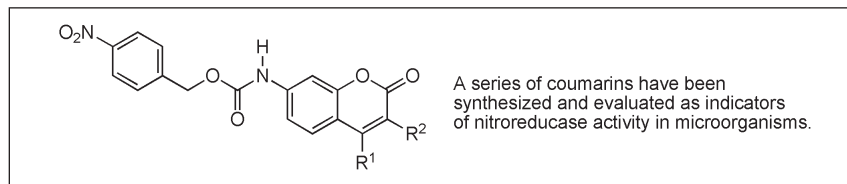


Arthur L. James^a, John D. Perry^b and Stephen P. Stanforth^{a*†}^a School of Applied Sciences, University of Northumbria, Newcastle upon Tyne, NE1 8ST, UK.^b Department of Microbiology, Freeman Hospital, Newcastle upon Tyne, NE7 7DN, UK.

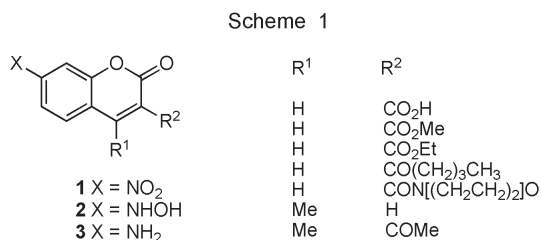
Received July 27, 2005



The carbamate **8** was reacted with appropriate β -keto esters in a Pechmann reaction giving the coumarin derivatives **9-11** bearing 4-nitrobenzylcarbamoyl triggers. These compounds were assessed as potential indicators of nitroreductase activity.

J. Heterocyclic Chem., **43**, 515 (2006).

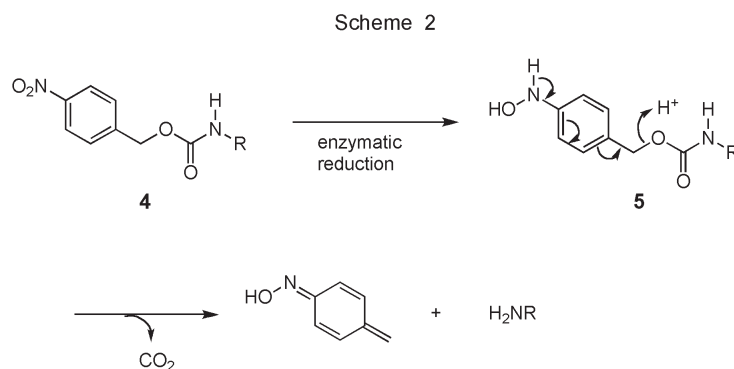
Many bacteria have the ability to reduce aromatic nitro compounds and the detection of nitroreductase activity may offer a useful means of analysing biological and environmental samples for the presence of microorganisms. The reduction of a series of seven non-fluorogenic nitro-coumarins **1** to their corresponding fluorogenic hydroxylamine derivatives **2** or amine derivatives **3** has been recently investigated for 30 strains encompassing 24 distinct microbial genera, including a wide range of clinically important species [1] (Scheme 1). The coumarin derivatives **2/3** are highly fluorogenic thus giving a sensitive method for the detection of nitroreductase activity.



The enzymatic reduction of nitro-groups in 4-nitrobenzylcarbamate derivatives **4** has also been used as a 'trig-

ger' mechanism in anti-cancer pro-drug design [2-7]. Reduction of the nitro-group in these inactive pro-drugs **4** gave the corresponding hydroxylamine derivatives **5** that underwent spontaneous fragmentation as shown in Scheme 2 releasing the active drug (RNH₂). In this note we report on our preliminary studies using a 4-nitrobenzylcarbamate trigger as a method for releasing fluorescent 7-hydroxylamine derivatives of coumarin thus giving a method for detecting nitroreductase activity. Substrates bearing triggers based upon 7-aminocoumarin derivatives and 7-hydroxycoumarin derivatives have recently found application in enzymatic profiling [8], image contrast agents [9], and probes for the detection of hydrogen peroxide [10], phosphatases [11] and Baeyer-Villigerases [12].

The 7-aminocoumarin derivatives **9-11** possessing 4-nitrobenzylcarbamate triggers were prepared by the method shown in Scheme 3. This route was chosen because the intermediate carbamate **8** could act as a precursor to a wide range of different coumarin derivatives in a Pechmann reaction [13]. Additionally, the majority of the nitro-compounds shown in Scheme 1 were prepared from 4-nitrosalicylaldehyde using a Knoevenagel condensation reaction to construct the coumarin ring system and the Pechmann route would therefore enable the preparation of an extended range



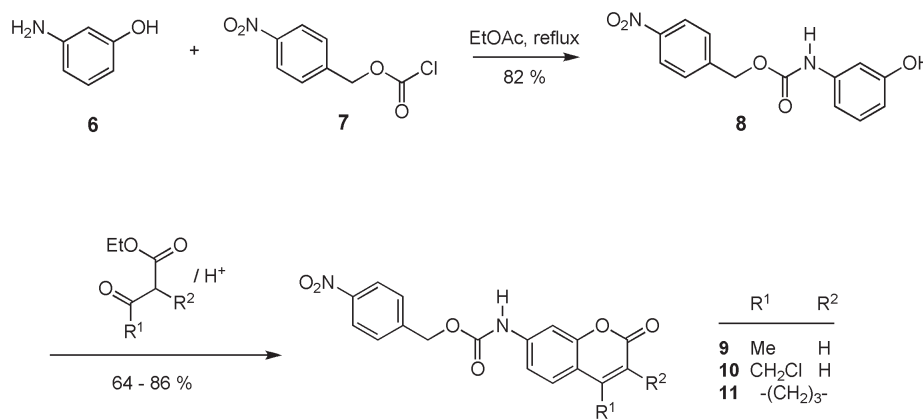
of potential nitroreductase substrates from readily available starting materials. Thus, 3-aminophenol **6** was reacted with 4-nitrobenzyl chloroformate **7** giving the carbamate derivative **8** in 82 % yield which has been previously obtained in 35 % yield [14]. Reaction of this carbamate **8** with the appropriate β -keto esters in the presence of 75 % sulphuric acid at room temperature afforded the coumarin derivatives **9** (73 %), **10** (86 %) and **11** (64 %). The carbamate **8** and all of the coumarin derivatives **9-11** had proton NMR spectral data, microanalytical and high resolution mass spectra consistent with their proposed structures.

spectra were determined at 270 MHz using a Jeol GX270 instrument. Elemental analyses were performed by the Department of Chemistry, University of Newcastle upon Tyne, UK. High resolution mass spectra were performed at the EPSRC national mass spectrometry service centre, Swansea, UK.

(3-Hydroxyphenyl)carbamic Acid 4-Nitrobenzyl Ester **8**.

To a boiling solution of 3-aminophenol **6** (1.0 g, 9.17 mmol) in ethyl acetate (10 mL) was added 4-nitrobenzyl chloroformate **7** (1.0 g, 4.65 mmol) in portions over 5 minutes. A white precipitate (3-aminophenol hydrochloride) formed as the 4-nitrobenzyl chloroformate was added. The mixture was stirred at reflux (5

Scheme 3



The nitroreductase substrates **9-11** (10 mg) were dissolved in *N*-methylpyrrolidinone (500 μ L) and the resulting solutions added to Columbia agar and then assessed against five organisms, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (MRSA). Substrates **9** and **11** did not produce any fluorescence in the presence of these organisms but compound **10** produced blue fluorescent colonies in the presence of *Serratia marcescens* and *Pseudomonas aeruginosa*. One of the consistent difficulties in working with these substrates is their inherent insolubility. Although they can be solubilized in *N*-methylpyrrolidinone, subsequent addition to the aqueous bacteriological media resulted in precipitation. Thus, we have been unable to produce at this stage quantitative data that demonstrates significant levels of fluorescence over and above those produced by substrate-free controls. Thus, the synthetic route to the coumarins described in this communication is now being applied to the production of coumarin derivatives that are expected to be more soluble in aqueous media.

EXPERIMENTAL

Infra-red spectra were recorded on a Perkin Elmer Paragon 1000 spectrophotometer using a diamond anvil. Proton NMR

(min), filtered while hot and the filtrate was allowed to cool to room temperature and then evaporated giving the carbamate **8** (1.1 g, 82 %) as a pale yellow solid, m.p. 170-172 °C (from methanol); lit. m.p. 168 °C [14]. Compound **8** had ir: 3354, 1706, 1600, 1558, 1511, 1439, 1346, 1238, 1212 and 1070 cm^{-1} ; ¹H-nmr: (DMSO-*d*₆): δ 9.76 (1H, broad s, >NH), 9.38 (1H, s, -OH), 8.24 (2H, d, *J* 8 Hz, Ar-H), 7.68 (2H, d, *J* 8 Hz, Ar-H), 7.02 (1H, t, *J* 8 Hz, Ar-H), 7.00 (1H, s, Ar-H), 6.84 (1H, d, *J* 8 Hz, Ar-H), 6.39 (1H, d, *J* 8 Hz, Ar-H), 5.28 (2H, s, >CH₂).

Anal. Calcd. for C₁₄H₁₂N₂O₅: C, 58.4; H, 4.1; N, 9.7. Found: C, 58.3; H, 4.2; N, 9.7 %.

Coumarins **9-11**: General Procedure.

In a typical procedure a mixture of the carbamate **8** (1.0 mmol), the β -keto ester (1 mL) and 75 % sulphuric acid (1 mL) were stirred at room temperature for 2 days. The mixture was diluted with water and the solid was collected and washed several times with water and then with ether giving the products **9-11**.

(4-Methyl-2-oxo-2H-chromen-7-yl)-carbamic Acid 4-Nitrobenzyl Ester **9**.

Compound **9** (73 %) had: m.p. >250 °C (from warm *ca.* 100 °C DMF); ir: 3266, 1703, 1583, 1520, 1348, 1232, 1203 and 1064 cm^{-1} ; ¹H-nmr: (DMSO-*d*₆): δ 10.42 (1H, broad s, >NH), 8.29 (2H, d, *J* 8 Hz, Ar-H), 7.72 (3H, 2 x overlapping d, *J* 8 Hz, Ar-H), 7.56 (1H, d, *J* 1 Hz, Ar-H), 7.42 (1H, dd, *J* 8 and 1 Hz, Ar-H), 6.26 (1H, s, Ar-H), 5.36 (2H, s, >CH₂) and 2.39 (3H, s, -CH₃); ms: m/z (electrospray positive) calcd. for C₁₈H₁₅N₂O₆ (M+H): 355.0925. Found: 355.0931.

Anal. Calcd. for $C_{18}H_{14}N_2O_6$: C, 55.6; H, 3.4; N, 7.2. Found: C, 55.3; H, 3.5; N, 7.1 %.

(4-Chloromethyl-2-oxo-2H-chromen-7-yl)-carbamic Acid 4-Nitrobenzyl Ester **10**.

Compound **10** (86 %) had: m.p. >250 °C (from warm *ca.* 100 °C DMF); ir: 3277, 1704, 1623, 1528, 1505, 1350, 1334 and 1236 cm^{-1} ; 1H -nmr: (DMSO- d_6): δ 10.47 (1H, broad s, >NH), 8.28 (2H, d, *J* 8 Hz, Ar-H), 7.78 (1H, d, *J* 8 Hz, Ar-H), 7.71 (2H, d, *J* 8 Hz, Ar-H), 7.59 (1H, d, *J* 1 Hz, Ar-H), 7.43 (1H, dd, *J* 8 and 1 Hz, Ar-H), 6.51 (1H, s, Ar-H), 5.33 (2H, s, >CH₂) and 4.98 (2H, s, >CH₂); ms: m/z (electrospray positive) calcd. for $C_{18}H_{17}^{35}ClN_3O_6$ (M+NH₄): 406.0804. Found: 406.0800.

Anal. Calcd. for $C_{18}H_{13}ClN_2O_6$: C, 61.0; H, 4.0; N, 7.9. Found: C, 60.9; H, 4.0; N, 7.85 %.

(2-Oxo-2H-cyclopentane[*c*]chromen-7-yl)-carbamic Acid 4-Nitrobenzyl Ester **11**.

Compound **11** (64 %) had: m.p. >250 °C (from warm *ca.* 100 °C DMF); ir: 3280, 1701, 1625, 1516, 1342 and 1238 cm^{-1} ; 1H -nmr: (DMSO- d_6): δ 10.40 (1H, broad s, >NH), 8.29 (2H, d, *J* 8 Hz, Ar-H), 7.71 (2H, d, *J* 8 Hz, Ar-H), 7.61 (1H, d, *J* 1 Hz, Ar-H), 7.56 (1H, d, *J* 8 Hz, Ar-H), 7.42 (1H, dd, *J* 8 and 1 Hz, Ar-H), 5.35 (2H, s, >CH₂), 3.05 (2H, t, *J* 7 Hz, -CH₂CH₂CH₂-), 2.73 (2H, t, *J* 7 Hz, -CH₂CH₂CH₂-) and 2.10 (2H, m, -CH₂CH₂CH₂-). ms: m/z (electrospray positive) calcd. for $C_{20}H_{17}N_2O_6$ (M+H): 381.1081. Found: 381.1081.

Anal. Calcd. for $C_{20}H_{16}N_2O_6$: C, 63.2; H, 4.2; N, 7.4. Found: C, 63.1; H, 4.3; N, 7.5 %.

Acknowledgement.

We thank the EPSRC mass spectrometry service for high reso-

lution mass spectra. We also thank Mr A. M. Lawrie for assistance in part of this project.

REFERENCES AND NOTES

- [1] A. L. James, J. D. Perry, C. Jay, D. Monget, J. W. Rasburn and F. K. Gould, *Letters in Appl. Microbiol.*, **33**, 403 (2001).
- [2] B. M. Sykes, M. P. Hay, D. Bohinc-Herceg, N. A. Helsby, C. J. O'Connor and W. A. Denny, *J. Chem. Soc., Perkin Trans 1*, 1601 (2000) and references therein.
- [3] P. L. Carl, P. K. Chakravarty and J. A. Katzenellenbogen, *J. Med. Chem.*, **24**, 479 (1981).
- [4] A. B. Mauger, P. J. Burke, H. H. Somani, F. Friedlos and R. J. Knox, *J. Med. Chem.*, **37**, 3452 (1994).
- [5] M. P. Hay, W. R. Wilson and W. A. Denny, *Bioorg. Med. Chem. Lett.*, **5**, 2829 (1995).
- [6] M. Tercel, W. A. Denny and W. R. Wilson, *Bioorg. Med. Chem. Lett.*, **6**, 2741 (1996).
- [7] M. Lee, J. E. Simpson, S. Woo, C. Kaenzig, G. M. Anlezark, E. Eno-Amooquaye, and P. J. Burke, *Bioorg. Med. Chem. Lett.*, **7**, 1065 (1997).
- [8] Q. Zhu, D. L. Uttamchandani, M. L. Lesaicherre and S. Q. Yao, *Org. Lett.*, **5**, 1257 (2003).
- [9] G. B. Jones, L. Xie, A. El-Shafey, C. F. Crasto, G. L. Bubleby and A. V. D'Amico, *Bioorg. Med. Chem. Lett.*, **14**, 3081 (2004).
- [10] L.-C. Lo and C.-Y. Chu, *Chem. Commun.*, 2728 (2003).
- [11] Q. Zhu, X. Huang, G. Y. J. Chen and S. Q. Yao, *Tetrahedron Lett.*, **45**, 707 (2004).
- [12] M. C. Gutierrez, A. Slegers, H. D. Simpson, V. Alphan and R. Furstoss, *Org. Biomol. Chem.*, **1**, 3500 (2003).
- [13] S. Sethna and R. Phadke, *Org. React.*, **7**, 1 (1953); G. P. Romanelli, D. Bennardi, D. M. Ruiz, G. Baronetti, H. J. Thomas and J. C. Autino, *Tetrahedron Lett.*, **45**, 8935 (2004).
- [14] E. R. Bissell, *Synthesis*, 846 (1982).