Kinase Inhibitors

Synthesis and Biological Evaluation of an Indomethacin Library Reveals a New Class of Angiogenesis-Related Kinase Inhibitors**

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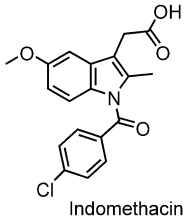
The combinatorial synthesis of small-molecule libraries on solid supports has emerged as a powerful and widely used method for the discovery of new biologically active compounds, in particular those for subsequent application in chemical biology and medicinal chemistry research. The choice of the underlying molecular framework of the individual library members is vital to the success of this approach. The hit rates in biochemical and cell biological assays, as well as the quality of the hits and the lead compounds derived from them are expected to be particularly high, even with relatively small libraries, if the compound class in question can be regarded as biologically prevalidated.[1] In this regard, indoles represent one of the most relevant structural classes. The plethora of indole-based biologically active natural products and indole-derived drugs spans an enormous range of biological activity. Consequently, the synthesis of indole-based compound libraries has received a great deal of attention, in particular in drug development.[2] Among the biologically active indole derivatives, indomethacin (**1**) is of particular interest. This compound belongs to the nonsteroidal antiinflammatory drugs (NSAIDs), which are widely applied in the treatment of, for example, pain, arthritis,[3] cardiovascular diseases,[4,5]



Indomethacin (**1**)

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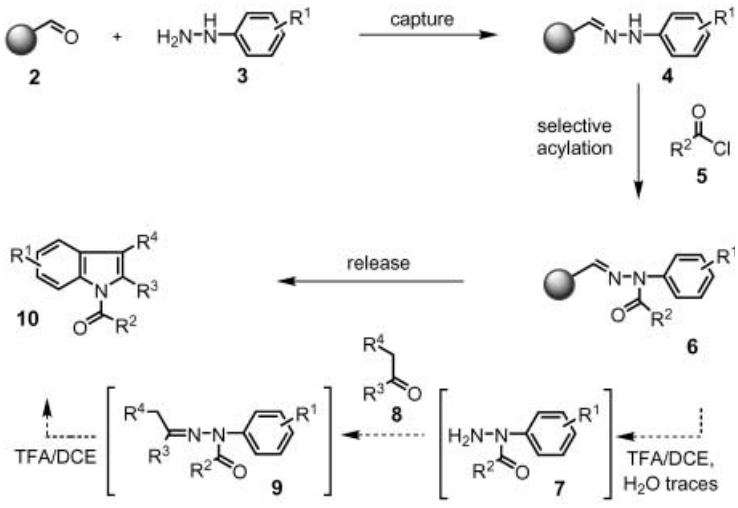
and Alzheimer's disease,[6,7] and in the treatment and prevention of cancer.[8,9]

It has been demonstrated that indomethacin induces G1 arrest and apoptosis of human colorectal cancer cells by influencing the Wnt signaling pathway,[10] downregulates the transcriptional activity of the peroxisome proliferation-activated receptor δ,[11] and inhibits the formation of new blood vessels from pre-existing ones (angiogenesis) through direct effects on endothelial cells mediated by the mitogen-activated protein (MAP) kinase signaling pathway.[9] Although the precise molecular targets of indomethacin in these processes have not been identified unambiguously, it is clear that indomethacin fulfils the criterium of biological prevalidation. In fact, its core may be regarded as a privileged structure.[12]

We have developed a synthesis of an indomethacin library and investigated the biological activity of the library compounds with respect to possible inhibition of receptor tyrosine kinases involved in angiogenesis. When planning the synthesis, we envisioned that application of the Fischer indole synthesis would allow a fairly diverse library to be built up from three readily available building blocks in a very short reaction sequence.[13] To establish a particularly practical and efficient method, we implemented a "resin-capture-release" strategy[14] that obviated the need for permanent attachment to and final detachment from the solid support, which would have required linker groups to be added and removed in two additional steps. The chosen strategy is summarized in Scheme 1. An aldehyde resin **2** is condensed with hydrazines **3** to yield polymer-bound hydrazones **4**. The polymer in intermediates **4** serves on the one hand as a reagent-capturing auxiliary, which allows easy removal of surplus reagent, and on the other hand as a temporary blocking function for the terminal NH_2 group of the hydrazine. Regioselective N-acylation of the second nitrogen atom in the subsequent step is thereby guaranteed. Surplus reagent is also readily removed from the selectively *N*-acylated hydrazones **6**. These intermediates are then subjected to treatment with acid and ketones **8** in the presence of traces of water to yield the desired indole derivatives **10**. In this reaction sequence, the water hydrolyzes the hydrazones and thereby releases selectively acylated hydrazines **7** into solution, where they condense with ketones **8** to give hydrazone intermediates **9**. These compounds then undergo a [3,3] sigmatropic rearrangement under the reaction conditions, which shifts the equilibrium between the hydrazones **6** and **9** irreversibly to the desired side. This strategy employs ketones, acid chlorides, and hydrazines as building blocks, all of which are either commercially available in great variety or readily prepared by numerous well-established synthesis methods. Use of these building blocks therefore allows ready construction of a fairly diverse compound collection.

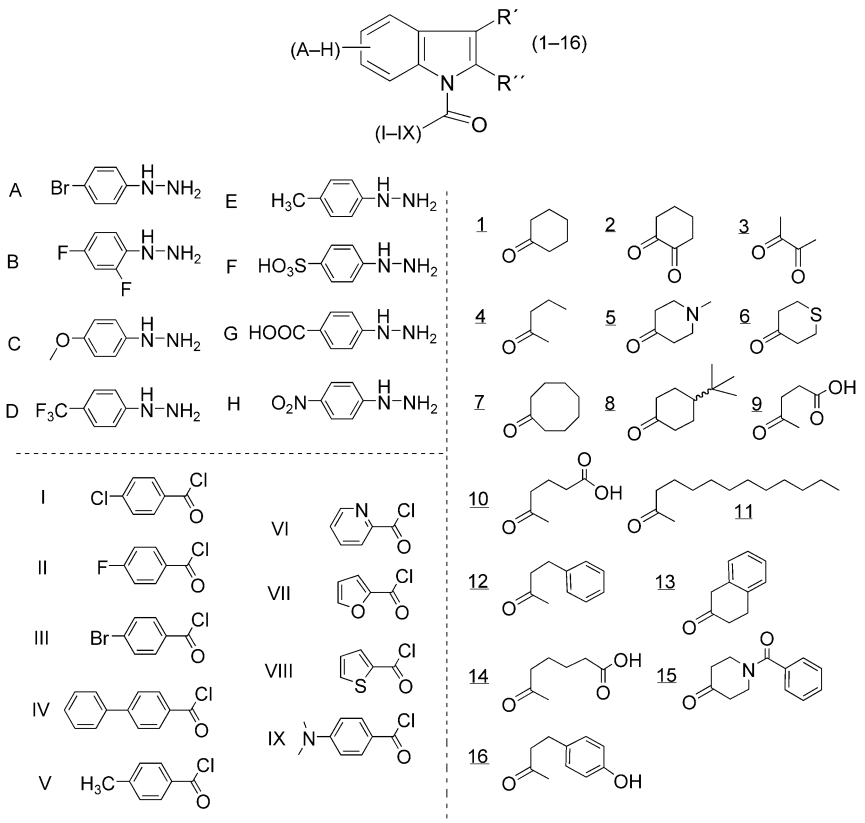
The building blocks shown in Scheme 2 were subjected to this resin-capture-release indole synthesis reaction sequence (for experimental details, see the Supporting Information). The solid support was a polystyrene aldehyde resin (Advanced Chemtech) with a loading of 0.9 mmol g^{-1}. A library of 197 indomethacin analogues was synthesized (see the Supporting Information) in overall yields ranging from 4% to quantitative (see Table 1). For example, indomethacin **1** was obtained by this four-step process in an overall yield of 63% after purification by chromatography.

These results demonstrate that the synthesis is compatible with a variety of different functional groups in each building block. Electron-withdrawing and electron-donating substituents can be incorporated in the hydrazine and the acid chloride, and electron-poor and electron-rich heterocycles are tolerated as well. The ketones may also incorporate different heteroatom substituents. Not unexpectedly, the overall yield was highest if activating electron-donating substituents were present in the hydrazines. This result indicates the importance of having two hydrazone-formation reactions in the whole process. However, the synthesis of *N*-acylated indoles by the resin-capture-release strategy is successful even in the presence of deactivating SO_3H, COOH, and NO_2 groups. The crude reaction products were obtained with purities of approximately 70 to over 99%, depending on the ketone employed. All compounds were isolated in more than 99% purity by simple chromatography.



Scheme 1. Concept of the "resin-capture-release" indole synthesis. DCE = 1,2-dichloroethane, TFA = trifluoroacetic acid.

Our initial biochemical investigation of the indomethacin-derived compound library was directed by the intriguing ability of the parent compound to inhibit angiogenesis. Angiogenesis, the development of new blood vessels from preexisting ones, is central to wound repair, inflammation, and embryonic development. Furthermore, aberrant angiogenesis is considered to be a key step in tumor growth, spread, and metastasis.[15,16] Vascular development depends on endothelium-specific receptor tyrosine kinases, in particular the vascular endothelial growth factor receptors 1–3 (VEGFR1–3) and the Tie-2 receptor.[17] All these receptors have been implicated in tumor angiogenesis[18–22] and antagonization of Tie-2, VEGFR-2, or VEGF-D (a ligand of VEGFR-3) inhibits tumor growth and tumor metastasis in vivo.[21,23,24] The development of low-molecular-weight inhibitors of these receptor tyrosine kinases is among the most promising approaches to



Scheme 2. Building blocks employed in the "capture-and-release" Fischer indole synthesis.

Table 1: Results of the library synthesis.

Building blocks	Yield[a] [%]	Building blocks	Yield[a] [%]
A-I	45–quant.	C-IX	20–47
A-V	9–26	D-I	47
A-VII	62–quant.	E-I	40–90
B-I	21–60	F-I	4–58
C-I	19–45	F-IV	4–52
C-II	16–63	F-VI	15–20
C-III	32–81	F-VII	7–55
C-IV	15–81	F-VIII	11–66
C-VI	17–49	F-IX	10–29
C-VII	9–99	G-I	4–26
C-VIII	21–46	H-I	11–29

[a] Yield ranges are given for the conversion of the respective *N*-acylated hydrazones by treatment with ketones 1–16. All yields are based on the initial loading of the aldehyde resin (0.9 mmol g^{-1}) and refer to purified products. The compounds were identified by LC-MS, GC-MS, NMR spectroscopy, and high-resolution mass spectrometry.

the development of new, alternative antitumor drugs, and several inhibitors of VEGFR-2 are in clinical trials.[25,26]

A high concentration (0.5 mM) of indomethacin inhibits angiogenesis in vitro after stimulation of endothelial cells with the angiogenic growth factors VEGF and basic fibroblast growth factor.[9] Analysis of the cells indicated that treatment with the drug led to reduced activity of MAP kinase, but it remained unclear whether this low activity was a result of direct inhibition of the enzyme or inhibition of a MAP-kinase-activating protein upstream in the signaling cascades that converge at MAP kinase.

Given the importance of the receptor tyrosine kinases mentioned above and the fact that they signal through MAP kinase, we speculated that the observed antiangiogenic effect of indomethacin might be mediated at least in part by inhibition of these kinases. This inhibiting effect appears to be relatively weak, since 0.5 mM indomethacin were required to inhibit angiogenesis in the cellular test system.[9] Screening of an indomethacin-derived compound library could therefore reveal analogues with higher biological activity.

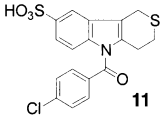
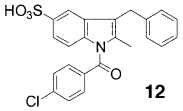
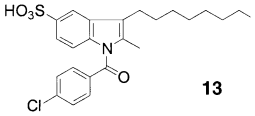
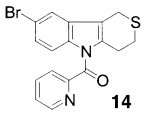
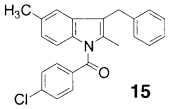
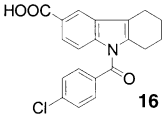
To investigate this hypothesis, 134 compounds considered to be representative of the entire library were selected and assayed as possible inhibitors of VEGFR-2, VEGFR-3, Tie-2, and fibroblast growth factor receptor 1 (FGFR-1). In addition, insulin-like growth factor 1 receptor (IGF1R) was included in the screen. 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.[27] The antiapoptotic effect induced by the IGF1/IGF1R system correlates with the induction of chemoresistance in various tumors.[28]

Of the 134 compounds investigated, 6 inhibit the kinases with IC_{50} values in the low-micromolar range (see Table 2).[29] Indomethacin itself does not inhibit any of the kinases at concentrations of up to 100 μM. Although a clear structure–activity relationship cannot be deduced from the limited number of compounds investigated, several preliminary conclusions may be drawn. A considerable degree of selectivity between the kinases can be achieved, and both selectivity and potency can be mediated by changing the substituents, in particular those on the indole ring. Among the *N*-acyl groups, the *p*-chlorobenzoic acid amide group appears to be particularly favorable, but not limiting. The presence of a polar substituent at the 3-position of the indole core structure is not beneficial for inhibitory activity. These initial findings indicate that it should be possible to develop more potent and selective inhibitors based on the molecular skeleton defined by indomethacin-type compounds.

Our data do not clearly prove a direct link between the antiangiogenic properties of indomethacin and inhibition of angiogenesis-related receptor tyrosine kinases. However, the finding that closely related analogues of the drug are active inhibitors of these 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 indomethacin concentrates in the plasma membrane and thereby creates local concentrations that are substantially higher than the overall concentration. These high local concentrations might lead to inhibition of the receptors.

The discovery that indomethacin-derived indole derivatives are inhibitors of angiogenesis-related receptor tyrosine

Table 2: Inhibition of angiogenesis-related receptor tyrosine kinases by members of the compound library.

Compound	IC_{50} for receptor tyrosine kinase [μM][a] VEGFR-2	VEGFR-3	Tie-2	IGF1R	FGFR-1
 **11**	21 ± 8.8	n.d.	3 ± 1.8	9 ± 3.5	6 ± 2.2
 **12**	20 ± 8.8	17 ± 6.2	9 ± 2.7	17 ± 4.9	n.d.
 **13**	9 ± 11.4	6 ± 4.6	4 ± 1.5	15 ± 3.6	7 ± 1.2
 **14**	n.a.	11 ± 1.4	n.d.	n.a.	n.a.
 **15**	19 ± 2.8	11 ± 1.5	n.d.	n.a.	n.a.
 **16**	10 ± 0.3	21 ± 0.8	n.d.	n.a.	n.a.

[a] To assay the inhibitory activity the kinase-catalyzed phosphorylation of poly(Glu_4-Tyr) was measured in the presence of varying concentrations of inhibitor. The kinases were employed in the form of fusion proteins of glutathione-*S*-transferase and the respective kinase domain. The relative amount of phosphorylated substrate was quantified by means of an antiphosphortyrosine enzyme-linked immunosorbent assay with an antiphosphotyrosine-antibody-conjugated horseradish peroxidase (POD). The bound antibody was detected by the light emitted after addition of a chemiluminescence substrate for POD. All IC_{50} values were calculated from at least four independent determinations. IC_{50}: concentration required for 50% inhibition. n.d.: not determined. n.a.: remaining enzyme activity more than 50% at 50 μM inhibitor concentration.

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.[25,30,31] The structural framework defined by the indomethacin core and the ease of its synthetic variation, for example, by the "resin-capture-release" strategy described herein, open up new opportunities for the development of antiangiogenesis drugs and antagonists of the IGF1 receptor. Compounds **11**–**13** are not particularly cytotoxic; exposure of T98g brain cells to these compounds at a concentration of 10 μM for four days did not lead to an increase in cell mortality.

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

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

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