Organic & Biomolecular Chemistry

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2014, **12**, 6080

Received 1st April 2014,
Accepted 1st July 2014

DOI: 10.1039/c4ob00686k

www.rsc.org/obc

TFAA/H_3PO_4 mediated unprecedented N-acylation of carbazoles leading to small molecules possessing anti-proliferative activities against cancer cells†

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For the first time TFAA/H_3PO_4 has facilitated the direct and metal-free N-acylation of carbazoles leading to a number of N-acylated derivatives. Several of these compounds were found to be promising when tested for their anti-proliferative properties against oral cancer cell lines.

The new and effective yet simple strategies for the rapid access to small molecules of potential pharmacological interest are of great demand both in academic and industrial R&D. Motivated by this fact we have developed a simple strategy for the N-acylation of carbazoles.

In addition to its natural occurrences in alkaloids isolated from plants, the carbazole framework has been found to be an integral part of many bioactive compounds and therefore (like indole) is considered as a privileged scaffold in the area of new drug discovery. For example, various carbazole derivatives have shown a wide range of biological activities, *e.g.* antioxidant,[1] antidiabetic,[1,2] antimitotic,[3] antimicrobial,[4–6] anti-vascular,[7] antitumor,[8–10] anticancer,[11–15] antipsychotic,[16] and anticonvulsant[17] activities. Carbazole derivatives are also found to be inhibitors of cyclin dependent kinases (CDKs)[18] and checkpoint kinase (Chk 1)[19] and promising peroxisome proliferation activated receptors (PPAR α, β and γ).[20] In view of the reported antitumor properties of condensed *N*-acylindoles[21a] the *N*-acyl carbazole framework attracted our particular attention due to their common structural features. We anticipated that small molecules based on *N*-acyl carbazoles (**A**, Fig. 1) might show anticancer properties. Our anticipation was further supported by an earlier report that the carbazole derivative **B** (Fig. 1) was found to be cytotoxic when tested against mouse mammary carcinoma FM3A cells.[21b]

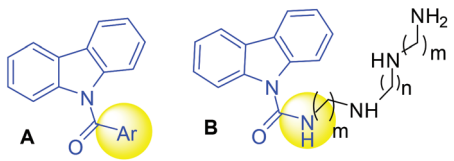

Fig. 1 Design of bioactive *N*-acyl carbazole (**A**) derived from **B**.

While N-acylation of indoles has been studied well, only a few reports are available on the N-acylation of carbazoles. The *N*-acyl carbazoles are generally prepared by the action of (i) acyl halides and anhydrides on carbazoles, (ii) acyl halides on potassium carbazole and (iii) acyl halides on carbazole magnesium halides.[22] These methods, however, have several drawbacks such as moderate yields, longer reaction times, and the use of volatile and environmentally harmful organic solvents. Moreover, the use of a common acylating agent, *i.e.* moisture sensitive acid chlorides, often causes environmental pollution and their preparation typically involves the use of thionyl chloride or oxalyl chloride. Thus, we were in need of an alternative and practical method for the preparation of compound **A**. The use of TFAA (trifluoroacetic anhydride)/H_3PO_4 as an efficient catalyst system for C-acylation of various aromatic compounds is reported in the literature.[23] However, we have observed that the reaction of carbazole (**1**) with various carboxylic acids (**2**) in the presence of TFAA and H_3PO_4 afforded the corresponding *N*-acyl carbazoles (**3** or **A**, path a) instead of C-acylated derivatives (path b, Scheme 1). Herein we present our preliminary findings on this mild, single-step and metal-free method for the synthesis of **3** *via* an unusual C–N bond forming reaction. A similar C–N bond formation under acidic conditions is not common in the literature and to the best of our knowledge the use of TFAA/H_3PO_4 for the N-acylation purpose has not been explored earlier.

To establish the optimized reaction conditions we used the reaction of **1** with benzoic acid (**2a**) as a model reaction that was performed in the presence of TFAA (2.093 mol) and varying amounts of 85% H_3PO_4 at room temperature (Table 1).

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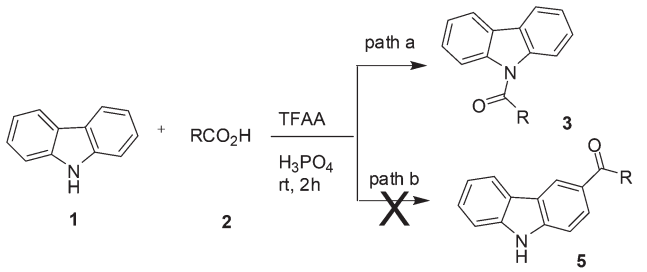
†Electronic supplementary information (ESI) available: Experimental procedures, spectral data for all new compounds, copies of spectra, results of *in vitro* and docking study. See DOI: 10.1039/c4ob00686k



Scheme 1 TFAA/H_3PO_4-mediated reaction of carbazole (**1**) with various carboxylic acids (**2**).

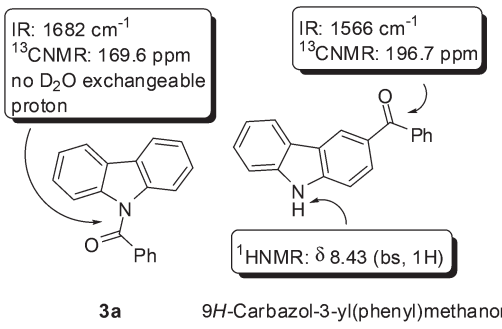
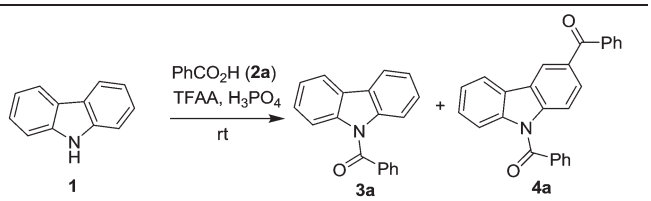


Fig. 2 Comparison of spectral data of **3a** and regioisomeric 9*H*-carbazol-3-yl(phenyl)methanone.[24]

Initially, the reaction was performed using 2.0 equivalents of **2a** and 0.1 equivalent of 85% H_3PO_4 (0.059 mmol) when a mixture of products *i.e.* **3a** and **4a** was isolated after 3 h (entry 1, Table 1). Based on spectral (NMR, IR, MS) data compound **3a** was identified as an N-acylated product (that contains an amide bond) rather than a C-acylated one[24] (Fig. 2). Compound **4a** was identified as a diacylated product *i.e.* (9*H*-carbazole-3,9-diyl)bis(phenylmethanone). We were delighted to observe the formation of **3a** in this reaction that was unprecedented in the literature under the conditions employed. An increase in the equivalent of H_3PO_4 used increased the yield of **3a** when the reaction was performed for 1 h (entry 2, Table 1). An increase in the reaction time afforded **4a** as the major product (entry 3, Table 1). Further increase of H_3PO_4 equivalents afforded **3a** as the major product when the reaction was performed for 1 h (entry 4, Table 1). However, increasing the reaction time (entry 5, Table 1) to 3 h afforded **4a** as the major product. We then performed the reaction using 1.0 equivalent of **2a** and 0.1 equivalent of 85% H_3PO_4 for 1 h when **3a** was isolated in 60% yield (entry 6, Table 1). An increase in reaction time to 2 h increased the yield of **3a** to 75% (entry 7, Table 1). However, in spite of several attempts we failed to find conditions where **3a** could be isolated as the only product. We therefore used the conditions of entry 7 of Table 1 for our further studies. It is worth mentioning that the reaction did not proceed in the absence of H_3PO_4, indicating its key role in the present reaction. All these reactions were performed at room temperature. While an increase in reaction temperature enhanced the reaction rate but decreased the yield of **3a**. The reaction was found to proceed in the absence of any additional organic solvent thereby decreasing the scope of environmental pollution. The excess of TFAA used perhaps played the role of a solvent in this reaction. After completion of the reaction TFAA converted to TFA can be recovered by distillation over a dehydrating agent. Nevertheless, the present reaction does not require the use of any inert or anhydrous atmosphere indicating the scale-up potential of this method.

Table 1 The effect of conditions on the TFAA/H_3PO_4-mediated reaction of **1** with **2a**[a]



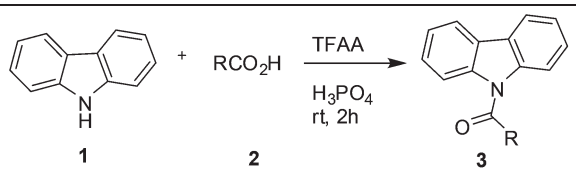
Entry	H_3PO_4 (equiv.)	**2a** (equiv.)	Time (h)	Yield[b] (%) **3a**	**4a**
1	0.1	2	3	42	45
2[c]	0.5	2	1	61	15
3	0.5	2	3	31	65
4[c]	1	2	1	65	18
5	1	2	3	26	68
6[c]	0.1	1	1	60	8
7	0.1	1	2	75	10

[a] All the reactions were carried out using carbazole **1** (0.598 mmol), benzoic acid **2a**, TFAA (2.093 mmol) and 85% H_3PO_4. [b] Isolated yield. [c] The presence of unreacted **1** was observed in this case.

With the optimum reaction conditions in hand we then examined the generality and scope of the present C–N bond forming reaction. Thus a range of carboxylic acids (**2**) were reacted with **1** under the conditions of entry 7 of Table 1 and yields of the corresponding products are shown in Table 2. It is evident from Table 2 that the acid **2** may contain a mono or disubstituted benzene ring and the substituent(s) may be present at the *o*-, *m*- or *p*-position or any two of these three positions. Aliphatic carboxylic acids *e.g.* **2p** and **2q** also participated well in the present reaction (entries 16 and 17, Table 2). However, the use of heteroaromatic acids *e.g.* thiophene-2-carboxylic acid was not successful as a mixture of unidentified products was isolated in this case. The use of a substituted carbazole *e.g.* 3-bromo-9*H*-carbazole **6** was examined and found to be effective under the conditions employed (Scheme 2).

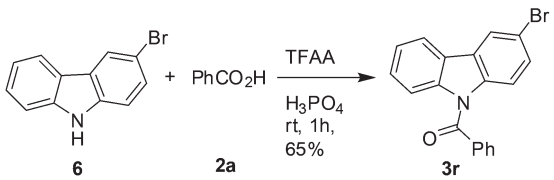
A plausible reaction mechanism for the present TFAA/H_3PO_4-mediated N-acylation of carbazoles is presented in Scheme 3.[23e,25] The reaction seems to proceed *via in situ* generation of the acylation precursor acyl trifluoroacetate **X** from TFAA and the carboxylic acid **2**. As a covalent catalyst the phosphoric acid then converts **X** into the arylacetyl bis(trifluoroacetyl)phosphate **Y** which then participates in N-acylation of carbazole **1**. Since the reaction does not proceed in the absence of H_3PO_4 and it is known that **Y** participates in a faster acylation reaction than **X**[25] the direct N-acylation of **1** by **X** seems to be unlikely. Moreover, acylation by **X** could lead to

Table 2 TFAA/H_3PO_4-mediated synthesis of *N*-acyl carbazole (**3**) (path a, Scheme 1)[a]

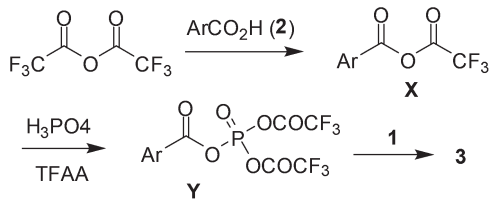


Entry	Carboxylic acid **2**; R	Product **3**	Yield[b] (%)
1	**2a**; Ph	**3a**	75
2	**2b**; 4-ClC_6H_4	**3b**	55
3	**2c**; 2-IC_6H_4	**3c**	60
4	**2d**; 2-Cl-5-BrC_6H_3	**3d**	64
5	**2e**; 2-BrC_6H_4	**3e**	60
6	**2f**; 3-ClC_6H_4	**3f**	75
7	**2g**; 2-ClC_6H_4	**3g**	60
8	**2h**; 2-MeC_6H_4	**3h**	60
9	**2i**; 4-MeC_6H_4	**3i**	68
10	**2j**; 4-IC_6H_4	**3j**	70
11	**2k**; 2,6-(di-Cl)C_6H_3	**3k**	60
12	**2l**; 3,4-(di-Cl)C_6H_3	**3l**	62
13	**2m**; 2,4-(di-Cl)C_6H_3	**3m**	70
14	**2n**; 2,5-(di-Cl)C_6H_3	**3n**	64
15	**2o**; 3,5-(di-Cl)C_6H_3	**3o**	60
16	**2p**; Ph_2CH	**3p**	61
17	**2q**; *n*-Pr	**3q**	63

[a] All the reactions were carried out using carbazole **1** (0.598 mmol), benzoic acid **2a** (0.598 mmol), TFAA (2.093 mmol) and 85% H_3PO_4 (0.059 mmol). [b] Isolated yield.

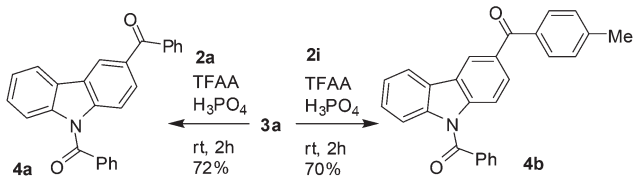


Scheme 2 TFAA/H_3PO_4-mediated reaction of **6** with **2a**.



Scheme 3 The proposed reaction mechanism for the TFAA/H_3PO_4-mediated N-acylation of carbazoles.

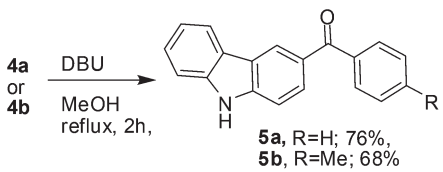
the trifluoroacetylated product of **1** (in addition to **3**) that was not detected in the reaction mixture. While the actual reason for the preferential N-acylation of **1** is not clear at this stage, the higher reactivity of the carbazole nitrogen over the ring carbon towards **Y** could be the reason for this observation. In fact the C-acylation of carbazoles was found to proceed smoothly after N-acylation separately. Thus, the *N*-benzoyl carbazole (**3a**) afforded the C-acylated product **4a**[26a] or **4b** when treated with aromatic acids in the presence of TFAA and



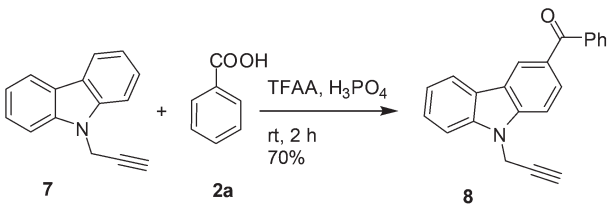
Scheme 4 TFAA/H_3PO_4-mediated C-acylation of **3a**.

H_3PO_4 (Scheme 4). Further, the selective deprotection of the *N*-benzoyl group of **4b** performed under the reported conditions[26b] to afford **5a**[24,26c] indicated the potential of this methodology for the generation of a compound library based on C-acylated carbazoles (Scheme 5). Indeed, the TFAA/H_3PO_4-mediated C-acylation also proceeded well when *N*-propargyl carbazole (**7**) was used as a substrate (Scheme 6).

All the synthesized *N*-acyl carbazoles were tested against cancer cell lines derived from tongue tissue *e.g.* CAL 27, breast cancer cell line *e.g.* MDA-MB231 and non-cancer cells *e.g.* HEK 293 T [Human Embryonic Kidney 293 cells] *in vitro* at 10 μM using the sulphorhodamine B (SRB) assay.[27] Gemcitabine,[28] a well known anticancer agent, was used as a reference com-



Scheme 5 Deprotection of **4a**/**4b** leading to the (9*H*-carbazol-3-yl)-(phenyl/*p*-tolyl)methanone (**5a**/**5b**).



Scheme 6 TFAA/H_3PO_4-mediated reaction of **7** with **2a**.

Table 3 Anti-proliferative activities of *N*-acyl carbazoles (**3**) against cancer and normal cells

	%inhibition[a]		
Compounds	CAL 27	MDA-MB231	HEK 293T
3a	>100	55.54	51.81
3b	100.84	72.98	57.74
3d	>100	78.28	58.73
3g	>100	80.42	65.22
3h	>100	76.63	65.47
3i	103.27	74.31	62.48
3l	93.18	68.10	46.70
3n	>100	78.24	41.61

[a] Average of at least three determinations.

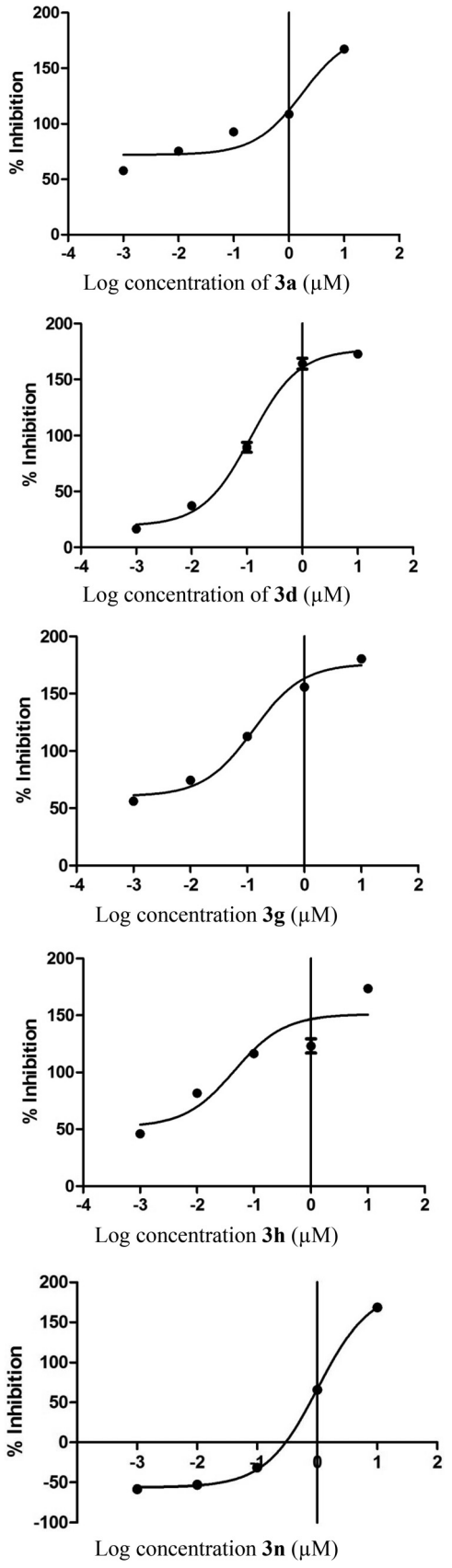


Fig. 3 Dose response study of compounds **3a**, **3d**, **3g**, **3h** and **3n** against CAL 27 cells.

pound in this assay. Several of these compounds *e.g.* **3a**, **3b**, **3d**, **3g**, **3h**, **3i**, **3l**, and **3n** showed promising growth inhibition especially of CAL 27 cells (>90% inhibition) after 72 h of treatment (Table 3).[30a] However, none of them showed notable effects on normal HEK 293 T cells (highest inhibition being ≤65%) when tested at 10 μM. Similarly, based on their effects on HEK 293 T cells, compounds **3b**, **3d**, **3g**, **3l** and **3n** were found to be active against MDA-MB231 cells (Table 3). Notably, gemcitabine[29] showed ~100% growth inhibition of CAL 27 cells at 10 μM. In a dose response study compounds **3a**, **3d**, **3g**, **3h** and **3n** showed consistent growth inhibition when tested against CAL 27 cells (Fig. 3).[30b] The IC_{50} value of **3d** and **3n** was found to be 0.028 ± 0.002 and 0.45 ± 0.03 μM, respectively. Thus these compounds were identified as the best active molecules in the present series. Nevertheless, since the death rate for oral cancer is higher than that of other cancers globally[31] the present class of compounds is of further interest.

In conclusion, TFAA/H_3PO_4 has been identified for the first time as an efficient coupling agent for the direct N-acylation of carbazoles. This metal-free methodology does not require the use of expensive reagents or catalysts and inert or anhydrous atmosphere and it works well at room temperature. A number of N-acylated derivatives were prepared using this methodology and tested for their anti-proliferative properties against oral and breast cancer cell lines. Several of them were found to be promising, indicating that the present series of *N*-acyl carbazoles represent a useful template for the discovery and development of potential anticancer agents.

BP thanks CSIR, for a research fellowship.

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29 Gemcitabine, generally used in various carcinomas such as non-small cell lung cancer, pancreatic cancer, bladder cancer and breast cancer, has shown *in vitro* GI_{50} value of 0.14 μM in the Human Tumor Cell Line Assay (NCI-60) and was validated; see: W. Wu, J. Sigmond, G. J. Peters and R. F. Borch, *J. Med. Chem.*, 2007, **50**, 3743.
30 (*a*) Compounds **3a**, **3d**, **3g**, **3h** and **3n** showed >100% inhibition of CAL 27 cells at 10 μM. The %inhibition more than 100% is considered as an indication of complete inhibition; see for example: Y. Hikichi, K. Honda, K. Hikami, H. Miyashita, I. Kaieda, S. Murai, N. Uchiyama, M. Hasegawa, T. Kawamoto, T. Sato, T. Ichikawa, S. Cao, Z. Nie, L. Zhang, J. Yang, K. Kuida and E. Kupperman, *Mol. Cancer Ther.*, 2012, **11**, 700. The possible reason for such observation is that the cells were not only inhibited but also were killed to some extent by these compounds. We thank one of the reviewers for pointing out this. (*b*) The dose in these curves is represented using a semi-logarithmic plot. On a semi-logarithmic plot, the amount of drug is plotted (on the *X* axis) as the log of drug concentration and response is plotted (on the *Y* axis) using a linear scale. For representative examples, see: A. Kumar, M. Zhang, L. Zhu, R. P. Liao, C. Mutai, S. Hafsat, D. R. Sherman and M.-W. Wang, *PLoS One*, 2012, 7, e39961, DOI: 10.1371/journal.pone.0039961.
31 For oral facts, see: http://www.oralcancerfoundation.org/facts/.

