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Novel nitroimidazole alkylsulfonamides as hypoxic cell radiosensitisers

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ABSTRACT

A novel class of nitroimidazole alkylsulfonamides have been prepared and evaluated as hypoxia-selective cytotoxins and radiosensitisers. The sulfonamide side chain markedly influences the physicochemical properties of the analogues: lowering aqueous solubility and raising the electron affinity of the nitroimidazole group. The addition of hydroxyl or basic amine groups increased aqueous solubility, with charged amine groups contributing to increased electron affinity. The analogues covered the range of electron affinity for effective radiosensitisation with one-electron reduction potentials ranging from −503 to −342 mV. Cytotoxicity under normoxia or anoxia against a panel of human tumour cell lines was determined using a proliferation assay. 2-Nitroimidazole sulfonamides displayed significant hypoxia-selective cytotoxicity (6 to 64-fold), while 4- and 5-nitroimidazole analogues did not display hypoxia-selective cytotoxicity. All analogues sensitised anoxic HCT-116 human colorectal cells to radiation at non-toxic concentrations. 2-Nitroimidazole analogues provided modest sensitisation due to the relatively low concentrations used while several 5-nitroimidazole analogues provided equivalent sensitisation to misonidazole and etanidazole at similar molar concentrations.

1. Introduction

Fractionated radiotherapy (FRT) is one of the main treatments for cancer patients with over 50% of patients receiving FRT (typically 60–70 Gy delivered in 2–3 Gy fractions over 7–8 weeks), mostly with curative intent.[1] FRT is a difficult regimen for both health service delivery and patient compliance and often normal tissue toxicity precludes delivery of sufficient doses of radiation to achieve local tumour control. Stereotactic body radiotherapy (SBRT) uses hypofractionated (1–5 doses) high dose (25–60 Gy total dose) radiation to treat tumours.[2] This new approach leverages recent advances in the accuracy and precision of radiation delivery to allow dose intensification to small tumours while minimising the effects to adjacent normal tissue. Clinical trials using SBRT to treat various solid tumours have demonstrated comparable control, toxicity and efficacy profiles to FRT.[2] The reduced treatment time and number of patient visits, combined with emerging potential to replace surgery with an outpatient procedure, indicates substantial health, social and economic advantages for SBRT and is driving increasing use of SBRT for treating cancer.

However, evidence is emerging that SBRT accentuates the role of hypoxia in radioresistance.[3,4] Hypoxia, which is a consequence of the inefficient vascularisation of tumours, contributes to altered tumour metabolism,[5] invasion,[6] and metastasis,[7] and is associated with poor prognosis and resistance to therapeutic agents.[8] Hypoxia is prevalent in a wide range of solid tumours[9,10] and patients with hypoxic tumours have significantly poorer outcomes than those with lower levels of hypoxia.[11,12] The significance of hypoxia in resistance to cytotoxic therapy has renewed interest in targeting these cells.[13] Hypoxic cells are less sensitive to radiation-induced DNA breakage, because oxygen is not available to oxidise radiation-induced DNA radicals to generate strand breaks.[14]

One approach to increase radiation response in hypoxic tumours is to use nitroimidazole radiosensitisers.[14] These radiosensitisers are relatively non-toxic molecules which react with radiation-induced DNA radicals and cause DNA strand breaks analogously to oxygen. They have to be present at the time of irradiation and are mechanistically distinct from hypoxia-selective cytotoxins which are enzymatically reduced under hypoxia to generate a cytotoxic moiety.[13] Misonidazole (**1**) (Fig. 1) was extensively trialled with FRT in the clinic and despite indications of

Abbreviations: DCM, dichloromethane; $E(1)$, one-electron reduction potential; FCS, foetal calf serum; FRT, fractionated radiotherapy; HCR, hypoxic cytotoxicity ratio; NCS, *N*-chlorosuccinimide; SAR, structure activity relationship; SBRT, stereotactic body radiotherapy.

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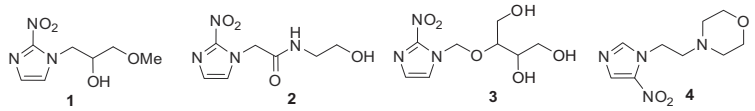


Figure 1. Clinically evaluated nitroimidazole radiosensitisers.

clinical benefit in some trials, failed to provide significant improvement on radiotherapy alone, with delayed peripheral neuropathy limiting treatment.[15] Attempts to design more polar analogues[16] with increased systemic clearance to minimise neurotoxicity[17] were only partially successful with etanidazole (**2**) failing to provide benefit in head & neck cancer.[18,19] A similar approach with the more polar doranidazole (**3**)[20] is currently being clinically evaluated. Although a recent meta-analysis has confirmed the clinical activity of nitroimidazole radiosensitisers,[21] only nimorazole (**4**) is in clinical use.[22] Nonetheless, the recent description of a hypoxic gene signature,[23] that in a retrospective study identifies head & neck cancer patients with hypoxic tumours and predicts the benefit of nimorazole in only those patients, has provided clinical validation of the use of nitroimidazole radiosensitisers with radiotherapy.[24] However, existing agents have no or limited intellectual property protection which restricts options for clinical development.

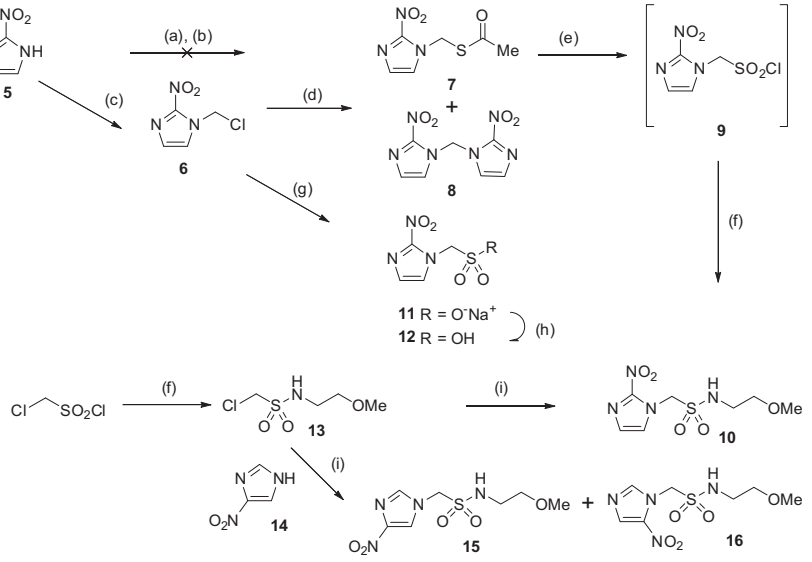
The advent of SBRT, combined with new approaches to identify patients with hypoxic tumours, heralds a new opportunity for the use of novel nitroimidazole radiosensitisers. We have addressed this opportunity and identified a class of nitroimidazole that incorporates an alkylsulfonamide moiety as a key element, with the objective of developing improved, novel radiosensitisers for SBRT. Here, we report the synthesis of a pilot set of compounds and in vitro appraisal of their potential as hypoxia-selective radiosensitisers. We also evaluate their hypoxia-selective cytotoxicity in culture as a measure of their sensitivity to bioreductive activation.

2. Results

2.1. Chemistry

Our synthetic strategy was directed towards nitroimidazoles bearing an alkyl sulfonamide as a moiety conferring both novelty and a strong modulating effect on the electron affinity of the nitroimidazole moiety. We initially explored a strategy previously used to prepare alkylaryl sulfonamides,[25] generating chloromethylthioacetate in situ to alkylate 2-nitroimidazole (**5**), but this failed to provide the key sulfonyl chloride **9** in our hands (Scheme 1). To avoid the use of the volatile chloromethylthioacetate we attempted a stepwise strategy to prepare **9**. Alkylation of **5** with bromochloromethane under a variety of conditions was explored; however formation of the dimer **8** predominated under most conditions. Optimum results were obtained using Cs_2CO_3 in the presence of an excess (20 equiv) of bromochloromethane giving chloride **6** which was condensed with potassium thioacetate to give thioester **7** in modest yield. Oxidation of **7** with NCS, gave the unstable sulfonyl chloride **9**. Efforts to isolate **9** resulted in significant loss of material and so **9** was used directly in subsequent reactions. Reaction of crude sulfonyl chloride **9** with 2-methoxyethylamine gave sulfonamide **10** in low yield. In an effort to avoid the unstable intermediates **7** and **9** we prepared the sulfonate salt **11** and sulfonic acid **12**. Attempts to prepare sulfonamides from either **11** or **12** with an array of coupling reagents (oxalyl chloride, EDCI, HOBt, HBTU, HATU) using multiple reaction conditions failed to provide positive results. We explored an alternative approach to **9** by condensing chloromethanesulfonyl chloride with 2-methoxyethylamine to give sulfonamide **13** which was used to alkylate 2-nitroimidazole. Although only providing a similar yield to the oxidative route, this approach was more direct and also provided access to the isomeric analogues **15** and **16**, albeit in low yield.

The direct oxidative approach provided low yields of **17**, reflecting losses of material in the aqueous workup, however use of a protected ethanolamine **18** gave no overall improvement in yield of **17** (Scheme 2). An alternative alkylation strategy using the protected chlorosulfonamide **19** provided a modest improvement in the yield of **20**, and subsequently **17**.



Scheme 1. Reagents and conditions: (a) $ClCH_2Br$, potassium thioacetate, MeCN; (b) iPr_2NEt, NaI, MeCN; (c) $ClCH_2Br$, Cs_2CO_3, DMF, 45%; (d) potassium thioacetate, DMF, 42%; (e) NCS, 2 M aq HCl, DCM; (f) $MeOCH_2CH_2NH_2$, Et_3N, DCM, 16% from **6**; (g) Na_2SO_3, acetone/H_2O; (h) DOWEX50 WX8, water, 87%; (i) **5** (18%) or **14** (7% and 3%), Cs_2CO_3, DMF.