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Novel indole inhibitors of IMPDH from fragments: Synthesis and initial structure–activity relationships

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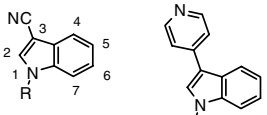
Abstract—The elaboration of previously reported indole fragments as inhibitors of inosine monophosphate dehydrogenase (IMPDH) is described. The synthesis, in vitro inhibitory values for IMPDH II, PBMC proliferation and physicochemical properties are discussed.

In the preceding article, we reported the results of our investigations into the inhibition of inosine monophosphate dehydrogenase (IMPDH) with low molecular weight indole fragments. As well as providing valuable insights into the requirements for potency against IMPDH II, two hit templates were highlighted for further investigation, namely 3-cyanoindoles and 3-(4-pyridyl)indoles.

Herein, we report our efforts to further develop representatives of these templates, namely the cyanoindoles **1a** and **1b**, and pyridylindole **2**, which have IMPDH II potencies (IC_{50}) of 20.9, 7.66 and 1.15 μM, respectively.



1a R=H
1b R=Me

2

Although SAR around a 3-(oxazol-5-yl)indole and 3-cyanoindole template has been previously reported, in both cases compounds were restricted to incorporation of a NHCONH or (2-oxazolyl)amino linker at the 6-position.[1,2] Based on binding mode hypotheses this was deemed necessary to allow formation of key H-bonds from the linker to Asp 274. From the observed SAR of the indole fragments, coupled with docking studies (see preceding paper), it was hoped that in the case of **1a** and **2** the indole NH could perform this function, thereby potentially allowing a greater range of substitution in the carbocyclic ring. Additionally, modelling studies suggested the indole 6- and 7-positions to be the optimum substitution points. Consequently, a range of 6- and 7-substituted indoles were prepared to test our hypotheses, and their syntheses are described in Schemes 1–5.

Indoles **3**, **7** and **8** were prepared via direct cyanation of their commercially available precursors with chlorosulfonylisocyanate (CSI) (Scheme 1). Catalytic hydrogenation of **3** afforded aminoindole **4**, which was further transformed into the urea **5** and the benzoxazole **6**. In the synthesis of *N*-1 alkylated cyanoindoles, nitroindoles (**3**) were alkylated and then reduced to versatile 6 or 7-aminoindole intermediates **I**. Employing isocyanate chemistry, indoles **I** were then converted to ureas (**9**, **10** and **16**), an *N*-hydroxy urea (**11**) and an *O*-amino carbamate (**12**). Sulfonamides (**13**–**15**) and the benzoxazole (**17**) were also prepared from aminoindoles **I** (Scheme 2). On the pyridylindole template, a phenyl group was introduced in the 6-position via Suzuki reaction upon bromoindole **II** to give **18** (Scheme 3). The ester **19**

Keywords: IMPDH; Inosine monophosphate dehydrogenase; Fragment elaboration.
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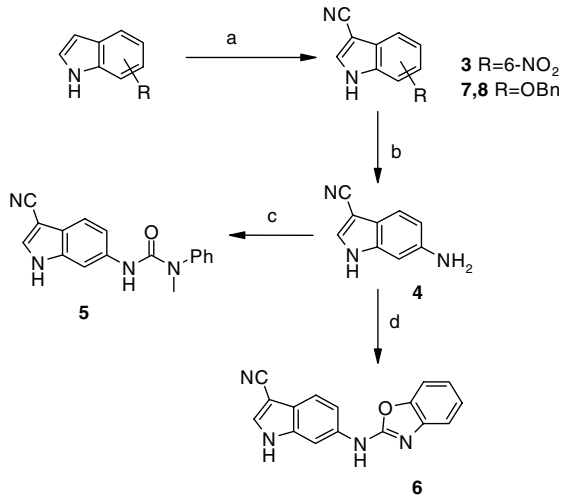
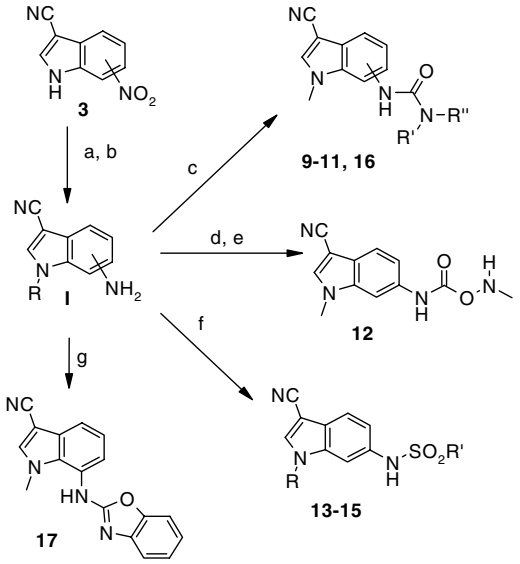
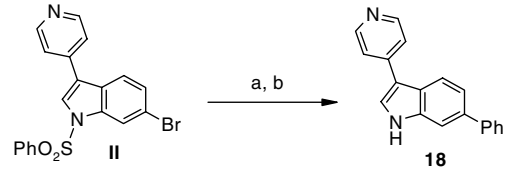


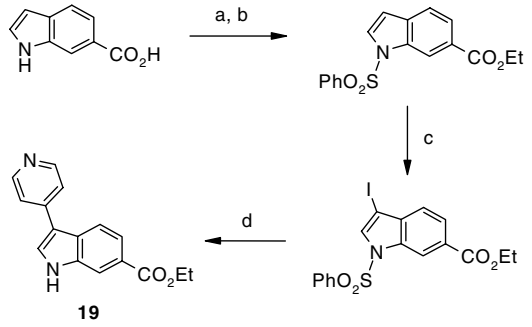
Scheme 1. Reagents and conditions: (a) CSI, MeCN, rt; then DMF, rt (22–100%); (b) (R = 6-NO_2) H_2, 10% Pd–C, MeOH, rt (100%); (c) triphosgene, Et_3N, DCM, 0 °C; then PhNHMe, rt (19%); (d) 2-chlorobenzoxazole, DMF, microwave, 120 °C (7%).



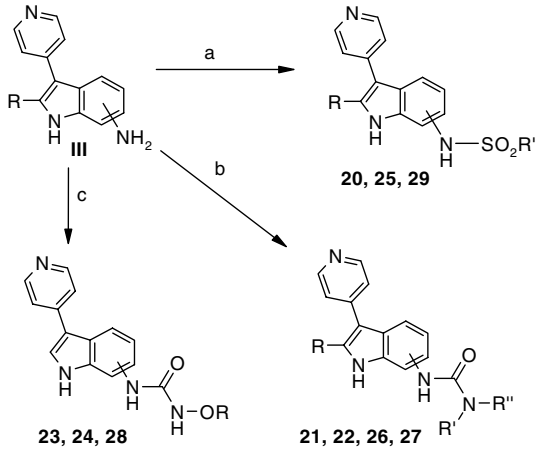
Scheme 2. Reagents and conditions: (a) MeI or EtI, KOH, acetone, rt (87%); (b) H_2, 10% Pd–C, MeOH, rt (100%); (c) PhNCO, DCM, rt (50%); or, triphosgene, Et_3N, DCM, 0 °C; then PhNHMe, rt (10–53%); or, $PhCH_2ONH_2$·HCl, triphosgene, Et_3N, DCM, −78 to 0 °C; then **I**, rt (25%); (d) triphosgene, Et_3N, DCM, 0 °C; then HONMeBoc (65%); (e) TFA, DCM, rt (89%); (f) R′SO_2Cl, DCM, pyridine, rt (9–35%); (g) 2-chlorobenzoxazole, DMF, microwave, 120 °C (33%).



Scheme 3. Reagents and conditions: (a) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, microwave, 150 °C; (b) KOH, MeOH, reflux (62%, 2 steps).



Scheme 4. Reagents and conditions: (a) EtOH, H_2SO_4, reflux; (b) $PhSO_2Cl$, K_2CO_3, methylethyl-ketone, reflux (51%, 2 steps); (c) *N*-iodosuccinimide, MeCN, rt (13%); (d) Pyridin-4-yl-boronic acid, $Pd(PPh_3)_4$, K_3PO_4, DME, H_2O, 80 °C (17%).

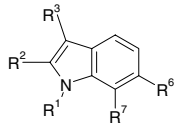


Scheme 5. Reagents and conditions: (a) $PhSO_2Cl$, DCM, pyridine, rt (8–49%); (b) triphosgene, THF, −78 to 0 °C; then R′R″NH, rt (7–34%); (c) $RONH_2$.HCl, triphosgene, Et_3N, DCM, −78–0 °C; then **III**, rt (4–33%).

was prepared from indole-6-carboxylic acid (Scheme 4). The aminoindole precursor **III**, described in the preceding paper, was elaborated to sulfonamides (**20**, **25** and **29**), ureas (**21**, **22**, **26** and **27**) and *N*-alkoxyureas (**23**, **24** and **28**) (Scheme 5).

The in vitro enzyme potencies and activity in a peripheral blood mononuclear cell (PBMC) proliferation assay are shown in Table 1. The first observation is that compared to the pyridylindoles, only moderate improvements in potency were achieved with *N*-methylcyanoindoles, and with the NH cyanoindoles no real improvements were found with the small set prepared. This may reflect a sub-optimal binding of the cyanoindole cores, as well as highlighting the sensitivity of the enzyme to the precise orientation of the substituents attached. Though simple 6-amino substitution on the NH cyanoindole in **4** was not tolerated, the urea **5** retained the potency of the fragment core. The benzoxazole **6** appeared to lose activity, failing to give an IC_{50}, and the 6-benzyloxyindole **7** was only weakly active. The 7-benzyloxyindole **8** was not tolerated, modelling predicting this position to be sterically restricted in the active site. In the case of *N*-methylcyanoindoles,

Table 1. SAR of indoles



Compound	R^1	R^2	R^3	R^6	R^7	IMPDH II[3] IC_{50} (μM)	PBMC[3] IC_{50} (μM)
1a	—	—	—	—	—	20.900	—
1b	—	—	—	—	—	7.660	—
2	—	—	—	—	—	1.150	28.0
4	H	H	CN	NH_2	H	IA[a]	—
5	H	H	CN	NHCON(Me)Ph	H	15.400	—
6	H	H	CN	Benzoxazol-2-ylamino	H	48% at 30 μM	—
7	H	H	CN	OBn	H	71.800	—
8	H	H	CN	H	OBn	IA[a]	—
9	Me	H	CN	NHCONHPh	H	0.722	14.0
10	Me	H	CN	NHCON(Me)Ph	H	17.000	—
11	Me	H	CN	NHCONHOBn	H	1.170	17.0
12	Me	H	CN	NHC(O)ONHMe	H	4.040	—
13	Me	H	CN	$NHSO_2Ph$	H	7.680	—
14	Me	H	CN	$NHSO_2Bn$	H	21.000	—
15	Et	H	CN	$NHSO_2Bn$	H	IA[a]	—
16	Me	H	CN	H	NHCON(Me)Ph	7.850	—
17	Me	H	CN	H	Benzoxazol-2-ylamino	58.400	—
18	H	H	4-Pyridyl	Ph	H	0.456	10.0
19	H	H	4-Pyridyl	CO_2Et	H	0.586	—
20	H	H	4-Pyridyl	$NHSO_2Ph$	H	0.364	14.0
21	H	H	4-Pyridyl	NHCONHPh	H	IA[a]	—
22	H	H	4-Pyridyl	NHCON(Me)Ph	H	0.084	12.0
23	H	H	4-Pyridyl	NHCONHOMe	H	1.110	—
24	H	H	4-Pyridyl	NHCONHOBn	H	0.076	4.6
25	H	Me	4-Pyridyl	$NHSO_2Ph$	H	0.222	—
26	H	Me	4-Pyridyl	NHCON(Me)Ph	H	0.128	—
27	H	H	4-Pyridyl	H	NHCON(Me)Ph	19.500	—
28	H	H	4-Pyridyl	H	NHCONHOBn	1.360	—
29	H	H	4-Pyridyl	H	$NHSO_2Ph$	6.210	—

[a] Inactive at 30 μM.

substitution at the 6-position with the monosubstituted urea **9** gave IMPDH potency in line with that previously reported,[2] but addition of a methyl in **10** resulted in a 23-fold reduction in activity. The hydroxyurea **11** was comparable to the urea **9**, however, attempts to significantly improve the potency through variation of the benzyl group were unsuccessful (data not shown). The *O*-aminocarbamate **12** and sulfonamides **13** and **14** gave low micrololar potency. Variation of the phenyl group of **13** for substituted phenyl/heteroaromatics also failed to improve potency (data not shown). Replacement of the *N*-methyl with an *N*-ethyl in **15** abolished activity, in agreement with expected steric restrictions at this position in our binding hypothesis. Only a small number of 7-substituted-*N*-methyl indoles were prepared. The urea **16** retained activity at a level similar to that of the fragment **1b**, whereas the benzoxazolylamino group in **17** lost activity.

The sub-micromolar IMPDH potency of the 6-phenyl and 6-ethoxycarbonyl pyridylindoles **18** and **19**, respectively, lends support to the hypothesis of the indole NH forming the key interaction with Asp 274. In this case, a functionalised 6-amino group leads to templates with consistantly better affinity. For example, sulfonamide **20** showed promising enzyme potency. In the case of ureas, the SAR appeared to completely contrast with the cyanoindoles, in that the simple urea **21** was inactive but the methyl-substituted analogue **22** showed potent inhibition (IC_{50} = 84 nM). In a further twist, although the simple methoxy urea **23** showed micromolar activity, the benzyloxy **24** again gave potent inhibition. In the case of the pyridylindole fragment **2** of the preceding article, addition of a 2-methyl on the indole increased affinity roughly threefold, however, as can be seen with **25** and **26** this effect was variable (compare to **20** and **22**, respectively). As seen with cyanoindoles, substitution at the 7-position of pyridylindoles in ureas **27** and **28**, and sulfonamide **29** led to a reduction in potency.

The activities in a PBMC proliferation assay showed a consistently high drop-off from the enzyme potency. The core fragments **1b** and **2** show good transport in a Caco-2 membrane permeability assay (Table 2), and the reasons for this observed drop-off remain unclear.

A number of physicochemical properties and Caco-2 transport were determined for cyanoindole **11** and pyridylindoles **20**, **22** and **24** (Table 2). All four compounds exhibited acceptable log *D*s and good Caco-2 transport. As expected, the solubility of the pyridyl derivatives was greatest at lower pH. The low molecular weights and PSAs allow significant scope for further optimisation of each template.

Table 2. Drug-like properties

Compound	Log *D*7.4[a]	Sol[b] (μg/ml)	Caco-2[c] (10^{-7} cm/s)		MW[d]	PSA[e] (Å^2)
			A-B	B-A		
1b	2.30	—	367	374	156	30
2	2.81	—	262	239	194	32
11	2.74	4.0 (4.5)	193	191	320	88
20	2.01	ND (8.0)	65	93	349	79
22	2.93	ND (42)	143	67	342	57
24	3.54	3.2 (17.5)	65	73	358	87

[a] Log *D*, measured at pH 7.4—Ref. 4.
[b] Aqueous solubility at pH 7.4, solubility at pH 5.0 in parentheses—Ref. 5. ND, not detected.
[c] Caco-2 Papp (apparent permeability), A-B (apical to basolateral) and B-A (basolateral to apical)—Ref. 6.
[d] Molecular weight.
[e] Polar surface area.

In summary, starting from a focused fragment based approach around an indole core we have gained valuable insights into the requirements for activity against IMPDH II. From this position, 3-cyanoindole and 3-(4-pyridyl)indole have been further elaborated, with the pyridylindole template providing sub-micromolar inhibitors of this enzyme with good drug-like properties. This represents a further departure from the 3-methoxy-4-(5-oxazolyl)aniline moiety of known IMPDH inhibitors, and efforts to optimise this interesting class will be reported elsewhere.

Acknowledgments

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4. Log $D_{pH7.4}$ was measured as follows. Compound (1–2 mg) was shaken with 1 ml of pH 7.4 buffer (0.1 M KH_2PO_4 adjusted to pH7.4 with 0.1 M NaOH, presaturated with octanol over 18 h) and 1 ml of octanol (presaturated with pH 7.4 buffer over 18 h) for 1 hour at 750 osc/min. The separate layers were filtered through 0.2 μm PTFE and analysed by HPLC. The log *D* was determined according to: $\log D_{pH\ 7.4} = \log$ (normalised peak area in octanol/normalised peak area in $buffer_{pH\ 7.4}$).
5. The aqueous solubility was measured as follows. Compound calibration standards were made up from DMSO stock solutions to produce a three-point calibration curve. Buffer (0.5 ml, 150 mM phosphate buffer) was added to 0.5 mg of compound sample and left to shake at room temperature for 90 mins. After this, the samples were filtered and the concentration of sample in the filtrate was measured using the calibration graph. Analysis of both calibration standards and filtrate samples was carried out by HPLC (UV detection at 254 nm). The solubility was measured in duplicate at two pHs.
6. Caco-2 transepithelial transport studies were performed using 24-well Transwell (Beckman Dickinson) format (10–21 day culture/passage 30–40). Once cells had grown to a confluent monolayer, confirmed by TEER measurement readings, the growth media (DMEM) were removed and replaced with Hanks' Buffer Salt Solutions (HBSS) pH = 7.4 (0.2 ml and 0.7 ml in apical and basolateral wells, respectively). Test compound (1 μM) cassetted with 2 reference compounds (10 μM) was spiked into the apical or basolateral wells ($n = 4$), and plates were incubated for 2 h at 37 °C, 5% CO_2 and 95% humidity. The basolateral or apical media were removed (if apically or basolaterally spiked, respectively) and transferred to a 96-well plate, containing calibration standards, and samples and standards were analysed by LC–MSMS. The measured concentrations of compound and reference compounds in each well were used to give a normalised compound Papp, based on the known Papps of the reference compounds.