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Facile synthesis and biological evaluation of assorted indolyl-3-amides and esters from a single, stable carbonyl nitrile intermediate

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ABSTRACT

The synthesis of biologically relevant amides and esters is routinely conducted under complex reaction conditions or requires the use of additional catalysts in order to generate sensitive electrophilic species for attack by a nucleophile. Here we present the synthesis of different indolic esters and amides from indolyl-3-carbonyl nitrile, without the requirement of anhydrous reaction conditions or catalysts. Additionally, we screened these compounds for potential in vitro antimalarial and anticancer activity, revealing 1*H*-indolyl-3-carboxylic acid 3-(indolyl-3-carboxamide)aminobenzyl ester to have moderate activity against both lines.

Amide and ester bonds have proven to be two of the most abundant and important functional groups in organic chemistry, featuring prominently in many bioactive natural products and pharmaceuticals.[1,2] The importance of amides in particular, from a medicinal chemistry viewpoint, is highlighted in a survey of GSK, AstraZeneca and Pfizer by Roughley and Jordan, which revealed that amide formation is the most common transformation used in their synthetic medicinal chemistry programmes.[3]

Amide and ester bonds are most commonly formed through activation of a carboxylic acid into an unstable acid halide,[2] followed by coupling with a nucleophile. However, this methodology often requires strictly adhered anhydrous conditions[4,5] in order to prevent the acid halide from reverting back into the organic acid through hydrolysis. Sophisticated new methods which enhance the reactivity of carboxylic acids towards nucleophilic attack are increasingly common in contemporary literature. While these methods have proven extremely versatile, they still rely on either the vigorous exclusion of moisture from the reaction environment,[6,7] or the concomitant use of numerous additional reagents.[8,9] A progressively more popular method of amide synthesis has emerged through the oxidative coupling of alcohols and amines. However, this requires the use of expensive rare earth metal catalysts.[10–12] Additionally, in all these cases, the complex reaction conditions have to be repeated for each synthesis of a desired amide or ester analogue.

Our interest in amide synthesis initially occurred upon the serendipitous formation of dimethyl-1*H*-indole-3-carboxamide (**1**) when we exposed indolyl-3-carbonylnitrile (**2**) to a DMF/HCl solution (Scheme 1).[13] In that study we, concluded that the DMF was being hydrolysed by HCl to form dimethylamine, which subsequently attacked the electrophilic carbonyl nitrile, thereby forming **1**. Examination of the literature revealed that Murahashi et al. had previously investigated the use of benzoyl cyanides to protect aliphatic amines,[14] which provided us with a sufficient precedent to investigate the potential of indolyl-amide and ester synthesis.

Consequently, we present here the synthesis of a diverse selection of indolyl-3-amides and esters (**3**–**13**), which were generated from a single stable activated electrophile **2**, along with three further analogues (**14**–**16**) synthesized from C-6 fluorinated (**17**) and brominated (**18**) analogues of **2**, respectively. This versatile reaction proceeds without the requirement of any protection strategy, additional reagents, or repeated anhydrous conditions. The indole moiety,[15,16] and its amide- and ester-containing analogues feature in many bioactive natural products[17] and are useful tools in drug discovery.[18–20] Accordingly, we evaluated our new compounds for activity against *Plasmodium falciparum* and a triple negative breast cancer cell line, revealing a single compound (**13**) with moderate activity against both cell lines.

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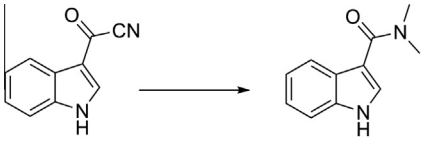


2 **1**, 37%

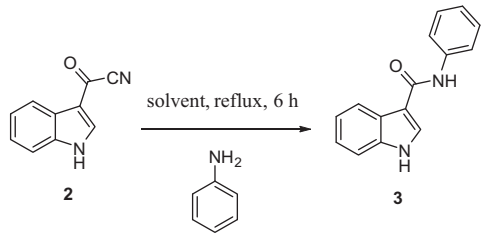
Scheme 1. Reagents and conditions: HCl saturated DMF, 80 °C, 1 h.

Due to the low solubility of indolyl-3-carbonylnitrile in non-polar solvents, we restricted our study to polar solvents while avoiding alcohols due to the possible formation of unwanted aliphatic esters (Table 1). We subsequently reacted **2** and aniline in acetone at reflux for 6 h (Table 1, entry 1). The reaction occurred smoothly to generate the *N*-phenyl carboxamide **3** in good yield. We proceeded to investigate three further polar solvents (Table 1, entries 2–4), with a particular interest in acetonitrile due to the reported stability of nitrile ions in acetonitrile,[21] which we hoped would render the carbonyl more susceptible to nucleophillic attack by facilitating removal of the leaving group. Reaction in MeCN resulted in an improved yield, although our highest yield was obtained in DMF, while the use of 1,4-dioxane resulted in a significant reduction in yield. We however decided to proceed with MeCN due to the potential difficulty in removing solvents with high boiling points.

Satisfied that we had adequate reaction conditions to yield our desired product in high yield, we proceeded with the synthesis of seven further amides (**4**–**10**), utilizing nucleophilic amines (R^2-H), varying in the nature of their respective chain lengths and attached rings (Table 2). Reactions with non indolic amines (Table 2, entries 1–5) proceeded smoothly in high yield, seemingly, with no observable preference for the nature of the respective nucleophiles. Reactions with indolic amines (Table 2, entries 6 and 7) proceeded in far lower yields, however, the reasons for this are not currently clear. We followed this study with an experiment to assess the relative reactivity of an amine or alcohol towards nucleophillic attack of the carbonyl nitrile (Table 2, entry 8). Murahashi et al.[14] had conducted a similar experiment with benzoyl cyanide and various aliphatic amino alcohols and found no evidence of ester formation. However, we were pleased to find that our method did allow the formation of an ester (**12**), albeit at a significantly lower rate than the corresponding amide (**11**), both of which were formed in the same reaction. Interestingly, a third compound from this reaction, the bisindole compound **13** featuring both amide and ester moieties was isolated in a higher yield than that of **12**.

Application of our synthetic method to the fluorinated indolyl-3-carbonyl nitrile **17** yielded carboxamide **14** in high yield, and again showed the preferential formation of amides over the corresponding ester (**15**) which was formed in the same pot (Table 2, entry 9). Interestingly, in this instance, no evidence of a bisindole derivative was observed. The brominated indolyl-3-carbonyl

Table 1
Optimized reaction conditions for the formation of *N*-phenyl carboxamide **3**

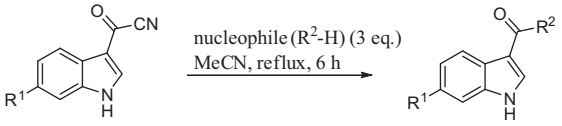


2 **3**

Entry	Solvent	Yield [a] (%)
1	Acetone	71
2	MeCN	85
3	DMF	95
4	1,4-Dioxane	23

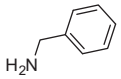
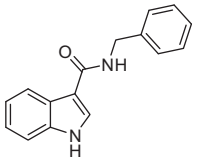
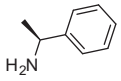
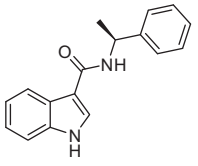
[a] Isolated yield.

Table 2
Synthesis of amides and esters **4**–**16**



2 R^1 = H
17 R^1 = F
18 R^1 = Br

4-13 R^1 = H
14, 15 R^1 = F
16 R^1 = Br

Entry	R^2-H	Product	Yield[a] (%)
1		 **4**	78
2		 **5**	97

(*continued on next page*)

Table 2 (*continued*)

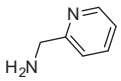
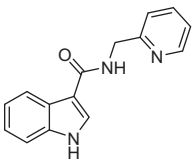
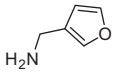
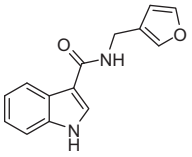
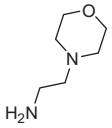
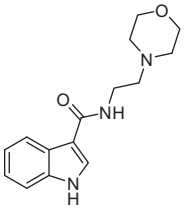
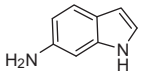
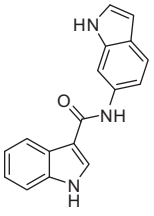
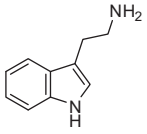
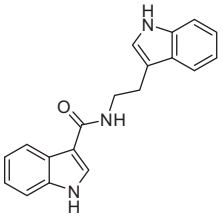
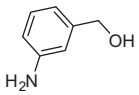
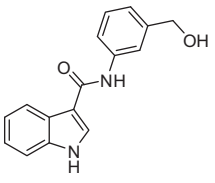
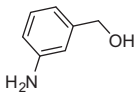
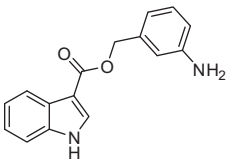
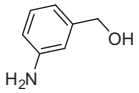
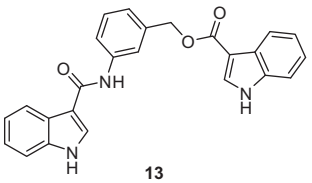
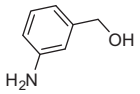
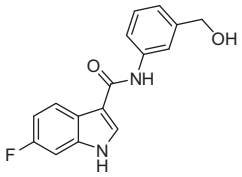
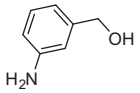
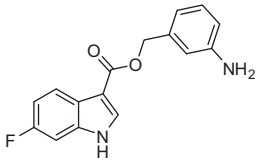
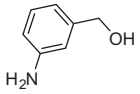
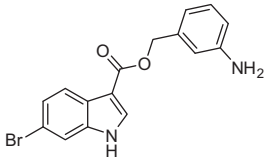
Entry	R^2-H	Product	Yield[a] (%)
3		 **6**	95
4		 **7**	72
5		 **8**	95
6		 **9**	33
7		 **10**	50
8		 **11**	41
		 **12**	11
		 **13**	23

Table 2 (*continued*)

Entry	R^2-H	Product	Yield[a] (%)
9		 **14**	71
		 **15**	7
10		 **16**	7

[a] Isolated yield.

nitrile analogue **18** was also able to generate an ester (**16**) in low yield, but unfortunately we were unable to purify the amide derivative to an acceptable level in order to accurately quantify the yield.

Following synthesis and characterization, our cohort of amides and esters was subjected to biological assessment against a chloroquine-sensitive malarial strain (NF54), a breast cancer cell line (Hs578T) and a non-malignant murine embryonic fibroblast cell line (MEF-1) as a control (Table 3). Chloroquine, artesunate and paclitaxel (PTX) were used as control compounds against the respective cell lines in order to assist interpreting the IC_{50} data. Antiplasmodial testing was performed according to previously published methods.[22] All compounds were considered inactive against malaria and breast cancer cells with the exception of **13** which displayed IC_{50} values in the low micromolar range against malaria, breast cancer and non-malignant cells. Interestingly, the compound library displayed generally greater cytotoxicity against non-cancerous MEF-1 cells in comparison to Hs578T cancer cells. Structure–activity relationship (SAR) analysis suggests that ester-containing analogues are more cytotoxic than their amide containing analogues, while C-6 halogenated analogues are more cytotoxic than their non halogenated counterparts. These observations have interesting implications in further SAR analysis of bisindole **13**.

Table 3
Growth inhibition assays of compounds **3**–**16**

Compound	NF54 IC_{50} (μM) ± sd[a]	Hs578T IC_{50} (μM) ± sd[a]	MEF-1 IC_{50} (μM) ± sd[a]
3	>100	83 ± 1.4	8.9 ± 1.4
4	>100	>100	27 ± 1.4
5	97 ± 20	>100	30 ± 1.5
6	>100	34 ± 1.5	59 ± 1.5
7	>100	36 ± 1.7	47 ± 1.7
8	>100	>100	63 ± 2.4
9	>100	>100	4.1 ± 1.2
10	72 ± 17	49 ± 1.6	32 ± 1.3
11	>100	>100	11 ± 2.2
12	48 ± 9.8	93 ± 1.2	71 ± 1.4
13	19 ± 5.4	10 ± 1.8	6.7 ± 1.2
14	>100	>100	57 ± 1.5
15	66 ± 14	35 ± 1.5	5.0 ± 1.4
16	28 ± 3.2	52 ± 1.3	4.8 ± 1.4
Chloroquine	28 ± 0.3[b]	—	—
Artesunate	8.6 ± 0.5[b]	—	—
PTX	—	75[b]	93[b]

[a] sd = standard deviation.
[b] nM values.

In this study, we have investigated the synthesis of indole-3-carboxamides and esters through a single stable intermediate. Currently, only two literature methods exist for the synthesis of indolyl-3-carbonylnitriles, both of which require anhydrous conditions.[13,23] However, the generation of a stable activated electrophile means that a variety of diverse chemical scaffolds can be generated under simple reaction conditions.[24] This is in contrast to most other amide synthetic strategies which require complex reaction conditions involving the addition of expensive catalysts for the generation of each new compound. Biological screening of these compounds revealed bisindole **13**, the unique structure of which warrants more extensive investigation into its SAR, to have moderate in vitro activity against both malarial and cancer cell lines.

Acknowledgments

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Supplementary data

Supplementary data (supplementary data relating to experimental procedures and spectral data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.02.090.

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24. General experimental procedure: Aniline (3 equiv) was added to a stirring solution of **2** (100 mg, 0.59 mmol, 1 equiv) in MeCN (4 ml). The mixture was heated to reflux and allowed to stir for 6 h, after which time the reaction was cooled and concentrated in vacuo to yield a brown amorphous solid. Flash chromatography (hexane/EtOAc, 1:1) afforded **3** (118 mg, 0.5 mmol, 85%) as a white amorphous solid. This representative method was applied to varying amounts of **2**, **17** and **18** using the same equivalents and solvent ratios above to afford products **4**–**16**. *N-Phenyl-1H-indolyl-3-carboxamide (3).* White amorphous solid (85% yield), ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.72 (1H, s, NH-1), 9.70 (1H, s, NH-2′), 8.29 (1H, d, J = 1.8 Hz, H-2), 8.20 (1H, d, J = 7.8 Hz, H-4), 7.77 (2H, d, J = 7.5 Hz, H-4′), 7.47 (1H, d, J = 7.8 Hz, H-7), 7.33 (2H, t, J = 7.5 Hz, H-5′), 7.19 (1H, t, J = 7.8 Hz, H-6), 7.15 (1H, t, J = 7.8 Hz, H-5), 7.04 (1H, t, J = 7.5 Hz, H-6′); ^{13}C NMR (DMSO-d_6, 150 MHz): δ 163.3 (q_c, C-1′), 139.8 (q_c, C-3′), 136.2 (q_c, C-7a), 128.7 (CH, C-5′), 128.5 (CH, C-2), 126.4 (q_c, C-3a), 122.6 (CH, C-6′), 122.1 (CH, C-6), 121.1 (CH, C-4), 120.7 (CH, C-5), 119.7 (CH, C-4′), 111.9 (CH, C-7), 110.5 (q_c, C-3) ppm; EIMS m/z (rel. int.): 144 $[M+H]^+$ (100), 118 (4), 116 (24), 94 (6), 89 (5); HREIMS: m/z 237.1025 $[M+H]^+$ (calcd for $C_{15}H_{13}N_2O$: 237.1028).