]propanoate (**6**).* This compound was synthesized in a manner similar as described for the ortho isomer starting from 0.7 g (2.22 mmol) of **4**. Compound **6** was obtained as a viscous clear and colorless oil. Yield: 92% (0.99 g, 2.0 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.57 (d, ^{1}H, CH_{aromat}), 7.54 (s, 1H, $CH_{aromatic}$), 7.45 (d, 1H, CH_{aromat}), 7.39 (t, 1H, CH_{aromat}), 5.37 (m, 1H, CH), 4.75 (dd, $^2J_{HH}$ = 5.9 Hz, $^4J_{HH}$ = 12.7 Hz, 1H, CH), 4.63 (dd, $^2J_{HH}$ = 5.9 Hz, $^4J_{HH}$ = 12.7 Hz, 1H, CH), 4.55 (dd, $^2J_{HH}$ = 5.9 Hz, $^4J_{HH}$ = 12.7 Hz, 1H, CH), 4.47 (dd, $^2J_{HH}$ = 5.9 Hz, $^4J_{HH}$ = 12.7 Hz, 1H, CH), 3.79 (q, $^3J_{HH}$ = 7 Hz, 1H, CH), 3.08 (s, 1H, $CH_{cluster}$), 1.54 (d, 3H, $^3J_{HH}$ = 7 Hz, CH_3), 3.29−1.45 (m, 10H, $BH_{cluster}$); ^{11}B{^{1}H} NMR ($CDCl_3$, 128 MHz): δ = −4.9 (s, 2B), −6.0 (s, 1B), −10.4 (s, 2B), −11.2 (s, 1B), −12.9 (s, 2B), −15.2 (s, 2B); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): 188.4 (CO), 172.7 (COOR), 139.5 (qC_{aromat}), 136.6 (qC_{aromat}), 131.4 (CH_{aromat}), 128.6 (CH_{aromat}), 127.9 (CH_{aromat}), 127.8 (CH_{aromat}), 69.4 (CH_2), 69.2 (CH_2), 66.9 (CH), 55.1 ($CH_{cluster}$), 45.0 (CH), 18.1 (CH_3); IR (KBr, cm^{-1}): $\tilde{\nu}$ = 3067 (m, ν(C−$H_{cluster}$)), 2617 (s, ν(B−$H_{cluster}$)), 1746 (m, v(C═O)), 1647 (s, v(C═O) or ν(N−O)), 1277 (s, ν(C−O) or ν(NO)), 851 (s, ν(N−O)) HR-ESI-MS (negative mode, ACN, FA) m/z [M + FA − H]$^-$: calculated for the formic acid (FA) adduct $C_{16}H_{25}{}^{10}B_{10}N_2O_{11}$: 521.2752, found: 521.2695; the observed isotopic pattern was in agreement with the calculated one. Elemental analysis: calcd for C: 37.81, H: 5.07, N: 5.87, found for C: 37.47, H: 5.06, N: 5.37.

4.2. Cell Viability Materials and Methods. *4.1.8. Reagents and Cells.* Reagents were for the most part obtained from Sigma (St. Louis, MO). Annexin V-FITC (AnnV) was obtained from Biotium (Hayward, CA), and apostat was from R&D (R&D Systems, Minneapolis, MN). The melanoma cell lines A375, B16 and its metastatic subclone B16F10 as well as the colon cancer cell lines HCT116, SW480, and CT26CL25 were cultivated in 10% fetal calf serum (FCS) Roswell Park Memorial Institute (RPMI)-1640 medium with 2 mM L-glutamine, 0.01% sodium pyruvate, and antibiotics at 37 °C in a humidified atmosphere with 5% CO_2. Upon trypsinization, cells were seeded in a certain number: 2.5 × 10^3 cells/well for 96-well plates and viability assessment and 2 × 10^4 cells/well for 24-well plates and flow-cytometric analysis. C57BL/6 mice, obtained from the animal facility at The Institute for Biological Research "Siniša Stanković", were sacrificed for isolation of peritoneal resident macrophages. For viability assessment, cells were isolated by peritoneal lavage, washed, counted, seeded in 96-well plates and incubated overnight before drug application. Before treatment, nonadherent cells were removed. Carborane-based ketoprofen analogues were dissolved in dimethyl sulfoxide (DMSO) and stored at −20 °C for a month. 10% FCS−RPMI-1640 working solutions were prepared before the experiment started. In each experiment, control cultures were exposed to equal amounts of DMSO.

4.1.9. Cell Viability Tests. For viability assessment, two of the most frequently used tests were employed: (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) and crystal violet (CV). Cells were exposed to **3**−**6**

DOI: 10.1021/acsomega.9b00412
ACS Omega 2019, 4, 8824−8833

in a wide range of doses for a certain time interval, and the number of viable cells was determined as described.[52] Nontreated cells were arbitrarily set to 100%, and according to this, results were expressed as the percentage of control.

4.1.10. Annexin V-FITC/PI, Acridine Orange Staining, and Caspase Detection. A375 cells were treated with **6** for 48 h and, after trypsinization, stained with Annexin V-FITC (Ann)/propidium iodide (PI) or apostat as proposed by the manufacturer. To evaluate the presence of acidic vesicles, markers of autophagy, in the cytoplasm of cells, after-treatment staining with a 10 μM AO solution for 15 min at 37 °C was done. At the end of incubation, cells were washed and finally resuspended in phosphate buffer solution (PBS). At the end of all staining procedures, cells were analyzed with CyFlow Space Partec using the PartecFloMax software.

4.1.11. Cell Staining with Carboxyfluorescein Succinimidyl Ester (CFSE). For cell division rate determination, cells were prestained with carboxyfluorescein diacetate succinimidyl ester (CFSE) (1 μM) for 10 min at 37 °C. After dye removal, cells were exposed to an IC_{50} dose of **6** and 48 h later analyzed by CyFlow Space Partec using the PartecFloMax.

4.1.12. Measurement of Intracellular Nitric Oxide. 4-Amino-5-methylamino-2′,7′-difluorofluorescein diacetate (DAF-FM, 5 μM) was used for quantification of the intracellular level of NO. After an incubation time of 48 h, the cells were incubated in phenol-red-free RPMI containing DAF-FM for 1 h at 37 °C, washed, and additionally incubated for 15 min in serum/phenol-red-free conditions to complete de-esterification of intracellular diacetate. Cells were resuspended in phosphate buffer solution (PBS) and analyzed as described.

4.1.13. Measurement of Intracellular Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). Dihydrorhodamine-123 (DHR) (1 μM) was used for detection of ROS/RNS production. To determinate the influence of **6** on the redox status of A375 cells, they were stained with DHR dye for 20 min prior to exposure to an IC_{50} dose of **6**. After 48 h, cells were collected, washed, and resuspended in PBS and analyzed with CyFlow Space Partec using PartecFloMax.

4.1.14. Nitrite Detection. For detection of NO release in the culture supernatant, nitrite accumulation, as an indicator of NO production, was measured after 48 h of cultivation in the presence of **6** using the Griess method.[53] For assessment of spontaneous release of nitric oxide, **6** was diluted in the culture medium or conditioned medium, collected from cell cultures after three days of incubation. Certain aliquots of culture supernatants were mixed with the same volume of the Griess reagent prepared from 0.1% naphthylenediamine dihydrochloride and 1% sulfanilamide in 5% H_3PO_4, mixed in a 1:1 ratio. Plates were incubated for 10 min at room temperature, and the absorbance was measured at 570 nm using a microplate reader. The nitrite concentration was calculated according to a standard curve derived from a range of $NaNO_2$ concentrations.

4.1.15. Statistics. The results were obtained in triplicate. IC_{50} concentrations were calculated from at least three independent experiments. The significance of the differences between various treatments was calculated by the analysis of variance, followed by the Student−Newman−Keuls test. A p value of less than 0.05 was considered significant in comparison to untreated control cells.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b00412.

COX inhibition assay: Measured COX-1 and COX-2 inhibition values for compounds **3**−**6**; Selectivity index values of **6** in melanoma and colon cancer cell lines (PDF)

Crystallographic data and crystal structure of **1** (CCDC 1880967); **3** (CCDC 1880968); **4** (CCDC 1880969) (CIF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: hey@uni-leipzig.de. Fax: (+49)341-9739319.

ORCID

Markus Laube: 0000-0003-4916-3794
Wilma Neumann: 0000-0002-1728-5140
Jens Pietzsch: 0000-0002-1610-1493
Evamarie Hey-Hawkins: 0000-0003-4267-0603

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Support from the Deutscher Akademischer Austauschdienst (DAAD, doctoral fellowship for A.B.), the DFG (HE 1376/38-1), the Graduate School BuildMoNa (A.B., W.N.), and the Ministry of Education, Science and Technological Development of the Republic of Serbia (project No. 173013) is gratefully acknowledged. The authors acknowledge the expert technical assistance of Mareike Barth and Johanna Wodtke (HZDR) and Stefanie Märcker and Ines Rein (Leipzig University). We acknowledge support from the German Research Foundation (DFG) and Leipzig University within the program of Open Access Publishing.

■ REFERENCES

(1) Shohin, I. E.; Kulinich, J. I.; Ramenskaya, G. V.; Abrahamsson, B.; Kopp, S.; Langguth, P.; Polli, J. E.; Shah, V. P.; Groot, D. W.; Barends, D. M.; Dressman, J. B. Biowaiver monographs for immediate-release solid oral dosage forms: ketoprofen. *J. Pharm. Sci.* **2012**, *101*, 3593−3603.

(2) Kantor, T. G. Ketoprofen: A Review of Its Pharmacologic and Clinical Properties. *Pharmacotherapy* **1986**, *6*, 93−102.

(3) Julou, L.; Guyonnet, J. C.; Ducrot, R.; Fournel, J.; Pasquet, J. Ketoprofen (19.583 R.P.) (2-(3-benzoylphenyl)-propionic acid). Main pharmacological properties-outline of toxicological and pharmacokinetic data. *Scand. J. Rheumatol., Suppl.* **1976**, 33−44.

(4) Dawson, W.; Boot, J. R.; Harvey, J.; Walker, J. R. The pharmacology of benoxaprofen with particular to effects on lipoxygenase product formation. *Eur. J. Rheumatol. Inflammation* **1982**, *5*, 61−68.

(5) Walker, J. L. Interrelationships of SRS-A production and arachidonic acid metabolism in human lung tissue. *Adv. Prostaglandin Thromboxane Res.* **1980**, *6*, 115−119.

(6) Gaitan, G.; Ahuir, F. J.; Del Soldato, P.; Herrero, J. F. Comparison of the antinociceptive activity of two new NO-releasing derivatives of the NSAID S-ketoprofen in rats. *Br. J. Pharmacol.* **2004**, *143*, 533−540.

(7) Fries, J. F.; Williams, C. A.; Ramey, D.; Bloch, D. A. The relative toxicity of disease-modifying antirheumatic drugs. *Arthritis Rheum.* **1993**, *36*, 297−306.

DOI: 10.1021/acsomega.9b00412
ACS Omega 2019, 4, 8824−8833

(8) Henry, D.; Lim, L. L.-Y.; Rodriguez, L. A. G.; Gutthann, S. P.; Carson, J. L.; Griffin, M.; Savage, R.; Logan, R.; Moride, Y.; Hawkey, C.; Hill, S.; Fries, J. T. Variability in risk of gastrointestinal complications with individual non-steroidal anti-inflammatory drugs: results of a collaborative meta-analysis. *BMJ* **1996**, *312*, 1563−1566.
(9) Mitchell, J. A.; Akarasereenont, P.; Thiemermann, C.; Flower, R. J.; Vane, J. R. Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11693−11697.
(10) Fiorucci, S.; Antonelli, E.; Burgaud, J.-L.; Morelli, A. Nitric Oxide—Releasing NSAIDs. *Drug Saf.* **2001**, *24*, 801−811.
(11) Chegaev, K.; Lazzarato, L.; Tosco, P.; Cena, C.; Marini, E.; Rolando, B.; Carrupt, P. A.; Fruttero, R.; Gasco, A. NO-donor COX-2 inhibitors. New nitrooxy-substituted 1,5-diarylimidazoles endowed with COX-2 inhibitory and vasodilator properties. *J. Med. Chem.* **2007**, *50*, 1449−1457.
(12) Brown, J. F.; Hanson, P. J.; Whittle, B. J. Nitric oxide donors increase mucus gel thickness in rat stomach. *Eur. J. Pharmacol.* **1992**, *223*, 103−104.
(13) Thun, M. J.; Henley, S. J.; Patrono, C. Nonsteroidal Anti-inflammatory Drugs as Anticancer Agents: Mechanistic, Pharmacologic, and Clinical Issues. *J. Natl. Cancer Inst.* **2002**, *94*, 252−266.
(14) Masferrer, J. L.; Leahy, K. M.; Koki, A. T.; Zweifel, B. S.; Settle, S. L.; Woerner, B. M.; Edwards, D. A.; Flickinger, A. G.; Moore, R. J.; Seibert, K. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. *Cancer Res.* **2000**, *60*, 1306−1311.
(15) Rüegg, C.; Zaric, J.; Stupp, R. Non steroidal anti-inflammatory drugs and COX-2 inhibitors as anti-cancer therapeutics: hypes, hopes and reality. *Ann. Med.* **2003**, *35*, 476−487.
(16) Damnjanovic, I.; Najman, S.; Stojanovic, S.; Stojanovic, D.; Veljkovic, A.; Kocic, H.; Langerholc, T.; Damnjanovic, Z.; Pesic, S. Crosstalk between possible cytostatic and antiinflammatory potential of ketoprofen in the treatment of culture of colon and cervix cancer cell lines. *Bratisl. Lek. Listy* **2015**, *116*, 227−232.
(17) Kamei, S.; Sakayama, K.; Tamashiro, S.; Aizawa, J.; Miyawaki, J.; Miyazaki, T.; Yamamoto, H.; Norimatsu, Y.; Masuno, H. Ketoprofen in topical formulation decreases the matrix metalloproteinase-2 expression and pulmonary metastatic incidence in nude mice with osteosarcoma. *J. Orthop. Res.* **2009**, *27*, 909−915.
(18) Reddy, B. S.; Tokumo, K.; Kulkarni, N.; Aligia, C.; Kelloff, G. Inhibition of colon carcinogenesis by prostaglandin synthesis inhibitors and related compounds. *Carcinogenesis* **1992**, *13*, 1019−1023.
(19) Sakayama, K.; Kidani, T.; Miyazaki, T.; Shirakata, H.; Kimura, Y.; Kamogawa, J.; Masuno, H.; Yamamoto, H. Effect of ketoprofen in topical formulation on vascular endothelial growth factor expression and tumor growth in nude mice with osteosarcoma. *J. Orthop. Res.* **2004**, *22*, 1168−1174.
(20) Hosmane, N. S. *Handbook of Boron Chemistry in Organometallics, Catalysis, Materials and Medicine*; World Scientific, 2018.
(21) Hosmane, N. S. *Boron Science: New Technologies and Applications*; Taylor & Francis, 2011.
(22) Valliant, J. F.; Guenther, K. J.; King, A. S.; Morel, P.; Schaffer, P.; Sogbein, O. O.; Stephenson, K. A. The medicinal chemistry of carboranes. *Coord. Chem. Rev.* **2002**, *232*, 173−230.
(23) Scholz, M.; Hey-Hawkins, E. Carbaboranes as Pharmacophores: Properties, Synthesis, and Application Strategies. *Chem. Rev.* **2011**, *111*, 7035−7062.
(24) Neumann, W.; Xu, S.; Sárosi, M. B.; Scholz, M. S.; Crews, B. C.; Ghebreselasie, K.; Banerjee, S.; Marnett, L. J.; Hey-Hawkins, E. *nido*-Dicarbaborate Induces Potent and Selective Inhibition of Cyclooxygenase-2. *ChemMedChem* **2016**, *11*, 175−178.
(25) Laube, M.; Neumann, W.; Scholz, M.; Lonnecke, P.; Crews, B.; Marnett, L. J.; Pietzsch, J.; Kniess, T.; Hey-Hawkins, E. 2-Carbaborane-3-phenyl-1H-indoles–synthesis via McMurry reaction and cyclooxygenase (COX) inhibition activity. *ChemMedChem* **2013**, *8*, 329−335.
(26) Scholz, M.; Blobaum, A. L.; Marnett, L. J.; Hey-Hawkins, E. Ortho-carbaborane derivatives of indomethacin as cyclooxygenase (COX)-2 selective inhibitors. *Bioorg. Med. Chem.* **2012**, *20*, 4830−4837.
(27) Scholz, M.; Bensdorf, K.; Gust, R.; Hey-Hawkins, E. Asborin: The Carbaborane Analogue of Aspirin. *ChemMedChem* **2009**, *4*, 746−748.
(28) Scholz, M.; Blobaum, A. L.; Marnett, L. J.; Hey-Hawkins, E. Synthesis and evaluation of carbaborane derivatives of indomethacin as cyclooxygenase inhibitors. *Bioorg. Med. Chem.* **2011**, *19*, 3242−3248.
(29) *Boron-Based Compounds: Potential and Emerging Applications in Medicine*; Hey-Hawkins, E.; Viñas Teixidor, C., Eds.; Wiley, 2018.
(30) Barth, R. F.; Mi, P.; Yang, W. Boron delivery agents for neutron capture therapy of cancer. *Cancer Commun.* **2018**, *38*, 35.
(31) Kallio, M.; Callaway, J.; Saario, E.; Kahl, S. Acute Toxicity of Elaidyl-1,2,-Carborane Carboxylate (ECC) in Rats. In *Cancer Neutron Capture Therapy*; Mishima, Y., Ed.; Springer: Boston, MA, 1996; pp 611−613.
(32) Butler, A. R.; Williams, D. L. H. The physiological role of nitric oxide. *Chem. Soc. Rev.* **1993**, *22*, 233−241.
(33) Houghton, G. W.; Dennis, M. J.; Rigler, E. D.; Parsons, R. L. Urinary pharmacokinetics of orally administered ketoprofen in man. *Eur. J. Drug Metab. Pharmacokinet.* **1984**, *9*, 201−204.
(34) Grimes, R. N. Open 12-Vertex and Supra-icosahedral Carboranes. In *Carboranes*, 2nd ed.; Grimes, R. N., Ed.; Academic Press: Oxford, 2011, Chapter 11; pp 675−699.
(35) Zakharkin, L. I.; L'vov, A. I. Synthesis of ketones of the barene series. *Bull. Acad. Sci. USSR, Div. Chem. Sci.* **1966**, *15*, 128−30.
(36) Boyd, L. A.; Clegg, W.; Copley, R. C.; Davidson, M. G.; Fox, M. A.; Hibbert, T. G.; Howard, J. A.; Mackinnon, A.; Peace, R. J.; Wade, K. Exo-pi-bonding to an ortho-carborane hypercarbon atom: systematic icosahedral cage distortions reflected in the structures of the fluoro-, hydroxy- and amino-carboranes, 1-X-2-Ph-1,2-$C_2B_{10}H_{10}$ (X = F, OH or NH_2) and related anions. *Dalton Trans.* **2004**, 2786−2789.
(37) Hussain, M.; Javeed, A.; Ashraf, M.; Zhao, Y.; Mukhtar, M. M.; Rehman, M. U. Aspirin and immune system. *Int. Immunopharmacol.* **2012**, *12*, 10−20.
(38) Bechmann, N.; Kniess, T.; Kockerling, M.; Pigorsch, A.; Steinbach, J.; Pietzsch, J. Novel (pyrazolyl)benzenesulfonamides with a nitric oxide-releasing moiety as selective cyclooxygenase-2 inhibitors. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 3295−3300.
(39) Denkert, C.; Köbel, M.; Berger, S.; Siegert, A.; Leclere, A.; Trefzer, U.; Hauptmann, S. Expression of Cyclooxygenase 2 in Human Malignant Melanoma. *Cancer Res.* **2001**, *61*, 303−308.
(40) Zhou, P.; Qin, J.; Li, Y.; Li, G.; Wang, Y.; Zhang, N.; Chen, P.; Li, C. Combination therapy of PKCzeta and COX-2 inhibitors synergistically suppress melanoma metastasis. *J. Exp. Clin. Cancer Res.* **2017**, *36*, 115.
(41) Valcárcel, M.; Mendoza, L.; Hernández, J.-J.; Carrascal, T.; Salado, C.; Crende, O.; Vidal-Vanaclocha, F. Vascular endothelial growth factor regulates melanoma cell adhesion and growth in the bone marrow microenvironment via tumor cyclooxygenase-2. *J. Transl. Med.* **2011**, *9*, No. 142.
(42) Pozzi, A.; Yan, X.; Macias-Perez, I.; Wei, S.; Hata, A. N.; Breyer, R. M.; Morrow, J. D.; Capdevila, J. H. Colon Carcinoma Cell Growth Is Associated with Prostaglandin E2/EP4 Receptor-evoked ERK Activation. *J. Biol. Chem.* **2004**, *279*, 29797−29804.
(43) Lin, P. C.; Lin, Y. J.; Lee, C. T.; Liu, H. S.; Lee, J. C. Cyclooxygenase-2 expression in the tumor environment is associated with poor prognosis in colorectal cancer patients. *Oncol. Lett.* **2013**, *6*, 733−739.
(44) Oláh, G.; Módis, K.; Törö, G.; Hellmich, M. R.; Szczesny, B.; Szabo, C. Role of endogenous and exogenous nitric oxide, carbon monoxide and hydrogen sulfide in HCT116 colon cancer cell proliferation. *Biochem. Pharmacol.* **2018**, *149*, 186−204.
(45) Pantovic, A.; Bosnjak, M.; Arsikin, K.; Kosic, M.; Mandic, M.; Ristic, B.; Tosic, J.; Grujicic, D.; Isakovic, A.; Micic, N.; Trajkovic, V.; Harhaji-Trajkovic, L. In vitro antiglioma action of indomethacin is

DOI: 10.1021/acsomega.9b00412
ACS Omega 2019, 4, 8824−8833

mediated via AMP-activated protein kinase/mTOR complex 1 signalling pathway. *Int. J. Biochem. Cell Biol.* **2017**, *83*, 84−96.
(46) Marjanović, M.; Zorc, B.; Pejnovic, L.; Zovko, M.; Kralj, M. Fenoprofen and ketoprofen amides as potential antitumor agents. *Chem. Biol. Drug Des.* **2007**, *69*, 222−226.
(47) Hixson, L. J.; Alberts, D. S.; Krutzsch, M.; Einsphar, J.; Brendel, K.; Gross, P. H.; Paranka, N. S.; Baier, M.; Emerson, S.; Pamukcu, R. Antiproliferative effect of nonsteroidal antiinflammatory drugs against human colon cancer cells. *Cancer Epidemiol. Biomarkers Prev.* **1994**, *3*, 433−438.
(48) Cheng, Y. T.; Ho, C. Y.; Jhang, J. J.; Lu, C. C.; Yen, G. C. DJ-1 plays an important role in caffeic acid-mediated protection of the gastrointestinal mucosa against ketoprofen-induced oxidative damage. *J. Nutr. Biochem.* **2014**, *25*, 1045−1057.
(49) Ignarro, L. J.; Lippton, H.; Edwards, J. C.; Baricos, W. H.; Hyman, A. L.; Kadowitz, P. J.; Gruetter, C. A. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: evidence for the involvement of S-nitrosothiols as active intermediates. *J. Pharmacol. Exp. Ther.* **1981**, *218*, 739−749.
(50) Kleinbongard, P.; Dejam, A.; Lauer, T.; Rassaf, T.; Schindler, A.; Picker, O.; Scheeren, T.; Godecke, A.; Schrader, J.; Schulz, R.; Heusch, G.; Schaub, G. A.; Bryan, N. S.; Feelisch, M.; Kelm, M. Plasma nitrite reflects constitutive nitric oxide synthase activity in mammals. *Free Radical Biol. Med.* **2003**, *35*, 790−796.
(51) Sako, M.; Oda, S.; Ohara, S.; Hirota, K.; Maki, Y. Facile Synthesis and NO-Generating Property of 4H-[1,2,5]Oxadiazolo[3,4-d]pyrimidine-5,7-dione 1-Oxides. *J. Org. Chem.* **1998**, *63*, 6947−6951.
(52) Mijatovic, S.; Maksimovic-Ivanic, D.; Radovic, J.; Miljkovic, D.; Harhaji, L.; Vuckovic, O.; Stosic-Grujicic, S.; Mostarica Stojkovic, M.; Trajkovic, V. Anti-glioma action of aloe emodin: the role of ERK inhibition. *Cell. Mol. Life Sci.* **2005**, *62*, 589−598.
(53) Green, L. C.; Wagner, D. A.; Glogowski, J.; Skipper, P. L.; Wishnok, J. S.; Tannenbaum, S. R. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Anal. Biochem.* **1982**, *126*, 131−138.

DOI: 10.1021/acsomega.9b00412
ACS Omega 2019, 4, 8824−8833