Bioisosteres of Indomethacin as Inhibitors of Aldo-Keto Reductase 1C3

Marco L. Lolli,[†] Irene M. Carnovale,[†] Agnese C. Pippione,[†] Weixiao Y. Wahlgren,[‡] Davide Bonanni,[†] Elisabetta Marini,[†] Daniele Zonari,[†] Margherita Gallicchio,[†] Valentina Boscaro,[†] Parveen Goyal,[‡] Rosmarie Friemann,[‡] Barbara Rolando,[‡] Renzo Bagnati,[§] Salvatore Adinolfi,[†] Simonetta Oliaro-Bosso,^{*,†} and Donatella Boschi^{*,†}

[†]Department of Science and Drug Technology, University of Turin, via Pietro Giuria 9, 10125 Turin, Italy

[‡]Department of Chemistry and Molecular Biology, University of Gothenburg, Box 462, S-40530 Gothenburg, Sweden

[§]Istituto di Ricerche Farmacologiche “Mario Negri” IRCCS, Via La Masa 19, 20156 Milan, Italy

Supporting Information

ABSTRACT: Aldo-keto reductase 1C3 (AKR1C3) is an attractive target in drug design for its role in resistance to anticancer therapy. Several nonsteroidal anti-inflammatory drugs such as indomethacin are known to inhibit AKR1C3 in a nonselective manner because of COX-off target effects. Here we designed two indomethacin analogues by proposing a bioisosteric connection between the indomethacin carboxylic acid function and either hydroxyfurazan or hydroxy triazole rings. Both compounds were found to target AKR1C3 in a selective manner. In particular, hydroxyfurazan derivative is highly selective for AKR1C3 over the 1C2 isoform (up to 90-times more) and inactive on COX enzymes. High-resolution crystal structure of its complex with AKR1C3 shed light onto the binding mode of the new inhibitors. In cell-based assays (on colorectal and prostate cancer cells), the two indomethacin analogues showed higher potency than indomethacin. Therefore, these two AKR1C3 inhibitors can be used to provide further insight into the role of AKR1C3 in cancer.

KEYWORDS: AKR1C3, Prostate cancer, Bioisosterism, Scaffold hopping, Indomethacin, X-ray crystallography

As a soluble enzyme member of the aldo-ketoreductase family, AKR1C3 is involved in numerous enzymatic reactions. It catalyzes the NADPH-dependent reduction of weak steroidal precursors, such as androstenedione, to the potent steroidal receptor ligand testosterone.¹ Elevated AKR1C3 expression has been identified in the sex hormone-dependent tissues of prostate and breast cancer as well as in sex hormone-independent tissues, such as adenocarcinoma, squamous cell carcinoma, columnar epithelium, and colorectal cancer.² Although a few recent studies have made controversial observations about the *in vivo* effectiveness of AKR1C3-based therapies,^{3,4} other studies have supported the use of AKR1C3 as a therapeutic target in castration-resistant prostate cancer (CRPC) and in Acute Myeloid Leukemia.^{5,6} AKR1C3's role in resistance to radiation and pharmacological therapies is also an emerging theme.⁷ AKR1C3 induction appears correlated with changes in the drug efficacy of important chemotherapeutic agents, such as abiraterone,⁸ enzalutamide,⁹ and anthracycline.¹⁰ Up-regulated AKR1C3 is also involved in colon cancer cisplatin resistance, and the treatment of colon-cancer cells with specific AKR1C3 inhibitors improved sensitivity to cisplatin toxicity.¹¹

AKR1C3 is inhibited by several classes of compounds,^{1,12} although no drugs have yet been approved for clinical use. Many AKR1C3 inhibitors were discovered by starting

investigations from nonsteroidal anti-inflammatory drug (NSAID) structures, such as flufenamic acid (FLU) and indomethacin (INDO) (Figure 1). Such compounds are usually carboxylic acids and their transport into cells is often carrier-mediated.¹³ The development of noncarboxylate inhibitors of AKR1C3 is therefore essential. The cross inhibition of AKR1C3 inhibitors, given their high amino acid sequence identity and structural similarity. AKR1C1 and AKR1C2 isoforms are closely related to AKR1C3 and are needed to inactivate DHT in the prostate.¹ Furthermore, COX inhibition has often been retained by NSAID-analogue AKR1C3 inhibitors. NSAID analogues that can inhibit AKR1C3 need to be designed without the structural features that are required for inhibition of COX, so to be devoid of gastrointestinal toxicity.¹² Furthermore, selective AKR1C3 inhibitors are an important tool to better understand AKR1C3's role in the development of cancer and other diseases.

Special Issue: Highlighting Medicinal Chemistry in Italy

Received: October 15, 2018

Accepted: January 28, 2019

Published: January 28, 2019

Figure 1. Chemical structures of AKR1C3 inhibitors: FLU, hydroxytriazoles bioisosteres of FLU (structures A and B), INDO and the INDO bioisosteres studied in this work (compounds 1 and 2).

The removal of the carboxylic acid moiety in fenamate derivatives coincides with a reduced COX1 activity, as demonstrated in our recent reports on the design of new AKR1C3 inhibitors.^{14,15} In those works, a *scaffold hopping* strategy based on the replacement of the FLU benzoate moiety with three different hydroxyazoles was used. The design of those ligands enabled us to demonstrate that hydroxyazoles are valid bioisosters of the carboxylic acid function versus AKR1C3 inhibitory activity, which can be deprotonated to various degrees at physiological pH, depending on their acidic properties.^{16–18} As part of this series of reports, we demonstrated how replacing the benzoic acid of the known NSAID and AKR1C3 inhibitor FLU (Figure 1) with a 4-hydroxy-N1-substituted triazolecarbonylic moiety (compounds A and B, Figure 1) led to a bioisosteric replacement for AKR1C3 activity, with no associated COX1/2 effect. Moreover, the regiosubstitution of the nitrogen atoms on the hydroxytriazole allowed us to perform a structural refinement,¹⁹ leading to improved AKR1C3 binding and to reduced AKR1C2 and COX1/2 off-target binding.

INDO (Figure 1), a potent but unselective AKR1C3 inhibitor with COX1/2 inhibitory activity, overcomes resistance to new agents (abiraterone,⁸ and enzalutamide⁹) that in turn have been shown to prolong survival in patients with metastatic CRPC. Considering the potential clinical use of INDO and the successful modulation of FLU, we report a bioisosteric strategy based on the replacement of the INDO carboxylic function with two hydroxyazoles with different pK_a values. Hydroxyfuran ($pK_a \cong 3$) and 3-hydroxy- *N*-methyl-1,2,5-triazole ($pK_a \cong 6$) were chosen to improve potency and selectivity toward AKR1C3 and remove COX1/2 off-target effects.

The present paper reports synthetic strategies and biochemical studies for AKR1C3 inhibitors **1** and **2** (Figure 1), also tested for *in vitro* cytotoxicity against two panels of human tumor cells: colorectal (HCT 116, HT-29) and prostate cancer (LNCaP and VCaP) cell lines. To study the inhibitor interactions within the AKR1C3 binding site, we

determined the high-resolution crystal structure of AKR1C3 in complex with compound **1**. In addition, physicochemical characterization and preliminary druglike properties are also presented.

Several creative methods for the assembly of N1-substituted indole compounds have been reported. These include variations of the Fischer Indole synthesis, as reported in the refined standard procedure to produce INDO.²⁰ In this procedure, *p*-methoxyphenylhydrazone **3** (Scheme 1) is

Scheme 1. Synthesis of Target Compounds 1 and 2^a

^aReagents and conditions: (a) Dry pyridine, dry toluene, rt; (b) HCl (gas), dry toluene, dry MeOH, 10 °C; (c) NaH (60% w/w), dry THF; (d) PTSA, dry toluene, 90 °C; (e) CH₃COOH, 80 °C; (f) H₂, Pd/C, dry THF.

benzoylated with **4** at the N1-position to give the N1,N1-disubstituted hydrazone **5** and then hydrolyzed with hydrogen chloride to give hydrazine hydrochloride salt **6**, which is cyclized, in the final reaction step, with levulinic acid to afford INDO. This procedure was modified by replacing levulinic acid with the appropriate 3-oxobutylazoles, **12** and **13**, giving the two desired hydroxyazole derivatives **1** and **2** in a convergent scheme (Scheme 1).

The benzyl-protected bromomethylhydroxyazoles **7**²¹ and **8**, were the key starting materials for the synthesis of the 3-oxobuthylazoles **12** and **13** and were prepared as previously reported.^{22,23} Their reaction with *tert*-butyl acetoacetate in the presence of NaH in dry THF furnished the *tert*-butyl esters **10** and **11**, which were hydrolyzed and decarboxylated via treatment with *p*-toluenesulfonic acid (PTSA) in dry toluene at 90 °C. It is important to note that the presence of the *tert*-butyl ester group in **10** and **11** is mandatory for decarboxylation, as the corresponding ethyl esters were observed to undergo deacetylation under these conditions. The key reagent for the underlying Fischer indolisation step, 4-chloro-*N*-(4-methoxyphenyl)-benzohydrazide hydrochloride (**6**), was prepared according to more recently reported

Table 1. Inhibitory Effect of Compounds 1, 2, and INDO against AKR1C3, AKR1C2, COX1, and COX2 Recombinant Purified Enzymes

compd	AKR1C3 IC ₅₀ ± SE (μM)	AKR1C2 IC ₅₀ ± SE (μM)	AKR1C2/AKR1C3 ^a	oCOX1 IC ₅₀ ± SE (μM)	hCOX2 IC ₅₀ ± SE (μM)	COX1/AKR1C3 ^a
INDO	7.35 ± 0.56	8.97 ± 0.22	1.2	0.10 ± 0.01	0.61 ± 0.09	0.01
1	0.30 ± 0.02	26.75 ± 2.89	89	>100 (0) ^b	>100 (12 ± 4) ^b	>333
2	0.94 ± 0.06	14.25 ± 3.39	15	>100 (37 ± 1) ^b	>100 (26 ± 2) ^b	>106

^aRatio of IC₅₀ value on the indicated enzymes. ^b% of inhibition ± SE at 100 μM.

literature procedures²⁴ and allowed to react with ketones **12** and **13** in glacial acetic acid at 80 °C for 1 h, giving indole derivatives **15** and **16**. Finally, the catalytic hydrogenation of **15** and **16** gave the desired hydroxyazoles **1** and **2**, respectively, in quantitative yields.

The selective targeting of AKR1C3 over 1C2 is considered critical for the development of effective prostate cancer therapy.² Not only do the two isoforms share over 86% sequence identity, but AKR1C2 is also involved in DHT inactivation making its inhibition undesirable. Accordingly, the inhibitory potencies of compounds **1** and **2** were determined for both AKR1C2 and AKR1C3 and were compared with INDO, which was used as a control. In order to investigate their selectivity, the IC₅₀ ratio of AKR1C2 and AKR1C3 inhibition was used as an indicator (Table 1).

The inhibitory activity assay was performed using recombinant purified enzymes with the oxidation of S-tetralol in the presence of NADP⁺ and compared to INDO. In our assays, INDO was shown to inhibit AKR1C3 with an IC₅₀ value of 7.35 μM and to be weakly selective against AKR1C2 (ratio AKR1C2/AKR1C3 IC₅₀ value 1.2). In order to evaluate the bioisosterism between its carboxylic acid function and new hydroxyazole moieties, the carboxylic acid function of INDO was replaced with the hydroxyfurazan and 3-hydroxy-*N*-1-methyl-1,2,5-triazole moieties, which modified both electronic properties and lipophilicity. Hydroxyfurazan is the more acidic heterocycle of the hydroxyazoles studied, as its experimental pK_a value was assessed to be around 3.^{25,26} It is therefore more acidic than INDO itself (pK_a 4.42), while 3-hydroxy-*N*-1-methyl-1,2,5-triazole is much less acidic, with an experimental pK_a value of around 6.^{16,23} The new hydroxytriazole indomethacin analogue **2** was more active than INDO and gave an IC₅₀ value (0.94 μM) that is 8-times lower than that of INDO (IC₅₀ 7.35 μM). Furthermore, it was more selective toward AKR1C3, with an AKR1C2 IC₅₀ value that is 15-times higher (IC₅₀ 14.25 μM). The new hydroxyfurazan indomethacin analogue **1** showed a better biochemical profile, presenting a IC₅₀ value (0.30 μM) that is 25-times lower than that of INDO (IC₅₀ 7.35 μM) and was also much more selective toward AKR1C3, with a AKR1C2 IC₅₀ value that is 90-times higher (IC₅₀ 26.75 μM). All these data support the bioisosterism between the carboxylic acid function of INDO and the 3-hydroxyfurazan and 3-hydroxyl-*N*-1-methyl-1,2,5-triazole moieties, as AKR1C activity was retained. Moreover, the bioisosteric replacement to these new scaffolds has a higher affinity for the AKR1C3 enzyme than for AKR1C2. The free carboxylic acid, which exists predominantly as the negatively charged carboxylate at physiological pH, is thought to contribute to the general inhibition of AKR1C enzymes by NSAIDs.²⁴ Accordingly, the replacement of the carboxylic acid function of INDO with the more highly acidic hydroxyfurazan led to new analogue **1** having higher AKR1C3 activity than the less acidic 3-hydroxyl-*N*(1)-methyl-1,2,5-triazole derivative **2**.

The compounds were also evaluated against COX1 and COX2 enzymes to prove that they are not active as NSAIDs and to ensure no COX related off-target effects occurred. INDO, compounds **1** and **2**, were assayed for their inhibitor effect against COX1 and COX2 enzymes using ovine COX1 (oCOX1) and human COX2 (hCOX2). The activity of the tested compounds is shown in Table 1. INDO, a nonselective, noncovalent slowly reversible inhibitor,²⁷ significantly inhibited COX1 and COX2 with IC₅₀ values of 0.10 and 0.61 μM, respectively. These values are consistent with the reported inhibitory potencies for INDO against COX.²⁴ Notably, neither of the two new hydroxyazole indomethacin analogues displayed significant inhibitory activity against either of the two COX isoforms at the highest concentration evaluated (100 μM) (Table 1). They showed high selectivity for AKR1C3 versus COX1/2. These data support the absence of bioisosterism between the carboxylic acid function and the 3-hydroxyfurazan and 3-hydroxyl-*N*-1-methyl-1,2,5-triazole moieties in the INDO scaffold for COX1/2 activity and demonstrate that these scaffolds display higher affinity for the AKR1C3 enzyme than for COX1/2. These data are in accordance with the known SAR of INDO, which highlights the importance of the distance between the carboxylate and the indole ring in obtaining COX activity.²⁴ In compounds **1** and **2**, the distance between the ionizable hydroxyazoles and indole is longer than the distance between the carboxylate and indole in INDO.

Its higher activity on the isolated enzyme led to compound **1** being selected for cocrystallization to rationalize the interactions of the new bioisostere INDO analogues within the AKR1C3 binding site. The crystal structure of AKR1C3 in complex with compound **1** (PDB ID 6GXX) was determined at a resolution of 1.7 Å. The AKR1C3 active site can be divided into five subsites: SP1, SP2, SP3, the oxyanion site (OS), and a steroid channel (SC). SP1 is lined by the amino acid residues Ser118, Asn167, Phe306, Phe311, and Tyr319. In all available structures cocrystallized with small molecules, SP1 is occupied with ligands bound.²⁸ SP2 is formed by residues Trp86, Leu122, and Ser129. SP3 is constituted of Tyr24, Glu192, Ser221, and Tyr305, and INDO is one of the few small molecules that interacts with this subpocket. The OS site is responsible for substrate's aldehyde or ketone reduction and is formed by the cofactor NADP⁺ and the catalytic residues Tyr55 and His117. The SC-site is an open channel that is gated by residues Tyr24, Leu54, Ser129, and Trp227.²⁸ The OMIT electron density map of the complex in the active site corresponds to compound **1** (Figure S2) and displays a unique binding conformation. It is well-known that INDO presents two different binding modes; the INDO carboxylate group establishes a hydrogen bond with the phosphate group of the cofactor at crystallization conditions at pH 6.0, while it is instead bound in the OS subsite at pH 7.5.²⁸ Liedtke et al.²⁴ have suggested that the binding mode of INDO is linked to the

protonation state of the NADP⁺ phosphate group, which shifts ionization state according to pH conditions. The binding mode of compound **1**, obtained from crystals grown at pH 6.0, clearly indicates that it interacts with the OS-site using the hydrofurazan portion (Figure 2); the interactions with the

Figure 2. AKR1C3 cocrystallized with compound **1** (orange sticks) (PDB ID 6GXX), superimposed with the AKR1C3 structure in complex with INDO (green sticks). NADP⁺ is represented in purple sticks. Molecular graphics and analyses were performed using the UCSF Chimera package.²⁹

protonated phosphate groups of NADP⁺, which are present in the INDO complex crystallized at the same pH, are missing (PDB ID 1S2A). The different steric encumbrances of the hydroxyfurazan and carboxyl groups, which hinder **1** from establishing a hydrogen-bond with the cofactor inside SP3, are a probable explanation. The overlay of compound **1** and INDO cocrystallized with AKR1C3 (Figure 2) shows two similar binding modes. The key binding interaction of **1** is the hydrogen bonding between the OS residues Tyr55 and His117 and oxygen atom of hydroxyfurazan motif, which overlays and mimic carboxylic oxygen of INDO. Interestingly, in many NSAIDs complexes with AKR1C3, an additional hydrogen bond interaction with a water molecule adjacent to carboxylic acid is present,²⁸ and actually that water is missing in the binding site of the compound **1**–AKR1C3 complex. The *p*-chlorobenzoyl ring is observed roughly perpendicular with respect to the plane of the indole ring and occupies the SP1, making few interactions with the protein. The indole ring extends toward SP3 subsite and is involved in a hydrogen bond between the methoxy oxygen and a water molecule present in SP3.

A molecular docking study was performed on COX-2 and AKR1C2 in order to provide further insight into compound **1**'s selectivity toward AKR1C3. The hydroxyfurazan motif is unable to overlay the INDO carboxylic group in the predicted conformation of compound **1** versus COX-2 and is not able to interact with its Arg89 and Tyr324 via a hydrogen bond (Figure S3). In fact, the (bio)isosteric modulation of INDO changes the link length between the anionic portion and the indole ring; this difference makes compound **1** unable to satisfy the requirements³⁰ for activity toward COX, making it selective against AKR1C3. Moving now to the C2 isoform, it is well-known that AKR1C2 is characterized by a smaller SP1 than the C3 isoform.² This difference allows the design of compounds selective for the latter isoform. Docking analyses of compound **1** with AKR1C2 (Figure S4) indicate that the

molecule, which places the fluorophenyl motif toward SC and the hydroxyfurazan into SP1, does not maintain the key interaction with the OS. This may explain its reduced activity toward the C2 isoform.

In the last phase of our study, we evaluated the antiproliferative effects of the compounds in two different panels of human cancer cells, colorectal and prostate cancer cells. Two cell lines that expressed (LNCaP and HCT 116 cells) or overexpressed (VCaP and HT-29 cells) AKR1C3 were selected for each tissue. AKR1C3 protein expression was confirmed by Western Blot results prior to chemosensitivity screening (Figure S5). The antiproliferative activity of the compounds was then investigated by assessing cell viability and dosing ATP content in a luminescent cell viability assay. Results are reported in Figure 3. The IC₅₀ values of the new

Figure 3. Effect of **1**, **2**, and INDO on selected cell lines. Compounds were utilized at 10–200 μ M. Cell viability was estimated by determining ATP content in three replicate wells. Results are normalized to the growth of cells that were treated with dimethyl sulfoxide (DMSO) and are represented as mean $\pm$ SEM of at least three independent experiments.

compounds were determined and are reported in Table 2. INDO inhibited cell proliferation with IC₅₀ values that ranged from about 313 μ M to 137 μ M, showing the lowest activity against HT-29 colon cancer cells. Both hydroxyazole indomethacin analogues **1** and **2** showed similar or greater activity than INDO in all the tested cell lines. Compound **2** showed higher potency than compound **1** against all the cell lines, except in HCT 116 (data were not statistically significant). The best effect was observed in both AKR1C3 overexpressing prostate cancer VCaP and colorectal cancer HT-29 cells. These results also confirmed the bioisosterism, in these cellular models, between the carboxylic acid function and the hydroxyfurazan and hydroxy-*N*-1-methyl-1,2,5-triazole moieties. In LNCaP, VCaP, and HT-29 cells, the behavior of the azole indomethacin analogues was opposite to the trend observed in the isolated enzyme experiments.

A cell-based system is a more complex environment than a purified enzyme, and multiple parallel outcomes that are connected to other pathways are possible. Moreover, the different lipophilicity and ionization grades of **1** and **2** may have an important role to play in their cellular internalization and consequently in their cellular activity. While compound **1** and INDO are largely ionized at physiological pH (pK_a values,

Table 2. Inhibitory Effect of Compounds 1, 2, and INDO on Cell Proliferation

compd	LNCaP IC ₅₀ ± SEM (μM)	VCaP IC ₅₀ ± SEM (μM)	HCT 116 IC ₅₀ ± SEM (μM)	HT-29 IC ₅₀ ± SEM (μM)
INDO	238.4 ± 1.1	136.9 ± 1.0	136.8 ± 1.0	312.6 ± 1.1
1	141.2 ± 1.0 ^b	138.1 ± 1.2	78.9 ± 1.2	134.7 ± 1.2 ^a
2	116.6 ± 1.1 ^c	69.1 ± 1.0 ^{d,e}	110.7 ± 1.2	82.0 ± 1.2 ^b

^a*p* < 0.05. ^b*p* < 0.01. ^c*p* < 0.001. ^d*p* < 0.0001 compound 1 or 2 vs INDO. ^e*p* < 0.0001 compound 2 vs 1.

Table 3. Ionization, Lipophilicity, and Solubility of Compounds 1, 2, and INDO

compd	pK _a ± SD ^a	<i>c</i> log <i>P</i> ^b	log <i>D</i> ^{7.4} ± SD	solubility (mg/L)	
				pH 2 SGF	pH 7 SIF
INDO	4.41 ± 0.04	4.18	1.01 ± 0.03	7.19	1819.52
1	4.09 ± 0.03	5.21	1.80 ± 0.03	0.48	276.33
2	6.10 ± 0.01	4.54	3.1 ± 0.2	0.12	2.40

^aPotentiometric titration. ^b*c* log *P* calculated using Bio-Loom for Windows, version 1.5.

respectively, 4.09 and 4.41, see Table 3, % ionization >99%), compound 2 is prevalently in not ionized form (pK_a value = 6.10, see Table 3, % ionization ~3%). Consequently, compound 2 is much more lipophilic than 1 and INDO at physiological pH (see log *D*^{7.4} in Table 3); therefore, it may cross membranes by passive diffusion more easily than 1 and INDO. Further membrane permeability assays would be necessary to confirm this hypothesis.

In view of testing in the near future the efficacy of these compounds *in vivo*, i.e., in a rodent model of prostate cancer by oral administration, we determined the solubility and the stability of compounds 1, 2, and INDO in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) (Table 3, Table S2, and Figure S1). As expected, the solubility of the tested compounds is higher at pH 7 than at pH 2, according to their measured log *D*^{7.4}. Serum stability and plasma protein binding (PPB) were also evaluated (Figure S1 and Table S2). These data on the whole prove that the behavior of 1 and 2 is similar to INDO, being stable in gastrointestinal fluids and in serum with a very high PPB (>99%).

In summary, we have presented a new generation of AKR1C3 inhibitors that have been designed using a bioisosteric approach and by replacing the carboxylic acid function of INDO with hydroxylated azoles. The two newly reported indomethacin analogues, compounds 1 and 2, were found to selectively inhibit AKR1C3 with greater potency than INDO and without any significant AKR1C2 and COX1/2 off-target effects. The crystal structure of AKR1C3 in complex with compound 1 allowed one to rationalize the interactions of the new inhibitors within the AKR1C3 binding site. Compounds 1 and 2 were also able to inhibit the cell proliferation of AKR1C3-expressing prostate and colorectal cancer cells in a dose-dependent manner. They demonstrated higher potency than INDO against most cell lines. Compound 2 showed higher potency than compound 1, especially in AKR1C3 overexpressing cancer cell lines. We are aware that the activities of 1, 2, and INDO itself are found only at relatively high concentration (IC₅₀ ≥ 70 μM). Anyway, this is the first report of indomethacin analogues, optimized for AKR1C3 inhibition, ever tested on cellular models of prostate and colorectal cancers. The novel chemical scaffolds reported herein provide a promising starting point for the design of more potent and selective AKR1C3 inhibitors and for the study of AKR1C3's role in cancer development and other disorders.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.8b00484.

Experimental procedures for the biological experiments, solubility and stability measures, synthetic procedures and characterization data for the compounds, X-ray diffraction details, and docking pose of compound 1 in COX2 and AKR1C2 binding sites (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*Phone: +39-0116707195. E-mail: donatella.boschi@unito.it.

*Phone: +39-0116706864. E-mail: simona.oliaro@unito.it.

ORCID

Marco L. Lolli: 0000-0002-3030-3163

Agnese C. Pippione: 0000-0003-3778-8420

Davide Bonanni: 0000-0002-8832-0492

Rosmarie Friemann: 0000-0002-5633-2894

Barbara Rolando: 0000-0001-6138-1503

Donatella Boschi: 0000-0003-4929-4460

Author Contributions

The manuscript was written through contributions of all authors.

Funding

This research was financially supported by the University of Turin (Ricerca Locale Grants 2015–2018) and Fondazione Cassa di Risparmio di Torino (Grant BOSD_CRT_17_2). P.G. is supported by the Wellcome Trust/DBT Alliance Fellowship (Grant IA/E/16/1/502999).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors wish to thank Dr. Dale Lawson for proofreading the final manuscript and Dr. Antonio Foti for the help in synthesis of compound 1 during his thesis training period.

■ ABBREVIATIONS

AKR1C3, aldo-keto reductase 1C3 isoform; CRPC, castration-resistant prostate cancer; DHT, dihydrotestosterone; FLU, flufenamic acid; INDO, indomethacin; COX, cyclooxygenase;

AKR1C2, aldo-keto reductase 1C2 isoform; PTSA, *p*-toluenesulfonic acid; NSAID, nonsteroidal anti-inflammatory drug

REFERENCES

- (1) Pippione, A. C.; Boschi, D.; Pors, K.; Oliaro-Bosso, S.; Lolli, M. L. Androgen-AR axis in primary and metastatic prostate cancer: chasing steroidogenic enzymes for therapeutic intervention. *J. Cancer Metastasis Treat* **2017**, *3*, 328–361.
- (2) Byrns, M. C.; Jin, Y.; Penning, T. M. Inhibitors of type 5 17 β -hydroxysteroid dehydrogenase (AKR1C3): overview and structural insights. *J. Steroid Biochem. Mol. Biol.* **2011**, *125*, 95–104.
- (3) Zhou, W.; Limonta, P. AKR1C3 inhibition therapy in castration-resistant prostate cancer and breast cancer: lessons from responses to SN33638. *Front. Oncol.* **2014**, *4*, 162.
- (4) Lorient, Y.; Fizazi, K.; Jones, R. J.; Van den Brande, J.; Molife, R. L.; Omlin, A.; James, N. D.; Baskin-Bey, E.; Heeringa, M.; Baron, B.; Holtkamp, G. M.; Ouatas, T.; De Bono, J. S. Safety, tolerability and anti-tumour activity of the androgen biosynthesis inhibitor ASP9521 in patients with metastatic castration-resistant prostate cancer: multicentre phase I/II study. *Invest. New Drugs* **2014**, *32*, 995–1004.
- (5) Seisen, T.; Roupert, M.; Gomez, F.; Malouf, G. G.; Shariat, S. F.; Peyronnet, B.; Spano, J. P.; Cancel-Tassin, G.; Cussenot, O. A comprehensive review of genomic landscape, biomarkers and treatment sequencing in castration-resistant prostate cancer. *Cancer Treat. Rev.* **2016**, *48*, 25–33.
- (6) Khanim, F. L.; Hayden, R. E.; Birtwistle, J.; Lodi, A.; Tiziani, S.; Davies, N. J.; Ride, J. P.; Viant, M. R.; Gunther, U. L.; Mountford, J. C.; Schrewe, H.; Green, R. M.; Murray, J. A.; Drayson, M. T.; Bunce, C. M. Combined bezafibrate and medroxyprogesterone acetate: potential novel therapy for acute myeloid leukaemia. *PLoS One* **2009**, *4*, No. e8147.
- (7) Sun, S. Q.; Gu, X.; Gao, X. S.; Li, Y.; Yu, H.; Xiong, W.; Yu, H.; Wang, W.; Li, Y.; Teng, Y.; Zhou, D. Overexpression of AKR1C3 significantly enhances human prostate cancer cells resistance to radiation. *Oncotarget* **2016**, *7*, 48050–48058.
- (8) Liu, C.; Armstrong, C. M.; Lou, W.; Lombard, A.; Evans, C. P.; Gao, A. C. Inhibition of AKR1C3 activation overcomes resistance to abiraterone in advanced prostate cancer. *Mol. Cancer Ther.* **2017**, *16*, 35–44.
- (9) Liu, C.; Lou, W.; Zhu, Y.; Yang, J. C.; Nadiminty, N.; Gaikwad, N. W.; Evans, C. P.; Gao, A. C. Intracrine androgens and AKR1C3 activation confer resistance to enzalutamide in prostate cancer. *Cancer Res.* **2015**, *75*, 1413–1422.
- (10) Zhong, T.; Xu, F.; Xu, J.; Liu, L.; Chen, Y. Aldo-keto reductase 1C3 (AKR1C3) is associated with the doxorubicin resistance in human breast cancer via PTEN loss. *Biomed. Pharmacother.* **2015**, *69*, 317–325.
- (11) Matsunaga, T.; Hojo, A.; Yamane, Y.; Endo, S.; El-Kabbani, O.; Hara, A. Pathophysiological roles of aldo-keto reductases (AKR1C1 and AKR1C3) in development of cisplatin resistance in human colon cancers. *Chem.-Biol. Interact.* **2013**, *202*, 234–242.
- (12) Penning, T. M. Aldo-Keto Reductase (AKR) 1C3 inhibitors: a patent review. *Expert Opin. Ther. Pat.* **2017**, *27*, 1329–1340.
- (13) Choi, J. S.; Jin, M. J.; Han, H. K. Role of monocarboxylic acid transporters in the cellular uptake of NSAIDs. *J. Pharm. Pharmacol.* **2005**, *57*, 1185–1189.
- (14) Pippione, A. C.; Giraudo, A.; Bonanni, D.; Carnovale, I. M.; Marini, E.; Cena, C.; Costale, A.; Zonari, D.; Pors, K.; Sadiq, M.; Boschi, D.; Oliaro-Bosso, S.; Lolli, M. L. Hydroxytriazole derivatives as potent and selective aldo-keto reductase 1C3 (AKR1C3) inhibitors discovered by bioisosteric scaffold hopping approach. *Eur. J. Med. Chem.* **2017**, *139*, 936–946.
- (15) Pippione, A. C.; Carnovale, I. M.; Bonanni, D.; Sini, M.; Goyal, P.; Marini, E.; Pors, K.; Adinolfi, S.; Zonari, D.; Festuccia, C.; Wahlgren, W. Y.; Friemann, R.; Bagnati, R.; Boschi, D.; Oliaro-Bosso, S.; Lolli, M. L. Potent and selective aldo-keto reductase 1C3 (AKR1C3) inhibitors based on the benzoisoxazole moiety: application of a bioisosteric scaffold hopping approach to flufenamic acid. *Eur. J. Med. Chem.* **2018**, *150*, 930–945.
- (16) Pippione, A. C.; Dosio, F.; Ducime, A.; Federico, A.; Martina, K.; Sainas, S.; Frolund, B.; Gooyit, M.; Janda, K. D.; Boschi, D.; Lolli, M. L. Substituted 4-hydroxy-1,2,3-triazoles: synthesis, characterization and first drug design applications through bioisosteric modulation and scaffold hopping approaches. *MedChemComm* **2015**, *6*, 1285–1292.
- (17) Sainas, S.; Pippione, A. C.; Giorgis, M.; Lupino, E.; Goyal, P.; Ramondetti, C.; Buccinna, B.; Piccinini, M.; Braga, R. C.; Andrade, C. H.; Andersson, M.; Moritzer, A. C.; Friemann, R.; Mensa, S.; Al-Kadaraghi, S.; Boschi, D.; Lolli, M. L. Design, synthesis, biological evaluation and X-ray structural studies of potent human dihydroorotate dehydrogenase inhibitors based on hydroxylated azole scaffolds. *Eur. J. Med. Chem.* **2017**, *129*, 287–302.
- (18) Sainas, S.; Pippione, A. C.; Giraudo, A.; Martina, K.; Bosca, F.; Rolando, B.; Barge, A.; Ducime, A.; Federico, A.; Grossert, S. J.; White, R. L.; Boschi, D.; Lolli, M. L. Regioselective N-Alkylation of Ethyl 4-Benzoyloxy-1,2,3-triazolecarboxylate: A Useful Tool for the Synthesis of Carboxylic Acid Bioisosteres. *J. Heterocycl. Chem.* **2018**, DOI: 10.1002/jhet.3426.
- (19) Lolli, M.; Narramore, S.; Fishwick, C. W.; Pors, K. Refining the chemical toolbox to be fit for educational and practical purpose for drug discovery in the 21st Century. *Drug Discovery Today* **2015**, *20*, 1018–1026.
- (20) Liedtke, A. J.; Kim, K.; Stec, D. F.; Sulikowski, G. A.; Marnett, L. J. Straightforward protocol for the efficient synthesis of varied N(1)-acylated (aza)indole 2-/3-alkanoic acids and esters: optimization and scale-up. *Tetrahedron* **2012**, *68*, 10049–10058.
- (21) Bonomo, S.; Tosco, P.; Giorgis, M.; Lolli, M.; Fruttero, R. The role of fluorine in stabilizing the bioactive conformation of dihydroorotate dehydrogenase inhibitors. *J. Mol. Model.* **2013**, *19*, 1099–1107.
- (22) Lolli, M. L.; Giordano, C.; Pickering, D. S.; Rolando, B.; Hansen, K. B.; Foti, A.; Contreras-Sanz, A.; Amir, A.; Fruttero, R.; Gasco, A.; Nielsen, B.; Johansen, T. N. 4-Hydroxy-1,2,5-oxadiazol-3-yl Moiety as Bioisoster of the Carboxy Function. Synthesis, Ionization Constants, and Molecular Pharmacological Characterization at Ionotropic Glutamate Receptors of Compounds Related to Glutamate and Its Homologues. *J. Med. Chem.* **2010**, *53*, 4110–4118.
- (23) Giraudo, A.; Krall, J.; Nielsen, B.; Sorensen, T. E.; Kongstad, K. T.; Rolando, B.; Boschi, D.; Frolund, B.; Lolli, M. L. 4-Hydroxy-1,2,3-triazole moiety as bioisostere of the carboxylic acid function: a novel scaffold to probe the orthosteric gamma-aminobutyric acid receptor binding site. *Eur. J. Med. Chem.* **2018**, *158*, 311–321.
- (24) Liedtke, A. J.; Adeniji, A. O.; Chen, M.; Byrns, M. C.; Jin, Y.; Christianson, D. W.; Marnett, L. J.; Penning, T. M. Development of Potent and Selective Indomethacin Analogues for the Inhibition of AKR1C3 (Type 5 17 β -Hydroxysteroid Dehydrogenase/Prostaglandin F Synthase) in Castrate-Resistant Prostate Cancer. *J. Med. Chem.* **2013**, *56*, 2429–2446.
- (25) Lolli, M. L.; Hansen, S. L.; Rolando, B.; Nielsen, B.; Wellendorph, P.; Madsen, K.; Larsen, O. M.; Kristiansen, U.; Fruttero, R.; Gasco, A.; Johansen, T. N. Hydroxy-1,2,5-oxadiazolyl Moiety as Bioisoster of the Carboxy Function. Synthesis, Ionization Constants, and Pharmacological Characterization of γ -Aminobutyric Acid (GABA) Related Compounds. *J. Med. Chem.* **2006**, *49*, 4442–4446.
- (26) Pippione, A. C.; Sainas, S.; Goyal, P.; Fritzson, I.; Cassiano, G. C.; Giraudo, A.; Giorgis, M.; Tavella, T. A.; Bagnati, R.; Rolando, B.; Caing-Carlsson, R.; Costa, F. T. M.; Andrade, C. H.; Al-Kadaraghi, S.; Boschi, D.; Friemann, R.; Lolli, M. L. Hydroxyazole scaffold-based Plasmodium falciparum dihydroorotate dehydrogenase inhibitors: Synthesis, biological evaluation and X-ray structural studies. *Eur. J. Med. Chem.* **2019**, *163*, 266–280.
- (27) Cena, C.; Tosco, P.; Marini, E.; Lazzarato, L.; Piccinini, M.; Ramondetti, C.; Lupino, E.; Fruttero, R.; Gasco, A. Nitrooxyacyl derivatives of salicylic acid: aspirin-like molecules that covalently inactivate cyclooxygenase-1. *ChemMedChem* **2011**, *6*, 523–530.

(28) Flanagan, J. U.; Yosaatmadja, Y.; Teague, R. M.; Chai, M. Z. L.; Turnbull, A. P.; Squire, C. J. Crystal structures of three classes of non-steroidal anti-inflammatory drugs in complex with aldo-keto reductase 1C3. *PLoS One* **2012**, 7, No. e43965.

(29) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. UCSF Chimera—a visualization system for exploratory research and analysis. *J. Comput. Chem.* **2004**, 25, 1605–1612.

(30) Kurumbail, R. G.; Stevens, A. M.; Gierse, J. K.; McDonald, J. J.; Stegeman, R. A.; Pak, J. Y.; Gildehaus, D.; Miyashiro, J. M.; Penning, T. D.; Seibert, K.; Isakson, P. C.; Stallings, W. C. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature* **1996**, 384, 644–648.