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Letter

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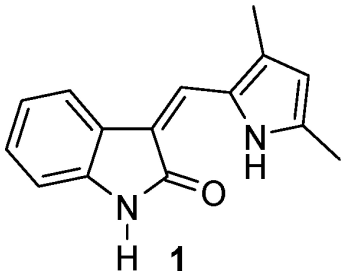

1

Inhib in the EAE model:
1, –98% ip
Prodrug of **1**, –56%, po

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Indolin-2-ones with High in Vivo Efficacy in a Model for Multiple Sclerosis[§]

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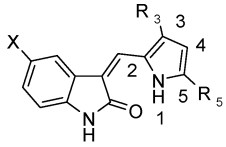
Abstract: The known KDR inhibitor SU5416 and several analogues of the indolin-2-one family were surprisingly found to be highly efficacious in the EAE model, an established model for multiple sclerosis. The high in vivo effect could be correlated to in vitro inhibition of the pro-inflammatory cytokine IL-2. Activity following po administration was obtained with several analogues and via the use of prodrugs.

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS). Both environmental and genetic factors are implicated in a misdirected immune response against myelin antigens, leading to axonal and nerve cell damage.[1,2] Existing treatments have limited efficacy on attack frequency and on slowing down the disease progression.[3−6] Besides, progressing forms of MS without attacks are poorly influenced by treatments and the sustained disability caused by the disease progression is irreversible.[7−9] For all drugs with documented effect, the administration mode is parenteral and no oral formulation, although needed, is available. Today, there is an obvious need for treatments of MS patients, which are more effective, better tolerated, and easier to administer. The most widely used animal model for MS is the rodent experimental autoimmune encephalomyelitis (EAE) model, where EAE can be induced by auto antigens such as proteolipid protein, myelin basic protein, or other components of the myelin sheaths.[10,11] Screening low molecular weight compounds with potential to fulfill the unmet clinical needs within MS, we found compound **1** to be very efficacious in the EAE model (up to 98% inhibition, dosed daily 50 mg/kg, ip, Chart 1). Compound **1** is an indolin-2-one that has previously been developed by Sugen as a tyrosine kinase inhibitor for the treatment of cancer.[12]

In this paper, we describe the synthesis of a series of indolin-2-ones and analogues and their evaluation in the EAE model. Analogues of compound **1** were first investigated. Among them, compounds **4** and **5** were synthesized (Scheme 1). Both compounds were prepared in one step starting from compound **1** using respectively NBS and SO_2Cl_2 as halogenating agents. Alternatively compound **4** could be prepared in two steps starting from the pyrrole **2**[13] in 84% overall yield. Compounds **7** and **8** were prepared in 87% and 62% overall yields, respectively, starting from the hydroxamic acid **6** prepared as described in the literature[14] (Scheme 2).

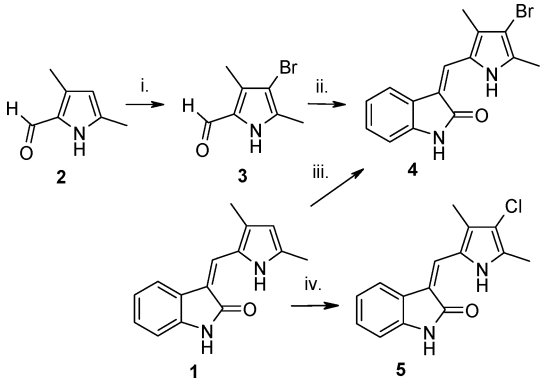
Chart 1. Compounds **1**, **14** and **16**: Structure and Activity in the EAE Model (ip Dosing)



1, X=H, R_3=R_5=Me, SU5416
Inhib EAE:−98%
14, X=OH, R_3=R_5=Me
Inhib EAE:n.s.[a]
16, X=R_3=R_5=H
Inhib EAE:n.s.[a]

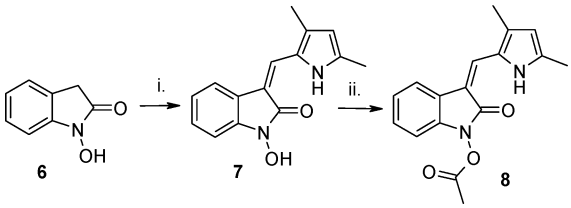
[a] n.s.: nonsignificant effect.

Scheme 1. Synthesis of Compounds **4** and **5**[a]



[a] Reagents and conditions: i. NBS, benzoyl peroxide, CCl_4, reflux (92%); ii. indolin-2-one, piperidine (cat.), EtOH, reflux (92%); iii. NBS, benzoyl peroxide, CCl_4, reflux (26%); iv. SO_2Cl_2, CH_2Cl_2, 0 °C to room temperature (34%).

Scheme 2. Synthesis of Compounds **7** and **8**[a]



[a] Reagents and conditions: i. 3,5-dimethyl-1*H*-pyrrole-2-carbaldehyde, piperidine (cat.), EtOH, reflux (87%); ii. acetic anhydride, Et_3N, room temperature (71%).

As compound **1** exhibited a high in vivo activity despite a low plasma concentration, we investigated whether reported primary and secondary metabolites[15] showed any activity in the EAE model. Metabolites **12** (major metabolite, Scheme 3), **13** (Scheme 3), and **14** (Chart 1, X = OH) have been isolated and characterized by Sugen. To obtain compounds **12** and **13** in the required quantities for testing in the EAE model, we developed the synthesis of both compounds in moderate to high yields: compound **12** was obtained in three steps in 67% overall yield using DIBAL-H for the reduction of the ester function of the intermediate **11**. Compound **13** was prepared in three steps from commercially available pyrrole **9** in 95% overall yield. In an attempt to increase solubility and to investigate the role of the substituents of the pyrrole ring, compounds **15** and **16**, bearing a polar amide at position 5 of the pyrrole and no substituent on the pyrrole ring,[16] respectively, were prepared. Compound **15** was prepared in one step from intermediate **11** using ethanolamine as both reactant

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[§] Dedicated to Lise and Ernst Binderup on the occasion of their retirement in December 2004.

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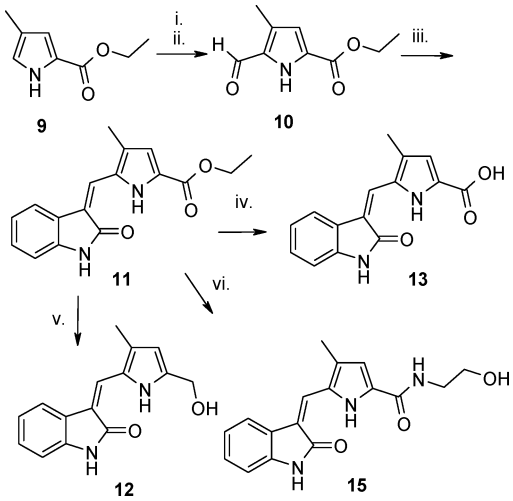
Scheme 3. Synthesis of the Primary and Secondary Metabolites of Compound **1**: **12** and **13**; Synthesis of Compounds **11** and **15**[a]



[a] Reagents and conditions: i. (chloromethylene)dimethylammonium chloride (Vilsmaier reagent), DCE; ii. NaOH (99%); iii. indolin-2-one, piperidine (cat.), EtOH, reflux (99%); iv. LiOH (1 N), THF (97%); v. DIBAL-H (1 M in toluene), toluene (68%); vi. ethanolamine (30 equiv), K_2CO_3, CH_3CN (85%).

Table 1. In Vivo and in Vitro Activities of Selected Analogues of Compound **1** in the EAE Model, the KDR Assay, and the IL-2 PBMC Assay, Respectively

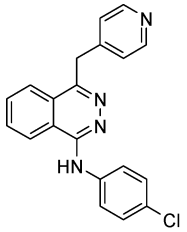
compd	EAE inhib, %[a]	significance[b]	IC_{50} KDR (10^{-6} M)	IC_{50} IL-2 (10^{-6} M)
1 (SU5416)	−98	$p < 0.0001$	0.23	0.005
4	−78	$p = 0.0014$	0.32	0.003
5	−96	$p < 0.0001$	1.0	0.004
7	−67	$p = 0.0008$	0.50	0.018
8	−48	$p = 0.0262$	0.40	0.011
11	−44	$p = 0.0019$	8.0	0.014
12 (SU9838)	−57	$p = 0.0002$	0.32	0.05
13 (SU6595)	−23	n.s.	>10	>1
14 (SU6689)	−12	n.s.	0.10	1.0
15	−41	$p < 0.0446$	>10	0.03
16	−9	n.s.	0.14	>1

[a] Inhibition in the EAE model; SJL/J mice were immunized for EAE induction and assessed clinically for 21 days. Area under the curve (AUC) of the disease score was calculated for all mice. Drug-treated groups were compared to the vehicle-treated group. Compounds were dosed ip, 50 mg/kg. [b] Significance; AUC of all groups was compared using the Kruskal−Wallis test. When $p < 0.05$, the Mann−Whitney test was used to compare drug-treated groups with the vehicle treated group ($p < 0.05$).

and solvent. The overall yield for the three-step synthesis of compound **15** was 83%.

The described analogues of compound **1** were all tested ip in the EAE model (Table 1). High in vivo efficacy was obtained with related analogues such as compounds **4** and **5** bearing a halogen substituent at position 4 of the pyrrole ring. A particularly high activity was observed with compound **5** (−96%). The amide function of the indolin-2-one scaffold was successfully replaced by a free or acetylated hydroxamic acid function while maintaining the in vivo activity as shown with compounds **7** (−67%) and **8** (−48%). The major metabolite **12** (hydroxylation of the methyl at position 5 of the pyrrole ring) exhibited in vivo efficacy (−57%). However, compound **12** was less active than its parental compound **1**. Ester and amide functions at position 5 of the pyrrole ring led to moderately active compounds as illustrated by compounds **11** and **15**, respectively, that both exhibit more than 40% inhibition.

Chart 2. Activity of a Known KDR Inhibitor in the EAE Model



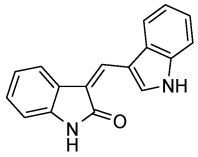
17, PTK-787
Inhib EAE (po): n.s.
IC_{50} (IL-2): >1μM
IC_{50} (KDR): 0.063μM

Indolin-2-ones have primarily been developed as KDR inhibitors and have been shown to have an anti-angiogenic effect. It has recently been suggested that angiogenesis would be an interesting approach for the treatment of MS.[17] Thus all new compounds and metabolites were tested for their ability to inhibit the KDR activity in an in vitro assay (Table 1). However, no obvious correlation was found between the in vivo efficacy in the EAE model and the in vitro potency in the KDR assay. For example the most potent compounds (**14** and **16**) did not exhibit any effect in the EAE model while one of the less in vitro active analogues, **5**, exhibited a high in vivo activity in the EAE model. Furthermore, no significant effect was obtained when mice were dosed with another known KDR inhibitor, which is currently undergoing clinical evaluation (compound **17**,[18] Chart 2). In conclusion, a mechanism of action other than KDR and angiogenesis inhibition or possibly a combination of several mechanisms accounts for the effect of compound **1** and its analogues in the EAE model.

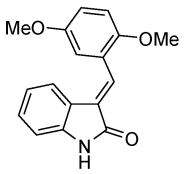
Cytokines are known to play an important role in the regulation of disease expression in EAE and in tolerance to disease induction. It is believed that the disease outcome can be modulated by tipping the balance between pro-inflammatory cytokines produced by Th1 cells (IL-2, IFN-γ, TNF-α) and antiinflammatory cytokines produced by Th2 cells (IL-4, IL-5, IL-10).[2,19] In EAE, the Th2 myelin specific cells suppress the disease whereas Th1 cells promote it.[10] To investigate the mechanism of action of indolin-2-ones, we tested the effect of compound **1** and analogues on the production of the pro-inflammatory cytokine IL-2 in a PBMC in vitro assay (Table 1). A clear correlation was found between the inhibition of the production of IL-2 and the in vivo activity in the EAE model: the most in vivo active compounds were also the most in vitro potent analogues (compounds **1**, **4**, **5**) and the non in vivo active compounds were also the less potent in vitro (compounds **11**, **15**, **16**). The IL-2 PBMC in vitro assay was then used as a tool for the selection of different indolin-2-ones for testing in the EAE model. Two other classes of indolin-2-ones were found to be both active in the IL-2 in vitro assay and in the EAE model (Chart 3): the indole-substituted analogues represented by compound **18** and the aryl-substituted analogues such as compound **19**.

Furthermore, compounds **18** and **19** were found orally active, showing an activity of, respectively, −32% and

Chart 3. Activity of Two Examples of Indole and Aryl Substituted Indolin-2-ones in the EAE Model

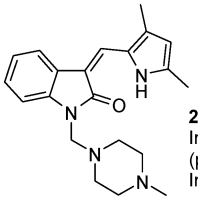


18, indole substituted
IC_{50}(IL-2)=0.008μM
Inhib EAE:–84%, ip
Inhib EAE:–32%, po



19, aryl substituted
IC_{50}(IL-2)=0.021μM
Inhib EAE:–90%, ip
Inhib EAE:–49%, po

Chart 4. po Activity in the EAE Model of Compound **20**, a Prodrug of **1**



20, prodrug of **1**
Inhib EAE: –56% po
(parent compound **1**
Inhib EAE: n.s., po)

−49%. This was surprising since all described in vivo active analogues exhibited both a poor aqueous solubility (<0.1 mg/mL) and a low oral absorption. To improve the oral bioavailability of the indolin-2-one family, we designed a series of prodrugs.[20,21] The Mannich base type prodrug **20** was synthesized as described in the literature[22] and tested in the EAE model. This prodrug of compound **1** showed significant po efficacy (−56%) as compared with the parent compound **1** which was not orally active (Chart 4).

In conclusion, our results suggest that the in vivo efficacy of the indolin-2-one family in the EAE model might be related to the inhibition of the pro-inflammatory cytokine IL-2. The IL-2 PBMC assay was successfully used as a screening assay to find indolin-2-ones exhibiting in vivo activity. Additionally, it was shown that po efficacy could be obtained by preparing prodrugs of active compounds. Further investigations should address the therapeutic dosing of orally active compounds in the EAE model as positive results could have important implications for the future treatment of MS.

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Supporting Information Available: experimental procedures and spectral data for the preparation and characterization of compounds **3**−**5**, **7**, **8**, **10**−**13**, **15**, **18**, and **19**, descriptions of the in vitro and in vivo models and in vivo results obtained with compound **1** are available free of charge at http://pubs.acs.org.

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