ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

The synthesis and structure revision of NSC-134754†

Cite this: *Chem. Commun.*, 2014, **50**, 1238

Received 28th October 2013,
Accepted 5th December 2013

DOI: 10.1039/c3cc48189a

www.rsc.org/chemcomm

Jennie A. Hickin,[a] Afshan Ahmed,[b] Katharina Fucke,[c] Margaret Ashcroft[b] and Keith Jones*[a]

The synthesis of emetine analogue NSC-134754, a potent inhibitor of the HIF pathway, has been accomplished and its structure reassigned. The stereochemistry of NSC-134754 has been assigned for the first time using X-ray crystallography and it has been demonstrated that only one diastereoisomer is active against HIF.

The emetine group of alkaloids, including non-natural derivative dehydroemetine **2** (Fig. 1), has a long and distinguished history of biological activity.[1,2] The closely related compound NSC-134754 **3** (Fig. 1) was first reported in the literature in 1971 where it was tested as part of a study into the effect of drugs related to (−) emetine on the lifespan of leukemic mice[3] and on protein synthesis in rat liver.[4] In these reports NSC-134754 was found to be inactive compared to (±)-2,3-dehydroemetine. This observation was supported in 1981 when NSC-134754 was tested alongside (−)-emetine and other analogues in Chinese hamster ovary cells.[5] More recently, NSC-134754 was identified as an inhibitor of hypoxia inducible factors (HIFs).[6,7] The HIFs are transcription factors that play a central role in tumour progression and metastasis and have been widely explored as a target for anti-cancer therapies.[8,9] Indeed, blocking HIF using NSC-134754 has been shown to significantly reduce recurrence of glioblastoma after irradiation in mice.[10]

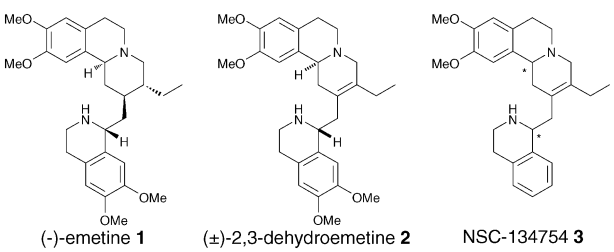


Fig. 1 Emetine and analogues.

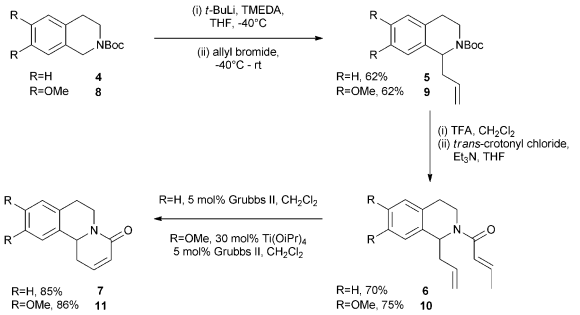
Although NSC-134754 is part of the National Cancer Institute (NCI) diversity set of compounds,[11] a synthetic route to this compound has not been previously reported. Analysis of a sample of NSC-134754 obtained from the NCI showed the compound to be a racemic mixture but a single diastereoisomer. The structure of NSC-134754 has been reported twice as a single diastereoisomer with the same stereochemistry as (±)-dehydroemetine **2**.[3,5] However, no proof of this stereochemistry has been described and its stereochemistry is not recorded in the NCI database.[11] We aimed to develop a synthetic route to both diastereoisomers of NSC-134754 and analogues in order to test their ability to inhibit HIF activity. Classical routes to emetine and dehydroemetine commonly involve Pictet–Spengler or Bischler–Napieralski reactions to form the key carbon–carbon bonds, relying on the presence of electron-donating aromatic substituents.[12] Owing to the lack of these substituents on one of the tetrahydroisoquinoline moieties in NSC-134754 and the desire to make the synthesis amenable to analogue generation, an alternative route was required.

We envisaged that α,β-unsaturated lactams such as **11** and its unsubstituted analogue **7** (Scheme 1) would be useful key intermediates in the synthesis of NSC-134754 and analogues as they could potentially be elaborated *via* conjugate addition and electrophile trapping.[13] Current routes to this type of tricyclic intermediate only exist for the methoxy-substituted analogue **11** and either rely on the electron-donating substituents on the aromatic ring to proceed or involve multiple steps, resulting in a low overall yield.[13,14]

A new route to α,β-unsaturated lactams **7** and **11** was developed employing directed lithiation and Ring-Closing Metathesis (RCM) (Scheme 1). *N*-Boc tetrahydroisoquinoline **4** was lithiated[15] and the resulting anion was treated with allyl bromide to yield **5**. *N*-Deprotection followed by acylation was used to give the diene precursor **6** for ring-closing metathesis. *trans*-Crotonoyl chloride was used for the acylation as the terminal methyl group was found to lead to a higher yield for this reaction, without having an adverse effect on the RCM. Diene **6** was efficiently converted to tricycle **7** in

[a] *CRUK Cancer Therapeutics Unit, The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5NG, UK. E-mail: keith.jones@icr.ac.uk*
[b] *Metabolism and Experimental Therapeutics, Division of Medicine, University College London, 5 University Street, London, WC1E 6JF, UK*
[c] *Department of Chemistry, Durham University, University Science Laboratories, South Road, Durham, DH1 3LE, UK*

† Electronic supplementary information (ESI) available. CCDC 968373. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3cc48189a



Scheme 1 Synthesis of the tricyclic core.

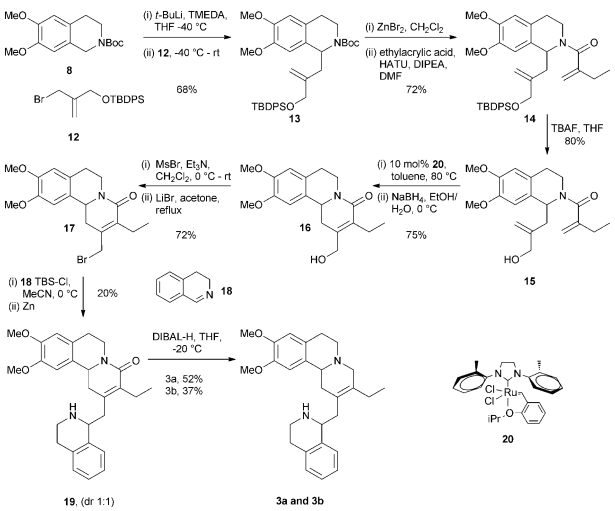
85% yield using Grubbs II catalyst. Synthesis of methoxy-substituted tricycle **11** was achieved using the same route starting from tetrahydroisoquinoline **8**, however the yield for the RCM was only 67%. Use of a $Ti(OiPr)_4$ additive was found to increase the yield to 86%.[16] The synthesis of both the dimethoxy-substituted and the unsubstituted tricyclic core was achieved in 33% and 26% overall yield respectively and on multi-gram scale.

Unfortunately, although intermediates **7** and **11** could be elaborated to generate analogues of NSC-134754 *via* conjugate addition, attempts to generate NSC-134754 itself using this route were unsuccessful. A new strategy was developed involving directed lithiation and RCM to install the tetrasubstituted double bond (Scheme 2).

Directed lithiation of tetrahydroisoquinoline **8** using our established conditions followed by reaction with protected bromo alcohol **12** was successful in 68% yield to give **13**. *N*-Boc deprotection in the presence of the silicon protecting group proved to be problematic. We were most successful using a hindered protecting group (TBDPS) and the mild removal of the Boc group with zinc bromide.[17] The resulting amine was directly acylated using ethyl acrylic acid to give diene **14**. After removal of the silicon protecting group, RCM was investigated. Following catalyst screening, recently developed catalyst **20** (Scheme 2)[18] was found to carry out this transformation in good yield. Surprisingly, the corresponding tricyclic aldehyde was formed as a side product (10%) in this reaction.[19] Nevertheless, the aldehyde could be quantitatively reduced to alcohol **16** using sodium borohydride, giving an overall yield of 75% for the formation of the tetrasubstituted double bond by RCM. Bromination of alcohol **16** proceeded smoothly to give tricyclic bromide **17**, which was used to investigate introduction of the tetrahydroisoquinoline moiety.

Our initial strategy for introduction of the tetrahydroisoquinoline was to employ the directed lithiation of tetrahydroisoquinoline **4**, followed by reaction with tricyclic bromide **17**. However, this reaction was unsuccessful under a wide range of conditions. A variety of transmetallations of the lithiated species were investigated along with a range of leaving groups to replace the bromide, with no success. Given this lack of reactivity, we next investigated forming the required carbon–carbon bond *via* generation of a reactive metal species from bromide **17** and its addition to 3,4-dihydroisoquinoline **18**. Both Grignard formation from bromide **17** and lithium–halogen exchange were unsuccessful, leading to a variety of unwanted side products. This problem was overcome by employing Barbier-type conditions in which a zinc species was formed from bromide **17** in the presence of the dihydroisoquinoline electrophile. TBS-Cl was found to be essential for the success of this reaction, possibly due to the ability of this group to activate the imine towards nucleophilic attack.[20] The desired carbon–carbon bond was formed in moderate yield (20%) and as expected a 1 : 1 mixture of diastereoisomers was obtained. The diastereoisomers were separated by column chromatography and reduction of the amide using diisobutylaluminium hydride led to both diastereoisomers of NSC-134754, **3a** and **3b** in 9 steps and 2% overall yield. However, comparison of the NMR data for the original sample of NSC-134754 obtained from the NCI with the synthetic samples **3a** and **3b** revealed that spectra for neither synthetic diastereoisomer matched with the original sample. Furthermore, both synthetic diastereoisomers were unable to inhibit HIF activity in a HRE-luciferase reporter assay (data shown in Fig. 4), leading to the conclusion that the reported structure for NSC-134754 was incorrect.

Similarities in the NMR data indicated that both the synthetic and NCI samples must have closely related structures and both had identical masses by high-resolution mass spectrometry. We hypothesized that NSC-134754 is actually a regioisomer, **21**, of the reported structure with the methoxy groups at the 6′ and 7′ positions on the tetrahydroisoquinoline (Fig. 2). This was supported by the differing nOes observed in both samples (Fig. 2). Further investigation revealed that this regioisomer **21** is also part of the NCI database with compound number NSC-134756.[11] A sample of NSC-134756 was obtained from the NCI and was shown to have the same structure as the sample of NSC-134754 by mixed NMR experiments.



Scheme 2 Synthesis of NSC-134754.

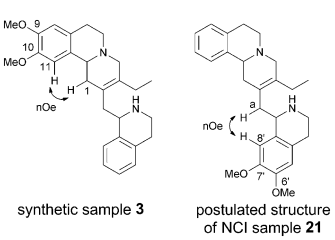
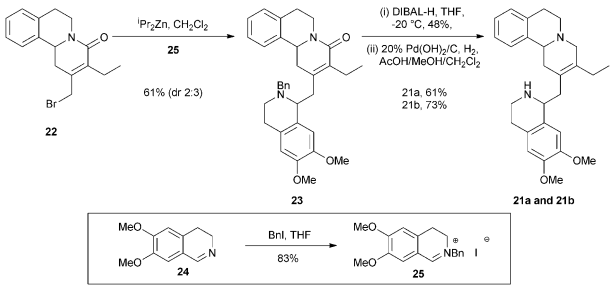


Fig. 2 Postulated structure of NSC-134754 and observed nOes.



Scheme 3 Synthesis of the reassigned structure.

To prove conclusively the postulated structure of NSC-134754, the synthesis of both diastereoisomers of the regioisomer **21** was carried out. We envisaged that this could be achieved using the previously developed chemistry starting from Boc-protected tetrahydroisoquinoline **4**. The required tricyclic bromide **22** was successfully synthesized in 12% yield in 6 steps using the same chemistry employed in the original synthesis. Unfortunately, the zinc-mediated reaction to introduce the tetrahydroisoquinoline was not successful using 6,7-dimethoxydihydroisoquinoline **24**. We postulated that this was due to the electron-donating methoxy groups reducing the electrophilicity of the dihydroisoquinoline. This problem was overcome by the formation of an iminium salt from dihydroisoquinoline **24**[21] and best results were seen using the benzyl iodide salt **25** (Scheme 3).

The zinc-mediated reaction was improved further by replacing solid zinc, which was found to be capricious, with a dialkylzinc reagent. Initially diethylzinc was used but addition of the ethyl group to **25** was found to compete with the desired reaction. This unwanted reaction was avoided by using the more sterically hindered diisopropylzinc and the required carbon–carbon bond was formed in 61% yield. Amide reduction and benzyl deprotection proceeded smoothly to give both diastereoisomers of the reassigned structure **21a** and **21b** in 10 steps. Although an extra deprotection step was required in this synthesis, the overall yield was maintained at 2% due to the improved yield of the zinc-mediated reaction.

NMR data for one of the synthetic diastereoisomers was identical to the NCI sample, proving that the correct structure of NSC-134754 is structure **21**, a regioisomer of the reported structure. The stereochemistry of NSC-134754 was also assigned for the first time by X-ray crystallography (Fig. 3, formula $C_{27}H_{36}N_2O_2{\cdot}H_2O{\cdot}HSO_4{\cdot}0.5SO_4$) and shown to be the same as for (±)-2,3-dehydroemetine **2**.

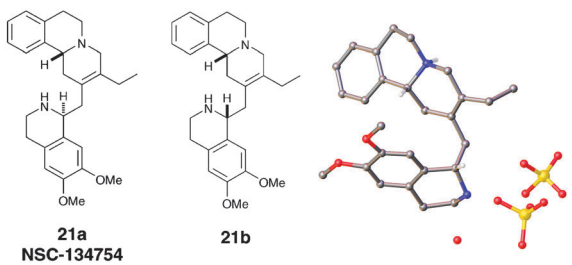


Fig. 3 Stereochemistry of NSC-134754 and X-ray crystal structure (relative stereochemistry of the cation in the asymmetric unit is SRS at N1, C13, C17 respectively, see ESI† for ORTEP plot).

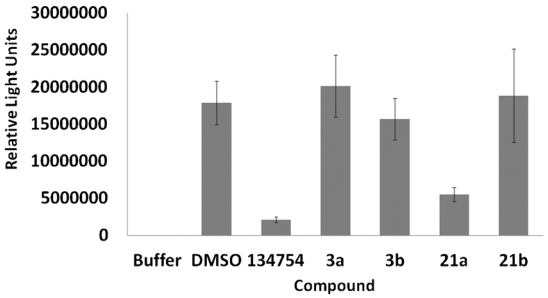


Fig. 4 Luciferase assay-U2OS-HRE-Luc cells,[6] 1% O_2 (16 h), compounds at 5 μM.

Both diastereoisomers of each regioisomer synthesized were tested for their HIF inhibitory activity using our previously described HRE luciferase reporter assay[6] (Fig. 4). Only **21a** was shown to exhibit inhibitory activity, demonstrating the importance of both the position of the methoxy groups and the stereochemistry for activity.

In summary, we have synthesized a compound with the reported structure of the HIF inhibitor NSC-134754 using an RCM reaction to prepare a key tricyclic intermediate and an allylzinc addition to a dihydroisoquinoline to complete the pentacycle. Both diastereoisomers of this compound **3a,b** are inactive. This has led to the reassignment of the structure of NSC-134754 and we further developed the allylzinc chemistry to prepare this compound **21a,b**. One of the diastereomers, **21a**, was shown to possess HIF inhibitory properties. Furthermore, we have shown that NSC-134754 and NSC-134756 have the same structure, **21a**. Our synthetic route will allow the synthesis of a variety of novel analogues to further investigate the mechanism of action of this interesting class of compounds.

Notes and references

1 W. Weigrebe, W. J. Kramer and M. Shamma, *J. Nat. Prod.*, 1984, **47**, 397.
2 R. Knight, *J. Antimicrob. Chemother.*, 1980, **6**, 577.
3 W. R. Jondorf, B. J. Abbott, N. H. Greenber and J. A. R. Mead, *Chemotherapy*, 1971, **16**, 109.
4 J. D. Donahue, R. K. Johnson and W. R. Jondorf, *Br. J. Pharmacol.*, 1971, **43**, P456.
5 R. S. Gupta, J. J. Krepinsky and L. Siminovitch, *Mol. Pharmacol.*, 1980, **18**, 136.
6 N. M. Chau, P. Rogers, W. Aherne, V. Carroll, I. Collins, E. McDonald, P. Workman and M. Ashcroft, *Cancer Res.*, 2005, **65**, 4918.
7 V. A. Carroll and M. Ashcroft, *Cancer Res.*, 2006, **66**, 6264.
8 E. Poon, A. L. Harris and M. Ashcroft, *Expert Rev. Mol. Med.*, 2009, **11**, 26.
9 G. L. Semenza, *Oncogene*, 2010, **29**, 625.
10 M. Kioi, H. Vogel, G. Schultz, R. M. Hoffman, G. R. Harsh and J. M. Brown, *J. Clin. Invest.*, 2010, **120**, 694.
11 http://dtp.cancer.gov/dtpstandard/dwindex/index.jsp.
12 M. Shamma, *The Isoquinoline Alkaloids*, 1972, p. 426.
13 E. Garcia, E. Lete and N. Sotomayor, *J. Org. Chem.*, 2006, **71**, 6776.
14 S. M Allin, D. G. Vaidya, S. L. James, J. E. Allard, T. A. D. Smith, V. McKee and W. P. Martin, *Tetrahedron Lett.*, 2002, **43**, 3661.
15 G. M. Coppola, *J. Heterocycl. Chem.*, 1991, **28**, 1769.
16 A. Furstner and K. Langemann, *J. Am. Chem. Soc.*, 1997, **119**, 9130.
17 T. J. Donohoe, R. E. Thomas, M. D. Cheeseman, C. L. Rigby, G. Bhalay and I. D. Linney, *Org. Lett.*, 2008, **10**, 3615.
18 I. C. Stewart, T. Ung, A. A. Pletnev, J. M. Berlin, R. H. Grubbs and Y. Schrodi, *Org. Lett.*, 2007, **9**, 1589.
19 G. R. A. Adair and J. M. J. Williams, *Tetrahedron Lett.*, 2005, **46**, 8233.
20 C. Jahangir, D. B. Maclean, M. A. Brook and H. L. J. Holland, *J. Chem. Soc., Chem. Commun.*, 1986, 1608.
21 T. Shono, H. Hamaguchi, M. Sasaki, S. Fujita and K. J. Nagami, *J. Org. Chem.*, 1983, **48**, 1621.

