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Oxazole and thiazole analogs of sulindac for cancer prevention

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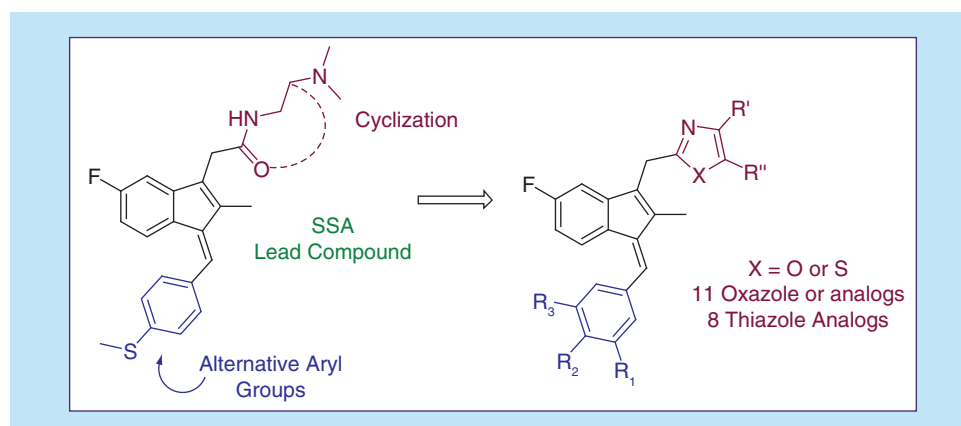
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Aim: Experimental and epidemiological studies and clinical trials suggest that nonsteroidal anti-inflammatory drugs possess antitumor potential. Sulindac, a widely used nonsteroidal anti-inflammatory drug, can prevent adenomatous colorectal polyps and colon cancer, especially in patients with familial adenomatous polyposis. Sulindac sulfide amide (SSA) is an amide-linked sulindac sulfide analog that showed *in vivo* antitumor activity in a human colon tumor xenograft model. **Results/methodology:** A new analog series with heterocyclic rings such as oxazole or thiazole at the C-2 position of sulindac was prepared and screened against prostate, colon and breast cancer cell lines to probe the effect of these novel substitutions on the activity of sulindac analogs. **Conclusion:** In general, replacement of the amide function of SSA analogs had a negative impact on the cell lines tested. A small number of hits incorporating rigid oxazole or thiazole groups in the sulindac scaffold in place of the amide linkage show comparable activity to our lead agent SSA.

Graphical abstract:



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Keywords: cancer • heterocycles • NSAIDs • oxazole • sulindac • thiazole

Since the introduction of the analgesic aspirin in the late 1800s for treating pain and inflammation, this class of drugs has become the most widely used drug category and is generally known as nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs have potent antipyretic and analgesic effects, and they constitute a relatively varied group of chemicals classified according to their chemical structures and shared pharmaceutical uses. The anti-inflammatory mechanism of the NSAIDs was elucidated by Vane in the 1970s and they are considered to act by inhibition of

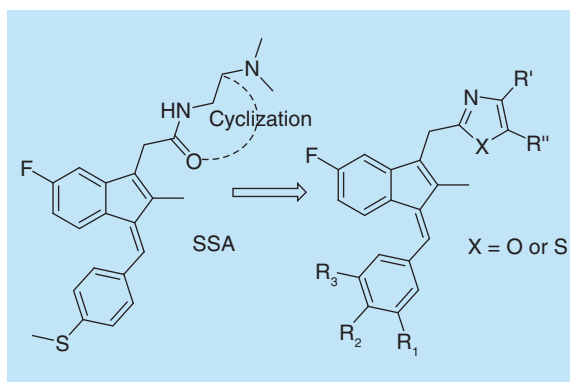


Figure 1. Outline of structural modifications on sulindac sulfide amide.

the COXs through attenuation of prostaglandin signaling molecules [1]. There are two COX isozymes, COX-1 and COX-2. Although COX-1 is constitutively expressed in most tissues and is crucial to tissue homeostasis, COX-2 is typically induced as a part of the acute inflammatory pathway after injury. Beyond their routine use for pain and inflammation, the NSAIDs have also been shown to have chemopreventive activity for several cancers such as colon, prostate and breast cancer. Numerous research studies have shown that routine application of certain NSAIDs can have anticancer effects *in vitro* and can also reduce the incidence and mortality of colorectal cancer by up to 50% in clinical/epidemiological studies [2–5]. Unfortunately, the dramatic effects demonstrated by the NSAIDs for chemoprevention come with potentially life-threatening side effects from chronic depletion of physiologically important prostaglandins, and these complications present clinically as gastrointestinal, renal and cardiovascular toxicity [6–10]. It has been observed that COX-2 can be selectively overexpressed at high levels in many different types of human tumors relative to surrounding normal tissue, and this altered expression profile may be one of the mechanisms of NSAID chemoprevention [11–13]. However, COX independent targets and mechanisms have also been implicated in the anticancer effects of NSAIDs [14–16]. Compounds structurally similar to various NSAIDs showing no or little activity against the COX isozymes also demonstrate potent chemopreventive and proapoptotic properties supporting the contention that the basic NSAID scaffold has multifarious beneficial properties against cancer [17–20].

Sulindac is a potent and regularly used NSAID developed at Merck in the early 1970s. It is nonselective versus the COX isozymes and has been utilized for chemoprevention of adenomatous colorectal polyps and colon cancer, particularly in patients with familial adenomatous polyposis [21–25]. Furthermore, a sulfone derivative of sulindac that has minimal COX-inhibitory activity inhibits chemical carcinogenesis and colon tumor growth *in vitro* and *in vivo*, albeit to a lesser degree [26–29]. A chemically modified analog of sulindac (sulindac sulfide amide [SSA], Figure 1) was reported to have significantly attenuated COX-related activity and toxicity while enhancing anticancer activity *in vitro* and showing *in vivo* xenograft activity [30]. These results support our hypothesis that preparation of diverse NSAID analogs can be useful to probe the chemical biology and the potentially diverse set of alternative activities represented by this broad class. Indeed, it may be possible to develop new analogs that show superior *in vitro* and *in vivo* activities without the adverse side effects caused by chronic inhibition of COX enzymes and loss of crucial prostaglandins required for normal tissue homeostasis. Through chemical modifications of NSAID compounds, a number of new scaffolds have been developed for the improvement of efficacy and safety profile of conventional NSAIDs [31].

Our earlier modeling results predicted that the carboxylate group of sulindac sulfide in the COX-1, COX-2 active site forms a key salt bridging interaction with R120, while the rest of the ligand is accommodated in a region rich in nonpolar amino acids [30]. Replacement of the carboxylate functionality with an amine group that has a pKa around physiological pH should yield a protonated and positively charged ammonium group as present in SSA, and this change was predicted to reduce COX-related activity due to the propinquity of positively charged amino acids in the enzyme active site. In this model, the benzylidene moiety of sulindac sulfide also occupies a deep-lying small, nonpolar pocket with limited cavity space available around 3-, 4-, 5- positions, suggesting that substitutions at these positions may also attenuate COX activity of derived analogs, which is also in line with the lack of COX activity of sulindac sulfone or sulfoxide. Thus, our earlier modeling suggested the carboxylate group and 3-, 4-, 5 phenyl positions in benzylidene as selectivity vectors to abrogate COX-1, COX-2 binding thus allowing us to probe the chemical biology and so called off-target activities of the scaffold without the complicating factor of COX inhibition.

A number of sulindac derived compounds have been reported with various anticancer activities that provide support for targeting these vectors for new designs aiming to avoid COX related activities. For example, the negatively charged carboxylate moiety is replaced by a basic substituent in SSA, an amide linked N, N-dimethylethylamine group (Figure 1). The tumor cell growth inhibitory activity of SSA has been studied extensively and it is known to act through a COX-independent mechanism [30,32]. A specific alteration of the carboxylate group in sulindac is exemplified by sulindac benzylamine, a potent colon tumor cell growth inhibitor in which the carboxylate moiety is replaced by an ethylbenzylamine group [33]. sulindac benzylamine inhibits cyclic-GMP phosphodiesterase isozyme PDE5 and activates cGMP-dependent protein kinase G at concentrations that suppress colon tumor cell growth, while it has no COX-related activity. An example of a 4-substituted analog of sulindac sulfide lacking cyclooxygenase activity is K-80003 [34]. This analog contains 4-isopropyl substituted benzylidene and shows increased affinity to retinoid X receptor- α compared with sulindac sulfide, but is not active against COXs. K-80003 inhibited tumor growth in animals through targeting N-terminally truncated retinoid X receptor- α present in several cancer cell lines and primary tumors. A recent example of a 3-, 4-, 5-substituted analog of sulindac sulfide showing potent anticancer properties is ADT-094 [35], containing trimethoxy substitutions of the phenyl ring of benzylidene. This compound contains an amide-linked furan in place of the carboxylate moiety of sulindac, while the fluorine is replaced by a methoxy group. ADT-094 acts through a COX-independent mechanism, suppressing colon tumor cell growth at concentrations at which it may inhibit cGMP phosphodiesterase isozymes PDE5 and 10 and activate cGMP-dependent protein kinase G [35].

As mounting evidence suggests, the carboxylate moiety of sulindac sulfide, as well as phenyl substitutions in the benzylidene moiety are reasonable vectors to explore for new sulindac based scaffolds designed to 'turn off' cyclooxygenase activity. In the present study, we aim to target these vectors, while introducing heterocycles, namely, oxazole or thiazole rings at the C-2 position in place of the amide linkage in our lead scaffold represented by SSA (Figure 1) in order to probe rigidification at the amide linkage. Thiazoles and oxazoles are common structural features of many biologically important natural products and are typically used as isosteric replacements for the amide linkage in medicinal chemistry [36–39]. These heterocyclic rings increase the rigidity of the molecule and may potentially alter binding potency by reducing mobility around the amide group. Incorporating an oxazole heterocycle in bacterial cell division protein FtsZ inhibitors was reported to produce scaffolds with improved pharmacokinetic properties and increased metabolic stability [40]. Furthermore, amide linkages are typically considered biologically labile groups, so alteration at this site may possibly increase metabolic stability toward nonspecific amidases *in vivo*. Metabolic site computations for selected compounds in the SSA series predict that oxazole or thiazole moieties present in our analogs are metabolically stable, while the dimethylaminoethyl amide substituent may have an associated metabolic lability (Supplementary Material). Thus, our *in silico* results suggest that incorporating oxazole or thiazole heterocycles would not reduce metabolic stability of any considered analogs. Replacement of the 4-methylthiophenyl of SSA with a methoxy substituted phenyl ring is advantageous since it would reduce metabolic vulnerability of this site (Supplementary Material). In the presented work, we aim to explore primarily methoxy substituents in 3-, 4-, 5-positions of the benzylidene. We synthesized and evaluated a series of analogs that explore the described vectors, probing activities stemmed from these new substitutions. The goal of this exercise has been the identification of new SSA analogs containing novel and more rigid side chains that may lead to interesting activity profiles, for future consideration in the development of safer NSAID analogs for cancer prevention.

Methodology

The synthetic strategy starts with a 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) or O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluoro phosphate catalyzed coupling of sulindac with the appropriately substituted amino acid methylester to form the corresponding amide in excellent yield. The amide ester was hydrolyzed to the acid by treating with KOH in EtOH/H₂O. Dakin–West reaction conditions (acetic anhydride, pyridine) were then utilized to introduce the methyl group at the –COOH group. Finally, Robinson–Gabriel cyclodehydration of the above compound with catalytic sulfuric acid in acetic anhydride provided oxazole derivatives 1–3 in moderate yields (Figure 2) [41].

The general synthetic route for the preparation of oxazole and thiazole carboxylic acids is given in Figure 3 [42]. The α -amido- β -ketoester, prepared by the HATU catalyzed amide coupling of sulindac and ethyl 2-amino-3-oxo-3-phenylpropanoate, was used as the common intermediate for the synthesis of both targets.

Cyclization of the α -amido- β -ketoester to oxazole was carried out with triphenylphosphine in the presence of iodine and triethylamine in dichloromethane at room temperature to give the oxazole ester in excellent yield.

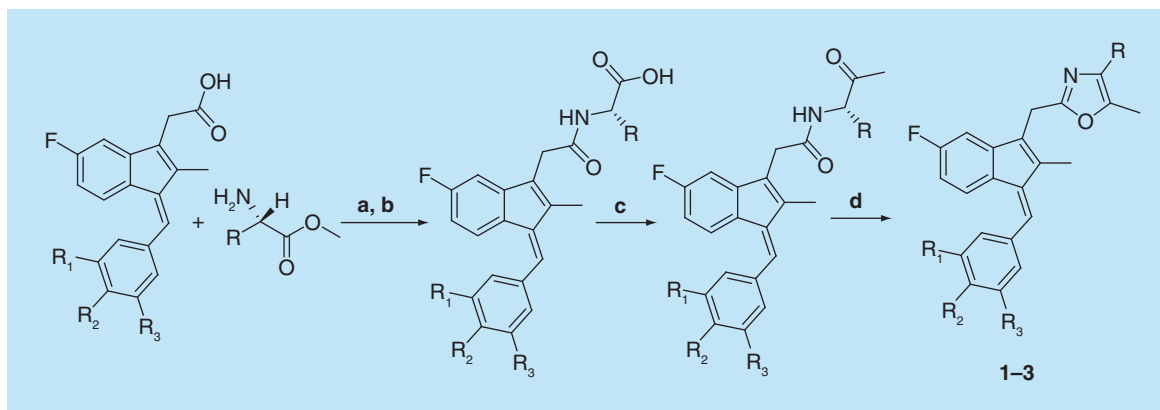


Figure 2. Synthetic pathways to analogs 1–3. Reagents and conditions: (a) HATU, DIEA, MeCN or HBTU, TEA, MeCN; (b) KOH, EtOH/H₂O; (c) Ac₂O, Pyridine; (d) Ac₂O, H₂SO₄.

HATU: 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate;
 HBTU: O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluoro phosphate.

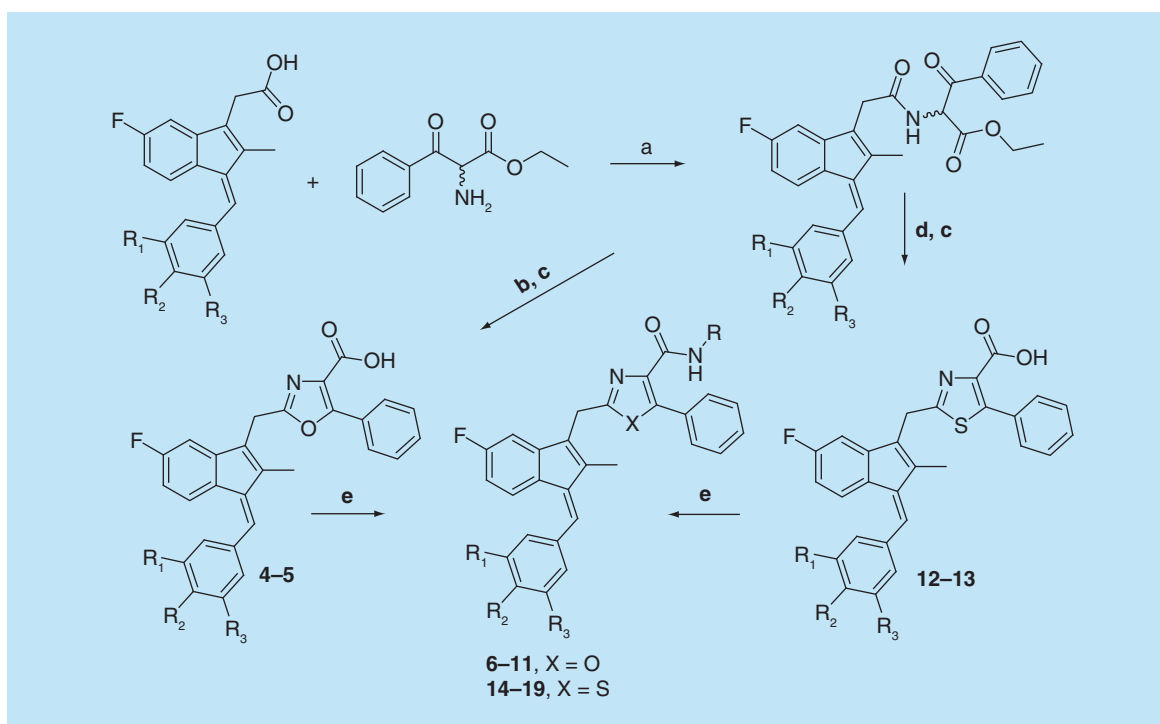


Figure 3. Synthetic pathways to analogs 4–19. Reagents and conditions: (a) HATU, DIEA, MeCN or HBTU, TEA, MeCN; (b) Et₃N, PPh₃, I₂, CH₂Cl₂, rt; (c) KOH, EtOH/H₂O; (d) Lawesson's reagent, THF, reflux; (e) RNH₂, HATU, DIEA, MeCN.

HATU: 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate;
 HBTU: O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluoro phosphate.

Similarly, the thiazole was made by the reaction of the α -amido- β -ketoester with Lawesson's reagent. Finally, deprotection of the oxazole and thiazole esters to the corresponding acids (4, 5, 12 and 13) was achieved by the basic hydrolysis (KOH in EtOH/H₂O). These acids were chemically modified to amides 6–11 and 14–19.

Cancer cell line screening methods

All target sulindac analogs were screened using quantitative high-throughput screen (qHTS) against prostate, colon and breast cancer cell lines using reported procedures [43].

Chemistry

Anhydrous solvents and reagents from Sigma-Aldrich were used without further drying. Reactions were monitored by thin-layer chromatography (TLC) on precoated E. Merck silica gel (60F254) plates (0.25 mm) and visualized using UV light (254 nm). Flash chromatography was carried out on Fischer silica gel G 60 (230–400 mesh). Purification of certain compounds was carried out by utilizing a Teledyne Isco Combiflash[®] Rf automated chromatography machine. Melting points, determined with a OptiMelt Automated Melting Point System and, are uncorrected. The exact mass spectral data were obtained with an Agilent LC-MSTOF or with Bruker Biotof II by ESI. ¹H-NMR spectra were recorded on a Nicolet NT-300 NB spectrometer operating at 300.635 MHz or on Agilent/Varian MR-400 spectrometer operating at 399.930 MHz. Chemical shifts in CDCl₃ and Me₂SO-d₆ are expressed in parts per million downfield from tetramethylsilane (TMS). All assigned structures were confirmed by ¹H-NMR and the chemical shifts (δ) for complex peaks (multiplets) were determined from the estimated centers. The integrated peak areas agreed with the expected values for the assigned structures. The percent purity was determined via HPLC on an Agilent 1100 LC attached to a diode array UV detector with monitoring over multiple wavelengths. ESI-MS spectra were obtained using a BioTof-2 TOF MS system.

General synthetic methods

Method 1

Amide formation was achieved using 1.2 equivalents of O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluoro phosphate. The coupling reagent was added to a solution of 1.0 equivalents of sulindac followed by 1.5 equivalents of the applicable amine with 2.0 equivalents of the general base triethylamine in 10 ml of dry CH₃CN (10 ml) in an argon atmosphere. The reaction mixture was stirred for up to 2 h and the acetonitrile was then removed via a rotary evaporator with a water aspirator. The resulting mixture was refined using flash column chromatography (60–200 mesh) to give an excellent overall yield of the desired amide.

Method 2

Alternatively, the amide coupling can be achieved using 1.2 equivalents of HATU. The coupling agent was added to a CH₃CN solution of 1.0 equivalent of the acid and 1.5 equivalents of the associated amine with 2.0 equivalents of the organic base di-isopropylethylamine at room temperature under argon atmosphere. The reaction was maintained at room temperature with stirring for 1–2 h followed by removal of the solvent on a rotary evaporator with water aspiration. The resulting mixture was purified using a Teledyne Isco Combiflash Rf machine to give an excellent yield of the desired amide.

Method 3

Ester hydrolysis was achieved by adding a solution of the sulindac ester in 20 ml of EtOH to 3.0 equivalents of the strong base KOH in 20 ml of deionized water followed by stirring at room temperature for 12 h. The reaction mixture was neutralized by washing with 2N HCl followed by extraction of the aqueous phase with CHCl₃ (3 × 25 ml). The combined chloroform washings were dried over anhydrous sodium sulfate and the solvent was evaporated using a rotary evaporator with a water aspirator. The resulting crude oil was purified on the Teledyne Isco Combiflash Rf instrument to give the desired acid analog of sulindac acid in a quantitative yield.

Method 4

Dakin–West reaction conditions were utilized to introduce the methyl group at the –COOH group. The acid (100 mg) was dissolved in 4.0 ml of pyridine and 2.0 ml of acetic anhydride followed by heating at 90°C for 2 h. The remaining reagents/solvents were removed via reduced pressure. Next the oil was dissolved in 20 ml CHCl₃ and the solution was washed with 20 ml of 1.0 N HCl followed by 25 ml water, and dried over Na₂SO₄ (anhydrous). The solvent was removed and flash column purification or application of the crude material to the Teledyne Isco Combiflash Rf purification machine provided the desired ketone in moderate yield.

Method 5

Robinson–Gabriel cyclodehydration of the products from method 4 with catalytic sulfuric acid in acetic anhydride provided the desired oxazole derivatives in moderate yields. The ketone (150 mg) was dissolved in 2 ml of acetic anhydride and 0.2 ml of concentrated H₂SO₄ was added carefully followed by heating at 90°C for 30 min and subsequent cooling to room temperature. Water (20 ml) was added to the cooled solution followed by extraction

Table 1. Screening data for sulindac sulfide amide and 1–3.

Compounds	R ₁ , R ₂ & R ₃	R	CC ₅₀ (μM)		
			HT29	PC3	MDA-MB-231
SSA			0.65 ± 0.03	3.12 ± 0.15	2.67 ± 0.08
1	R ₁ & R ₃ = H, R ₂ = SCH ₃	CH ₃	>50.00	>50.00	>50.00
2	R ₁ & R ₃ = H, R ₂ = SOCH ₃	CH ₃	29.75 ± 2.07	47.30 ± 5.37	24.46 ± 2.99
3	R ₁ , R ₂ & R ₃ = OCH ₃	Phenyl	>50.00	>50.00	>50.00

SSA: Sulindac sulfide amide.

with chloroform – 3 × 25 ml. The CHCl₃ containing phase was further rinsed with saturated aqueous NaHCO₃ and dried over anhydrous Na₂SO₄. Evaporation of the organic phase using rotary evaporation gave a crude oil that was purified by flash column chromatography or the use of the Teledyne Isco Combiflash Rf purification instrument to provide a moderate yield of the desired oxazole.

Method 6

The common amide ester intermediates were cyclized to the desired oxazoles using 4.0 equivalents of Et₃N mixed with a solution of 2.0 equivalents of Ph₃P and 2.0 equivalents of I₂ in 6.0 ml of dry chloroform that was stirred for 5 min to ensure dissolution of all reagents. A chloroform solution (4.0 ml) and the starting amide ester (1 equivalent) was added to the above solution followed by stirring of the reaction for 3 h. Removal of the solution and purification via Teledyne Isco Combiflash Rf provided the oxazole in excellent yield.

Method 7

Thiazoles were prepared using Lawesson's reagent by this method. A solution of 10 ml dry tetrahydrofuran, 1.0 equivalent of the common amide esters and cyclization reagent (2 equivalents) was heated to reflux for 3 h. Next, the reaction was cooled and 20 ml of water was added followed by chloroform extraction (3 × 25 ml) using a separatory funnel. Drying over anhydrous sodium sulfate and removal of the solvent with a rotary evaporator gave the crude product that was further purified using Teledyne Isco Combiflash Rf purification machine to give the desired thiazole in excellent yield.

Results & discussion

Prostate, colon & breast cancer cell line screening

All synthesized compound series were screened against HT29 colorectal carcinoma, PC3 prostate and MDA-MB-231 breast cancer cell lines using qHTS [43]. Solubility issues have been noted with oxazoles and thiazoles, but these heterocycles are commonly used for successful drug discovery work (for an example by Stokes [40]). As part of the qHTS process for this study, it is determined if there are issues with precipitation of materials through visual inspection for clouding of the wells in the sample plates. Furthermore, a significant advantage of qHTS is determination of a full dose response from the outset rather than a typical primary screening filter at a higher concentration (where samples are likely to be less soluble) prior to selection of the best candidates for a full dose response format. Hence, the qHTS screening concentrations start from very low levels that would certainly include soluble concentrations and working up to higher concentrations. No issues with the dose response curves at higher concentrations were noted for this small library suggesting solubility issues did not arise in our qHTS screen of these samples. Screening data for all new compounds are summarized in Tables 1 & 2. Oxazole analogs 1–3 were found to be less active than our lead compound SSA. Among these three compounds, dimethyl substituted oxazole analog of sulindac, 2 was modestly active against all three cell lines *in vitro* (Table 1).

Table 2 lists the anticancer activity of oxazole and thiazole carboxylic acids and their amide derivatives. Among the carboxylic acid analogs 4, 5, 12 and 13, 3,4,5-trimethoxybenzylidene derivatives 5 and 13 were the only active carboxylates with very modest activity compared with sulindac.

We also explored the activity of benzyl, furan-2-ylmethyl and N,N-dimethylaminoethyl amides of oxazole and thiazole carboxylic acids against HT29, PC3 and MDA-MB-231 cell lines. Amide derivatives displayed more activity than their parent acids. Among all the amides (6–11 and 14–19), N,N-dimethylaminoethyl derivatives 8, 11, 16 and 19 were found to be significantly more active than other amides in all the three assays. 3,4,5-Trimethoxybenzylidene-N,N-dimethylaminoethyl amide derivatives 11 and 19 were observed to be about twofold

Table 2. Screening data for compounds 4–19.

Compounds	R ₁ , R ₂ & R ₃	CC ₅₀ (μM)		
		HT29	PC3	MDA-MB-231
4	R ₁ & R ₃ = H, R ₂ = SCH ₃	>50	>50	43.14 ± 3.22
5	R ₁ , R ₂ & R ₃ = OCH ₃	28.49 ± 4.59	33.02 ± 6.70	25.13 ± 4.50
6	R ₁ & R ₃ = H, R ₂ = SCH ₃ , R = benzyl	>50	>50	>50
7	R ₁ & R ₃ = H, R ₂ = SCH ₃ , R = furan-2-ylmethyl	>50	>50	>50
8	R ₁ & R ₃ = H, R ₂ = SCH ₃ , R = N,N-dimethylaminoethyl	4.57 ± 0.14	7.67 ± 0.45	7.41 ± 0.33
9	R ₁ , R ₂ & R ₃ = OCH ₃ , R = benzyl	>50.00	>50.00	>50.00
10	R ₁ , R ₂ & R ₃ = OCH ₃ , R = furan-2-ylmethyl	>50.00	>50.00	>50.00
11	R ₁ , R ₂ & R ₃ = OCH ₃ , R = N,N-dimethylaminoethyl	2.05 ± 0.19	5.43 ± 0.21	3.88 ± 0.68
12	R ₁ & R ₃ = H, R ₂ = SCH ₃	>50	>50	>50
13	R ₁ , R ₂ & R ₃ = OCH ₃	31.39 ± 5.75	35.46 ± 6.35	22.19 ± 2.90
14	R ₁ & R ₃ = H, R ₂ = SCH ₃ , R = benzyl	>50	>50	>50
15	R ₁ & R ₃ = H, R ₂ = SCH ₃ , R = furan-2-ylmethyl	>50	>50	>50
16	R ₁ & R ₃ = H, R ₂ = SCH ₃ , R = N,N-dimethylaminoethyl	2.96 ± 0.07	5.30 ± 0.34	4.20 ± 0.27
17	R ₁ , R ₂ & R ₃ = OCH ₃ , R = benzyl	7.73 ± 3.07	25.14 ± 13.80	5.36 ± 1.06
18	R ₁ , R ₂ & R ₃ = OCH ₃ , R = furan-2-ylmethyl	7.44 ± 3.22	21.23 ± 12.97	6.45 ± 1.69
19	R ₁ , R ₂ & R ₃ = OCH ₃ , R = N,N-dimethylaminoethyl	1.86 ± 0.19	4.76 ± 0.25	3.98 ± 0.34

Table 3. Leukemia/lymphoma/BJ cell line screening data.

Compounds	EC ₅₀ (μM)				
	JURKT	REH	RAJI	697	BJ
SSA	NA	NA	NA	NA	>7.57
8	ND	ND	NA	NA	>7.57
11	23 (23–23)	16 (16–16)	NA	NA	>22.73
16	NA	NA	NA	NA	>7.57
17	NA	NA	25 (25–25)	NA	>22.73
18	NA	NA	NA	16 (16–16)	>22.73

Confidence intervals are shown in parentheses.

NA: Not active; ND: Undetermined due to questionable curve fit; SSA: Sulindac sulfide amide.

more active than 4-thiomethylbenzylidene-N,N-dimethylaminoethyl amide derivatives **8** and **16**. After comparing all the amides prepared, thiazole derivatives appeared slightly more active than their corresponding oxazole amides.

Leukemia/lymphoma cancer cell line screening of selected analogs

Out of the presented series compounds **8**, **11**, **16**, **17**, **18** and **19** show the most potent activity against the cancer cell lines screened and these analogs of interest (except for 19) were also evaluated against several acute lymphoblastic leukemia cell lines and a lymphoma line as available at St Jude Children's Research Hospital. The following cell lines were used for screening: acute T cell leukemia Jurkat e6–1 cells (JURKT), precursor B-cell ALL patient-derived cell line expressing only wild-type MLL and wild-type AF4 (REH cells), Burkitt's lymphoma, with FAB L3 (RAJI cells) and a cell line established from bone marrow cells obtained from children with acute lymphoblastic leukemia in relapse (697). Compounds were counter-screened for cytotoxicity at 10 μM drug concentration using a normal human foreskin fibroblast (BJ) cell line described previously [44]. Screening results are listed in Table 3.

Interestingly, compound **11** is modestly active against two acute lymphoblastic leukemia cell lines on this panel, while compounds **17** and **18** were also found to inhibit one of these leukemic cell lines. Results of cytotoxicity evaluations are listed in Table 3. Compounds **11**, **17**, **18** show $EC_{50} > 22.73 \mu\text{M}$ values against BJ cells, which suggests that these compounds are not simply broad cytotoxins. Compounds **8**, **16** are not overtly cytotoxic either, having $EC_{50} > 7.57 \mu\text{M}$ in the BJ assay.

Conclusion

We have synthesized and screened new sulindac derivatives containing oxazole and thiazole heterocycles against colon, prostate and breast cancer. In general, incorporating these more rigid groups into the sulindac scaffold in place of the original amide linkage decreases activity relative to their corresponding open chain analogs and the control lead SSA. Several derivatives, however, show comparable activity, namely compounds **8**, **11**, **16**, **19**, which are oxazole and thiazole amide derivatives with either 4-methylthiobenzylidene or trimethoxybenzylidene at the C-1 position (Table 2). The N,N-dimethylaminoethyl amide analogs of 3,4,5-trimethoxybenzylidene sulindac, **11** and **19** have the most promising activity in HT29, PC3 and MDA-MB-231 proliferation assays. It is notable that these new analogs have increased molecular weights relative to SSA while showing no improvement in potency against the three cancer cell lines compared to SSA. Even so, these compounds are of interest in terms of representing more rigid scaffolds, which can alter their target binding profiles. Potentially a number of enzymatic targets may be relevant to their anticancer activities [45]. Indicative that even within this small set there may be altered activities/targets, compound **11** inhibited two acute lymphoblastic leukemia cell lines, while compounds **17** and **18** showed distinct activity against other, lymphoma/leukemia cancer cell lines. This result may be interesting to others in the field as a potential alteration in similar or other NSAID scaffolds in order to probe structure–activity relationships and demonstrates the value of using small diversity sets built around an active NSAID scaffold to probe the chemical biology of this class [46].

Computed physicochemical properties predict that compounds **8**, **11** are slightly more soluble than SSA while compounds **16**, **19** and SSA are comparably soluble (Supplementary Material). LogD values of compounds **8**, **11**, **16**, **19** are comparable to that of SSA. Common to their structures is a basic amine group, although the neutral analogs **17** and **18** also maintain good inhibitory potency at HT29 (colon) and MDA-MB-231 (breast) cell lines, while modest activity against the PC3 (prostate) cell line. Compounds **17**, **18** are predicted to have less desirable solubility and LogD properties while improved metabolic stability relative to SSA. Relative to this issue and the aforementioned calculated parameters, compound activities for new drug candidates can be optimized using two measures called ligand efficiency and lipophilic efficiency. Both are measures of molecular weight/heavy atom numbers versus changes in compound activity or drug lipophilicity, and ideally one would want a drug candidate with the highest activity and the lowest numbers of heavy atoms and reduced/optimum lipophilicity for crossing biological barriers/membranes. Since this study involves whole cell screening and a specific target activity or ligand is not known, lipophilic efficiency is a more relevant assessment of compound optimization (see [47] for an example of improving lipophilic efficiency in order to optimize a HIV drug candidate). Our goal in this lead finding study, however, was to probe the chemical biology of focused libraries built around the sulindac scaffold while minimizing COX activity in order to discover compounds that showed interesting cellular activity against common cancer cell lines: active compounds in this set may be useful for further mechanism of action work. Hence, we have not attempted to optimize drug-like properties such as lipophilic efficiency, or even ligand efficiency should a non-COX target be identified, and improving that calculated measure of compound activity will certainly be a focus for later improvement of interesting compounds. Metabolic site predictions suggest that introducing oxazole or thiazole heterocycles as in compounds **8**, **11**, **16**, **19** does not reduce the metabolic stability of these compounds relative to SSA (Supplementary Material). The most potent compounds from the presented series (**8**, **11** and **16–19**), particularly compound **11** may be further considered for advanced bioavailability, toxicology and efficacy studies *in vivo*, and mechanistic studies *in vitro*.

Future perspective

Although it is clear that COX-2 is a critical player in cancer and a wide variety of NSAID scaffolds likely have anticancer/chemopreventive activity acting on this cyclooxygenase, it is also evident that the broad class has multifarious other activities through mechanisms that are independent of the COX enzymes. Future work with the numerous NSAID chemical classes through diversity generation and chemical biology can further elucidate the role of other non-COX targets in the anticancer and other activities of this broad class relevant to cancer

treatment/prevention and other therapeutic areas. Potentially, further medicinal chemistry optimization of the various and diverse compounds versus other identified target activities can lead to potent and selective inhibitors to further probe the chemical biology of the NSAIDs.

Summary points

- Sulindac can effectively prevent adenomatous colorectal polyps and colon cancer, especially in patients with familial adenomatous polyposis.
- A new series of neutral or positively charged sulindac derivatives with oxazole or thiazole rings at the C-2 position were prepared.
- In general, addition of an oxazole or thiazole group had a negative impact on activity relative to sulindac sulfide amide (SSA).
- A small number of hits incorporating these rigid groups (oxazoles or thiazoles) in place of the amide group of SSA showed reasonable activity versus established cancer cell lines (HT29 [colon], PC3 [prostate] and MDA-MB-231 [breast] cell lines) *in vitro* relative to our control SSA.
- Four derivatives gave comparable activity in all three cell lines to the lead active agent SSA.
- Three compounds also showed activity against leukemia cell lines.
- Modest changes to the sulindac scaffold led to a range of inhibition (CC₅₀ ranges from ca. 1–2 μM to >50 μM) against the three cell lines.
- Future work further generating additional diversity to elucidate SAR in the series and optimize medicinal chemistry properties, *in vivo* bioavailability and antitumor activity is merited.

Supplementary materials

Analytical data, metabolic stability predictions and computed physicochemical properties are provided for selected compounds.

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References

Papers of special note have been highlighted as: ● of interest; ●● of considerable interest

1. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat. New Biol.* 231, 232–235 (1971).
2. Thun MJ, Henley SJ, Patrono C. Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. *J. Natl Cancer Inst.* 94(4), 252–266 (2002).
3. Chan TA. Nonsteroidal anti-inflammatory drugs, apoptosis, and colon-cancer chemoprevention. *Lancet Oncol.* 3(3), 166–174 (2002).
4. Reeder MK, Pamakcu R, Weinstein IB, Hoffman K, Thompson WJ. In: *Cancer Chemoprevention, Promising Cancer Chemoprevention Agents (Volume 1)*. Hawk ET, Kelloff GJ, Sigman CC (Eds). Humana Press, Inc., NJ, USA, 401 (2004).
5. Soh JW, Weinstein IB. Role of COX-independent targets of NSAIDs and related compounds in cancer prevention and treatment. *Prog. Exp. Tumor Res.* 37, 261–283 (2003).
6. Cannon CP, Cannon PJ. COX-2 inhibitors and cardiovascular risk. *Science* 336(6087), 1386–1387 (2012).
7. Yu Y, Ricciotti E, Scalia R *et al.* Vascular COX-2 modulates blood pressure and thrombosis in mice. *Sci. Transl. Med.* 4(132), 132ra54 (2012).

8. Mukherjee D. Selective cyclooxygenase-2 (COX-2) inhibitors and potential risk of cardiovascular events. *Biochem. Pharmacol.* 63(5), 817–821 (2002).
9. Vane JR, Botting RM. Mechanism of action of antiinflammatory drugs. *Int. J. Tissue React.* 20(1), 3–15 (1998).
10. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. *Annu. Rev. Pharmacol. Toxicol.* 38, 97–120 (1998).
11. Brown JR, DuBois RN. COX-2: a molecular target for colorectal cancer prevention. *J. Clin. Oncol.* 23(12), 2840–2855 (2005).
12. Husain SS, Szabo IL, Tarnawski AS. NSAID inhibition of GI cancer growth: clinical implications and molecular mechanisms of action. *Am. J. Gastroenterol.* 9(3), 542–553 (2002).
13. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 107(4), 1183–1188 (1994).
14. Gurpinar E, Grizzle WE, Piazza GA. NSAIDs inhibit tumorigenesis, but how? *Clin. Cancer Res.* 20(5), 1104–1113 (2014).
15. Liggett JL, Zhang X, Eling TE, Baek SJ. Anti-tumor activity of non-steroidal anti-inflammatory drugs: cyclooxygenase-independent targets. *Cancer Lett.* 346(2), 217–224 (2014).
16. Li N, Xi Y, Tinsley HN *et al.* Sulindac selectively inhibits colon tumor cell growth by activating the cGMP/PKG pathway to suppress Wnt/ β -catenin signaling. *Mol. Cancer Ther.* 12(9), 1848–1859 (2013).
17. Piazza GA, Keeton AB, Tinsley HN *et al.* NSAIDs: old drugs reveal new anticancer targets. *Pharmaceuticals* 3, 1652 (2010).
- **Summarizes the anticancer activity of nonsteroidal anti-inflammatory drugs (NSAIDs) particularly so-called off target activities distinct from the COX isozymes demonstrating the value of selected small diversity libraries to probe the chemical biology of the NSAIDs.**
18. Elder DJ, Halton DE, Hague A, Paraskeva C. Induction of apoptotic cell death in human colorectal carcinoma cell lines by a cyclooxygenase-2 (COX-2)-selective nonsteroidal anti-inflammatory drugs: independence from COX-2 protein expression. *Clin. Cancer Res.* 3, 1679–1683 (1997).
19. Hanif R, Pittas A, Feng Y *et al.* Effects of nonsteroidal anti-inflammatory drugs on proliferation and on induction of apoptosis in colon cancer cells by a prostaglandin-independent pathway. *Biochem. Pharmacol.* 52(2), 237–245 (1996).
20. Alberts DS, Hixson L, Ahnen D *et al.* Do NSAIDs exert their colon cancer chemoprevention activities through the inhibition of mucosal prostaglandin synthetase? *J. Cell Biochem. Suppl.* 59(S22), 18–23 (1995).
21. Labayle D, Fischer D, Vielh P *et al.* Sulindac causes regression of rectal polyps in familial adenomatous polyposis. *Gastroenterology* 101(3), 635–639 (1991).
22. Stoner GD, Budd GT, Ganapathi R *et al.* Sulindac sulfone induced regression of rectal polyps in patients with familial adenomatous polyposis. *Adv. Exp. Med. Biol.* 470, 45–53 (1999).
23. Keller JJ, Offerhaus GJ, Polak M *et al.* Rectal epithelial apoptosis in familial adenomatous polyposis patients treated with sulindac. *Gut* 45(6), 822–828 (1999).
24. Agarwal B, Rao CV, Bhendwal S *et al.* Lovastatin augments sulindac-induced apoptosis in colon cancer cells and potentiates chemopreventive effects of sulindac. *Gastroenterology* 117(4), 838–847 (1999).
25. Rahman MA, Dhar DK, Masunaga R, Yamano H, Nagasue N. Sulindac and exisulind exhibit a significant antiproliferative effect and induce apoptosis in human hepatocellular carcinoma cell lines. *Cancer Res.* 60(8), 2085–2089 (2000).
26. Pasricha PJ, Bedi A, O'Connor K *et al.* The effects of sulindac on colorectal proliferation and apoptosis in familial adenomatous polyposis. *Gastroenterology* 109(3), 994–998 (1995).
27. Shiff SJ, Qiao L, Tsai LL, Rigas B. Sulindac sulfide, an aspirin-like compound, inhibits proliferation, causes cell cycle quiescence, and induces apoptosis in HT-29 colon adenocarcinoma cells. *J. Clin. Invest.* 96(1), 491–503 (1995).
28. Piazza GA, Alberts DS, Hixson LJ *et al.* Sulindac sulfone inhibits azoxymethane-induced colon carcinogenesis in rats without reducing prostaglandin levels. *Cancer Res.* 57, 2909–2915 (1997).
29. Piazza GA, Rahm AK, Finn TS *et al.* Apoptosis primarily accounts for the growth-inhibitory properties of sulindac metabolites and involves a mechanism that is independent of cyclooxygenase inhibition, cell cycle arrest, and p53 induction. *Cancer Res.* 57(12), 2452–2459 (1997).
30. Piazza GA, Keeton AB, Tinsley HN, Maxuitenko, Reynolds RC *et al.* A novel sulindac derivative that does not inhibit cyclooxygenases, but inhibits colon tumor cell growth and induces apoptosis with antitumor activity. *Cancer Prev. Res.* 2, 572 (2009).
- **Antitumor activity of the lead agent sulindac sulfide amide and sulindac was described as well as the design rationale for a new set of agents designed to attenuate/eliminate COX binding activity while maintaining/potentiating anticancer activity in order to probe the chemical biology of the indene NSAID scaffold.**
31. Rigas B, Tsioulis GJ. The evolving role of nonsteroidal anti-inflammatory drugs in colon cancer prevention: a cause for optimism. *J. Pharmacol. Exp. Ther.* 353(1), 2–8 (2015).
32. Gurpinar E, Grizzle WE, Shacka JJ *et al.* A novel sulindac derivative inhibits lung adenocarcinoma cell growth through suppression of Akt/mTOR signaling and induction of autophagy. *Mol. Cancer Ther.* 12(5), 663–674 (2013).

33. Whitt JD, Li N, Tinsley HN *et al.* A novel sulindac derivative that potently suppresses colon tumor cell growth by inhibiting cGMP phosphodiesterase and β -catenin transcriptional activity. *Cancer Prev. Res. (Phila.)* 5(6), 822–833 (2012).
34. Zhou H, Liu W, Su Y *et al.* NSAID sulindac and its analogs bind RXR α and inhibit RXR α -dependent AKT signaling. *Cancer Cell* 17(6), 560–573 (2010).
35. Li N, Chen X, Zhu B *et al.* Suppression of β -catenin/TCF transcriptional activity and colon tumor cell growth by dual inhibition of PDE5 and 10. *Oncotarget* 6(29), 27403–27415 (2015).
36. Roy RS, Gehring AM, Milne JC, Belshaw PJ, Walsh CT. Thiazole and oxazole peptides: biosynthesis and molecular machinery. *Nat. Prod. Rep.* 16, 249–263 (1999).
37. Yeh VSC. Recent advances in the total syntheses of oxazole-containing natural products. *Tetrahedron* 60(52), 11995–12042 (2004).
38. Riego E, Hernández D, Albericio F, A'lvarez M. Directly linked polyazoles: important moieties in natural products. *Synthesis* 12, 1907–1922 (2005).
39. Jin Z. Imidazole, oxazole and thiazole alkaloids. *Nat. Prod. Rep.* 23, 464–496 (2006).
40. Stokes NR, Baker N, Bennett JM *et al.* Design, synthesis and structure–activity relationships of substituted oxazole–benzamide antibacterial inhibitors of FtsZ. *Bioorg. Med. Chem. Lett.* 24, 353–359 (2014).
41. Godfrey AG, Brooks DA, Hay LA, Peters M, McCarthy JR, Mitchell D. Application of the dakin–west reaction for the synthesis of oxazole-containing dual PPAR α / γ agonists. *J. Org. Chem.* 68(7), 2623–2632 (2003).
42. Sanz-Cervera JF, Blasco R, Piera J, Cynamon M, Ibanez I, Murguia M, Fustero S. Solution versus fluorous versus solid-phase synthesis of 2,5-disubstituted 1,3-azoles. Preliminary antibacterial activity studies. *J. Org. Chem.* 74(23), 8988–8996 (2009).
43. Mathew B, Hobrath JV, Lu W, Li Y, Reynolds RC. Synthesis and preliminary assessment of the anticancer and Wnt/ β -catenin inhibitory activity of small amide libraries of fenamates and profens. *Med. Chem. Res.* 26(11), 3038–3045 (2017).
- **Reports the anticancer and Wnt/ β -catenin inhibitory activity of other NSAID series demonstrating that selected small diversity libraries built around the sulindac scaffold can unlock unique and non-COX-related anticancer activities.**
44. Mathew B, Hobrath JV, Ross L *et al.* Screening and development of new inhibitors of FtsZ from *M. tuberculosis*. *PLoS ONE* 11(10): e0164100 (2016).
45. Gurpinar E, Grizzle WE, Piazza GA. NSAIDs inhibit tumorigenesis, but how? *Clin. Cancer Res.* 20(5), 1104–1113 (2014).
46. Mathew B, Hobrath JV, Connelly MC, Guy RK, Reynolds RC. Diverse amide analogs of sulindac for cancer treatment and prevention. *Bioorganic Med. Chem. Lett.* 27, 4614 (2017).
- **The anticancer activity of diverse sulindac amide series was described in this paper demonstrating that unique small libraries of sulindac analogs can be used to probe alterations in phenotypic cancer cell line activity with small structural changes and that these activities may indicate new avenues of study in the chemical biology of the NSAIDs.**
47. Zhi-Yuan Wu Z-Y, Liua N, Qina B *et al.* Optimization of antiviral potency and lipophilicity of halogenated 2,6-diarylpyridinamines (DAPAs) as a novel class of HIV-1 NNRTIs. *Chem. Med. Chem.* 9(7), 1546–1555 (2009).

