Inhibition of Angiogenesis-Relevant Receptor Tyrosine Kinases by Sulindac Analogues

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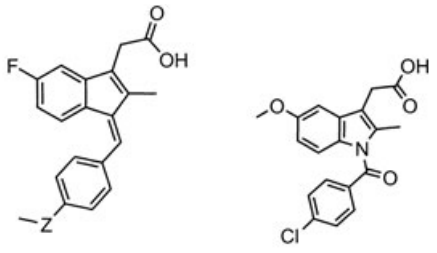
Nonsteroidal anti-inflammatory drugs (NSAIDs), like Sulindac **1** and Indomethacin, have a long history in the treatment of



1: Z = SO Sulindac
2: Z = SO_2 Sulindac sulfone
3: Z = S Sulindac sulfide

Indomethacin

pain and inflammation. Their effect is due to the ability of the NSAIDs to inhibit the enzymatic activity of cyclooxygenases (COX), which convert arachidonic acid to prostaglandins (PGs).[1] There are two known isoforms of the COX enzyme. COX-1 is expressed constitutively in most tissues and plays an important role in homeostasis.[2] COX-2 is absent in normal tissues, and its expression is induced by inflammatory cytokines and cellular transformation.[3,4] Prostaglandins are known promoters in the development of colon cancer.[5] It has been shown that NSAIDs have protective effects against colon cancer[6,7] and cardiovascular disease.[8,9]

Sulindac and its metabolites **2** and **3** also influence various biological phenomena besides the inflammatory-relevant cyclooxygenase pathway, such as an apoptosis-inducing pathway and the tumour-relevant Wnt pathway.[10,11] It has also been demonstrated that NSAIDs like Sulindac cause antiproliferative effects independent of COX inhibition.[12] Sulindac sulfone (**2**), the oxidized metabolite of Sulindac, which inhibits neither COX isoform, inhibits angiogenesis.[13] Angiogenesis,[14] the development of new blood vessels from pre-existing ones, is central to wound repair, inflammation and embryonic development.

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Furthermore, aberrant angiogenesis is considered a key step in tumour growth, spread and metastasis.[15,16] Vascular development depends on endothelial-specific receptor tyrosine kinases, in particular the vascular endothelial growth factors 1–3 (VEGFR-1–3) and the Tie-2 receptor.[17] All these receptors have been implicated in tumour angiogenesis,[18–22] and antagonization of Tie-2, VEGFR-2 or VEGF-D (a ligand for VEGFR-3) inhibits tumour growth and tumour metastasis in vivo.[21,23,24]

Although the exact mechanism of angiogenesis inhibition by Sulindac remains unclear, it has shown been that Indomethacin, a NSAID structurally related to Sulindac, inhibits VEGF-induced mitogen-activated protein (MAP) kinase/ERK2 activity.[13] MAP kinase is an important intermediate of several signalling pathways and is involved in the activation of transcription factors leading to cell proliferation. Furthermore it is known that NSAIDs suppress $\alpha v\beta 3$-dependent activation of the small GTPases Cdc42 and Rac, resulting in endothelial-cell spreading and migration in vitro and suppression of fibroblast growth factor 2-induced angiogenesis in vivo. Recently we have shown that members of an indomethacin-based compound library inhibited angiogenesis-related receptor tyrosine kinases like VEGFR-2, VEGFR-3, Tie-2 and the fibroblast growth factor-1 receptor (FGF1R).[25]

We have also shown that Sulindac and synthetic analogues of Sulindac interfere with the Ras pathway.[26,27] The findings detailed above demonstrate that Sulindac and Indomethacin display similar biological activities, such as inhibition of COX and inhibition of angiogenesis. Given this fact and the observation that Indomethacin-derived compounds inhibit angiogenesis-related receptor tyrosine kinases, we hypothesized that Sulindac-derived compounds might be inhibitors of these enzymes as well.

Herein we report on the ability of Sulindac analogues to inhibit the tyrosine kinases VEGFR-2, VEGFR-3, Tie-2 and FGF1R.

Results and Discussion

For the synthesis of Sulindac analogues, preparative routes were developed that yielded the desired compounds in a fast and effective manner and in 10–20 mg amounts with a purity high enough to avoid laborious purification prior to subsequent screening. As described previously,[26] a method was developed that combines the advantages of both solution- and solid-phase chemistry. First, differently substituted indenylacetic acids **4** were synthesized in solution by employing known methods. These intermediates were then attached to a polymeric support in order to carry out the final synthesis steps.

Briefly, differently substituted indenylacetic acids were attached to 2-chlorotrityl chloride resin (obtained from Calbiochem-Novabiochem, loading 1.08 mmol g^{-1}, or from CBL-Patras, loading 1.6 mmol g^{-1}). Resin-bound intermediates **5** were then subjected to a Knoevenagel condensation with aromatic aldehydes in the presence of 10 equivalents of DBU at 60 °C in DMF or toluene to yield, after release from the resin under acidic conditions, 188 Sulindac analogues **6** in overall yields of 47–98% (calculated based on the initial loading of the resin; Scheme 1). The library was further expanded by sub-

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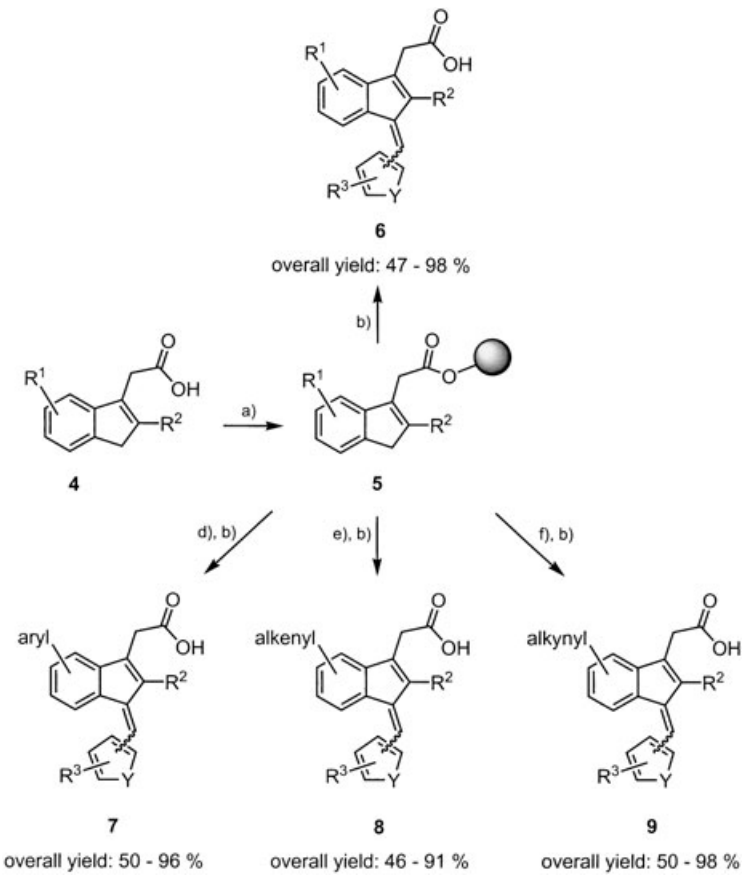


Scheme 1. *Solid-phase synthesis of Sulindac analogues. a) 2-chlorotrityl chloride resin, CH_2Cl_2, $(iPr)_2NEt$, 2 h, 21 °C; b) DBU, DMF or toluene, 60 °C, 16–48 h, aromatic aldehyde R^3CHO; c) alkene (5 equiv), $Pd_2[dba_3]$ (0.5 equiv), P(o-Tol)$_3$ (2 equiv), Et_3N/dioxane (1:1), nBu_4NBr (5 equiv), 85 °C, 20 h; d) boronic acid (10 equiv), $[Pd(PPh_3)_4]$ (0.35 equiv); $K_3PO_4 \cdot H_2O$ (20 equiv), DMF, 82 °C, 22 h; e) alkyne (20 equiv), CuI (0.3 equiv), DMF/Et_3N (1:1), PPh_3 (0.5 equiv), $Pd[PPh_3]_4$ (0.5 equiv), 85 °C, 20 h; f) 2 % CF_3COOH in CH_2Cl_2. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; DMF = dimethylformamide; dba = trans,trans-dibenzylideneacetone.* ***6****: R^1 = F, Cl, Br, I, OMe, OH, Me, H;* ***6–9****: R^2 = Me, Et; R^3 = halogen, alkyl, OR, NR′R″; Y = CH=CH, S, O.*

jecting immobilized indenylacetic acids **5** that carry a bromine or a iodine in the aromatic ring to a Heck, Suzuki or Sonogashira coupling, followed by the Knoevenagel condensation under the conditions described above.

Final release from the solid support by treatment with acid delivered Sulindac analogues **7**–**9**, which contain an aromatic substituent, an alkene or an alkyne attached to the indene benzene ring, in overall yields ranging from about 50 % to over 90 % (Scheme 1). After flash chromatography on silica gel with ethylacetate/cyclohexane (1 % acetic acid) 1:12 (*v*/*v*) as eluent, all compounds were obtained with >80 % purity. In total 239 compounds were prepared. NMR spectroscopic investigation employing NOE techniques revealed that, in general, the *Z* isomers were formed predominantly in the Knoevenagel reactions with *Z*/*E*-ratios of $>9/1$.

A total of 142 Sulindac analogues deemed to be representative for the entire collection were investigated as possible inhibitors of receptor tyrosine kinases (Table 1). The screen included the receptors mentioned above as well as fibroblast growth factor 1, which is also involved in angiogenesis. In addition, the prototypical epidermal growth factor receptor (EGFR) tyrosine kinase and insulin-like growth factor 1 receptor (IGF1R) were part of the assay.

IGF1R affects cell mitogenesis, survival, transformation and insulin-like activities by binding its ligands, IGF1 and IGF2. This receptor influences postnatal growth physiology, and its activity has been associated with malignant disorders such as breast cancer.[28] The antiapoptotic effect induced by the IGF1/IGF1R system correlates to the induction of chemoresistance in various tumours.[29]

Of the compounds investigated, 15 inhibited at least one of the kinases with IC_{50} values of about 10 μM or less (see Table 1).

Sulindac (**1**) and Sulindac sulfide (**3**) do not inhibit any of the investigated kinases at concentrations of up to 100 μM. With one exception, all compounds shown in Table 1 are inhibitors of the Tie-2 receptor. Compounds **10**, **12**, **16** and **18** were selective inhibitors, compound **13** was the most potent Tie-2 inhibitor with an IC_{50} value of 600 nM, but analogue **18** was similarly active and more selective. A fluorine at position 6 of the indenylacetic acid moiety is favourable for Tie-2 inhibition, but other halogens at position 6 or 5 are also tolerated. In addition, introduction of large substituents into the indene or arylidene part is compatible with Tie-2 inhibition (see **11**, **22**–**24**). Ten of the 15 compounds carry a five-membered electron-rich aromatic ring as arylidene substituent. An ethyl instead of a methyl group at position 2 of the indenylacetic acid moiety seems to increase inhibitory activity against Tie-2, the EGF receptor and FGFR-1 (compare compounds **14** and **15**). The profiles of the investigated Sulindac analogues for kinase inhibition are quite varied, and clear structure–activity relationships cannot conclusively be delineated. In this context, it is worth noting that whereas the 2-substituted thiophene derivative **12** selectively inhibits Tie-2 the 3-substituted furan derivative **14** is a selective inhibitor of VEGFR2.

We also investigated the difference in activity of the *E* and *Z* isomers **19** and **20**. The IC_{50} value for Tie-2 for compound **19** is one order of magnitude lower than for the corresponding isomer **20**. As mentioned above, the most potent inhibitor of Tie-2, with an IC_{50} of 0.6 ± 0.18 μM, is derivative **13**, which also inhibits VEGFR-2 and VEGFR-3 in the low-micromolar range. These combined properties make this derivative an interesting starting point for the development of inhibitors of angiogenesis and lymphangiogenesis.[30]

Our data do not clearly prove a direct link between the antiangiogenic properties of Sulindac and its metabolites and kinase inhibition. However, the finding that closely related analogues of the drug are active inhibitors of angiogenesis-related kinases suggests that such a link might indeed exist. It is possible that under the conditions of the cellular assay mentioned above, the fairly hydrophobic Sulindac metabolites concentrate in the plasma membrane and thereby create local concentra-

Table 1. *Inhibition of receptor tyrosine kinases by Sulindac analogues*

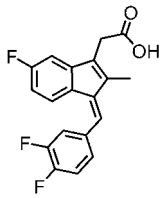
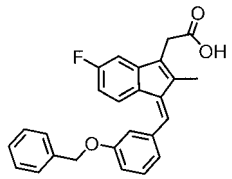
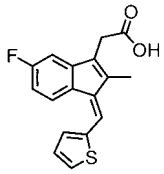
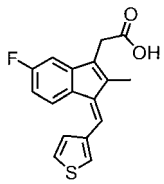
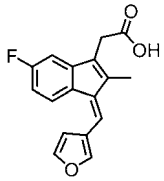
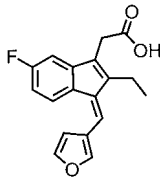
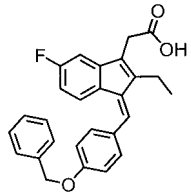
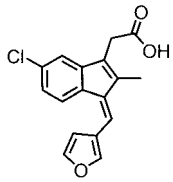
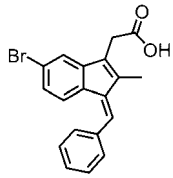
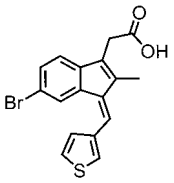
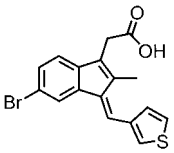
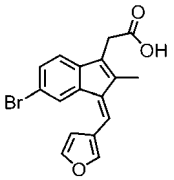
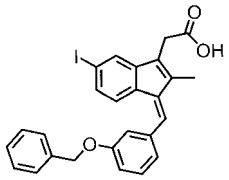
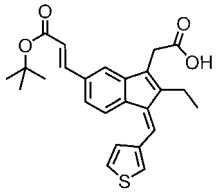
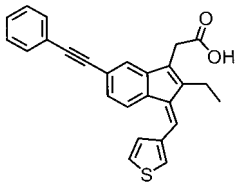
No.	Structure	IC_{50} for receptor tyrosine kinase [μM]					
		VEGFR-2	VEGFR-3	EGFR	IGF1R	FGFR-1	Tie-2
10		n.a.	n.a.	n.a.	n.a.	n.a.	5 ± 0.3
11		n.a.	n.a.	n.a.	n.a.	n.a.	11 ± 1.7
12		n.a.	n.a.	n.a.	n.a.	n.a.	5 ± 0.2
13		14 ± 0.9	15 ± 2.4	n.a.	n.a.	n.a.	0.6 ± 0.18
14		8 ± 4.5	n.a.	n.a.	n.a.	n.a.	n.a.
15		5 ± 0.9	n.a.	0.9 ± 0.27	n.a.	3 ± 1.6	1 ± 0.2
16		n.a.	n.a.	n.a.	n.d.	n.a.	2 ± 1.4
17		n.a.	14 ± 8.1	8 ± 1.7	7 ± 1.3	n.a.	5 ± 1.2
18		n.a.	n.a.	n.a.	n.a.	n.a.	1.0 ± 0.4

Table 1. (Continued)

No.	Structure	IC_{50} for receptor tyrosine kinase [μM]					
		VEGFR-2	VEGFR-3	EGFR	IGF1R	FGFR-1	Tie-2
19		3 ± 0.5	4 ± 1.1	12 ± 1.2	4 ± 1.0	23 ± 3.1	1 ± 0.2
20		n.a.	n.a.	n.a.	71 ± 6.2	n.a.	11 ± 3.2
21		24 ± 9.5	15 ± 8.0	22 ± 3.6	13 ± 2.5	n.a.	3 ± 1.1
22		n.a.	n.d.	n.a.	n.d.	n.a.	7 ± 3.5
23		n.a.	n.d.	n.a.	n.d.	n.a.	5 ± 1.6
24		n.a.	n.d.	n.a.	n.d.	n.a.	5 ± 2.3

[a] To assay the inhibitory activity, the kinase-catalyzed phosphorylation of poly(Glu_4-Tyr) in the presence of varying concentrations of inhibitor was determined. The kinases were employed as fusion proteins of glutathion-S-transferase (GST) and the respective kinase domain. The relative amount of phosphorylated substrate was quantified by means of an antiphosphotyrosine enzyme-linked immunosorbent assay, which employed an antiphosphotyrosine antibody conjugated to horseradish peroxidase (POD). The bound antibody was detected by light emission after addition of a chemiluminescence substrate for POD. All IC_{50} values were calculated from at least four independent determinations. n.d.: not determined. n.a.: remaining enzyme activity $>50\%$ at 100 μM inhibitor concentration.

tions that are substantially higher than the overall concentration. These high local concentrations might lead to inhibition of the receptors.

The discovery that Sulindac derivatives are inhibitors of angiogenesis-related receptor tyrosine kinases, in particular Tie-2, VEGFR-3 and FGFR-1, as well as the IGF1 receptor is of interest because only very few classes of inhibitors are known for Tie-2, VEGFR-3, FGFR-1 and IGF1R (see ref. [25] and references therein). The structural framework defined by the Sulindac core and the ease of its synthetic variation open up new opportunities for the development of antiangiogenesis drugs and antagonists of the IGF1 receptor.

In addition to the opportunity for the development of antiangiogenesis drugs, the finding that the Sulindac core structure defines a new class of kinase inhibitors is of general relevance to medicinal chemistry and chemical biology.

Acknowledgements

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Keywords: angiogenesis · combinatorial chemistry · kinase inhibitors · medicinal chemistry · Sulindac

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