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Original article

Synthesis, radioiodination and *in vivo* screening of novel potent iodinated and fluorinated radiotracers as melanoma imaging and therapeutic probes

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ABSTRACT

In order to develop new iodinated and fluorinated matched-pair radiotracers for Single-Photon Emission Computed Tomography (SPECT)/Positron Emission Tomography (PET) imaging and targeted radionuclide therapy of melanoma, we successfully synthesized and radiolabelled with iodine-125 seven new derivatives, starting from our previously described lead structure **3**. The relevance of these radiotracers for gamma scintigraphic imaging of melanoma in rodent was assessed. The tumoural radioactivity uptake was most often high and specific even at early time points (12.1–18.3% ID/g at 3 h p.i. for [^{125}I]**39**–**42**) and a fast clearance from the non-target organs was observed. Also, calculated effective doses that could be delivered to tumours when using corresponding [^{131}I]-labelled analogues were generally higher than 100 cGy/MBq injected (98.9–150.5 cGy/MBq for [^{131}I]**39**–**42**). These results make compounds **39**–**42** suitable candidates for (i) PET imaging of melanoma after labelling with fluorine-18 and (ii) targeted radionuclide therapy of disseminated melanoma after labelling with iodine-131.

1. Introduction

In contrast to many other malignancies, the incidence of melanoma, the most lethal form of skin cancer, is steadily increasing worldwide [1–4]. In the United States the estimated lifetime risk of developing cutaneous melanoma is currently 1 in 58 overall against 1 in 250 in 1980 [5,6]. Survival largely depends on the stage of detection [7,8]. At the earliest invasive stages, melanoma is usually cured by surgical excision and the estimated 5-year overall survival rate is 97% [6]. Unfortunately, this cancer can rapidly spread to almost all the organs, and for patients with advanced-stage disease, the survival rate decreases dramatically (12% at 5 years) [9]. This poor prognosis is due to the ineffectiveness of existing chemo- or immunotherapies [6,9,10]. In addition, although promising results in terms of responses to treatment are obtained with new targeted therapy approaches, median progression-free survival is still disappointingly low [11–13]. Powerful tools for both early detection and new efficient treatment strategies of melanoma are therefore urgently needed.

An interesting area of melanoma research is the development of radiopharmaceuticals specific to this pathology. Among all the

Abbreviations: DAFBA, *N*-(2-diethylaminoethyl)-4-fluorobenzamide; DAST, (diethylamino)sulphur trifluoride; DME, dimethoxyethane; ESI-MS, electrospray ionization mass spectra; ID, injected dose; i.v., intravenous injection; PET, positron emission tomography; p.i., post injection; RCP, radiochemical purity; RCY, radiochemical yield; ROI, region of interest; RP-HPLC, reverse-phase high performance liquid chromatography; SA, specific activity; SPECT, single-photon emission computed tomography; TBDMSCl, *tert*-butyldimethylsilyl chloride; TBAF, tetrabutylammonium fluoride.

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approaches investigated, melanins are relevant molecular targets due to the capacity of these amorphous, irregular natural polymers to bind with many drugs, and especially those with coplanar fused aromatic rings [14]. Based on the work done on the benzamide family [15–20], recent studies using radiohalogenated heteroaromatic analogues have revealed that this class of compounds is suitable for positron emission tomography (PET) imaging (e.g. [^{18}F]**1**, [^{18}F]DAFBA [21–26]) or targeted radionuclide therapy (e.g. [^{131}I]**2**, [^{131}I]MIP-1145) of melanoma (Fig. 1) [27–30]. These findings prompted us to investigate a new approach consisting in using iodinated and fluorinated matched-pair radiotracers targeting melanins and offering potential for both diagnosis *via* single-photon emission computed tomography (SPECT, iodine-123) or PET imaging (iodine-124 or fluorine-18), and therapy (iodine-131), depending on the type of radionuclide introduced on the same chemical scaffold [31].

Fourteen iodinated compounds bearing a 2- or 6-fluoropyridine moiety on the *N*,*N*-diethylethylenediamine side chain were recently synthesized, radiolabelled with iodine-125 and screened by gamma scintigraphic imaging to evaluate their *in vivo* biodistribution profiles in B16F0 primary melanoma-bearing mice [31,32]. This screening allowed the selection of the tracer **3** (Fig. 1) with high, specific and long-lasting tumoural uptake. This compound was then radiolabelled with fluorine-18 in a three-step procedure. A first PET imaging experiment using the same murine model confirmed the promising results obtained by gamma scintigraphic imaging, and the utility of combining such tracer specificity with the performance of PET technology. Compound **3** was finally radiolabelled with iodine-131 and evaluated in B16F0 primary melanoma-bearing mice for a first targeted radionuclide therapy assay. This treatment induced a significant tumoural growth inhibition. Promising properties of **3** radiolabelled with either fluorine-18 or iodine-131 gave a first validation of our concept and underscored the potential of this class of iodinated and fluorinated matched-pair radiotracers for both diagnosis and targeted radionuclide therapy of melanoma [32]. To further improve pharmacokinetic profiles of the tracers, and facilitate radiofluorination processes, we planned to extend this strategy to fluoroaliphatic derivatives of the lead compound **3** as depicted in Fig. 2. Here we describe the synthesis, and iodine-125 radiolabelling of these new analogues and their preliminary *in vivo* screening in a melanoma-bearing mice model.

2. Results and discussion

2.1. Chemistry

The synthesis of seven new derivatives showing structural similarity to **3** was undertaken, based on the replacement of the (2-fluoropyridin-3-yloxy)ethyl moiety by various fluoroaliphatic groups (Fig. 2). As a first approach, we designed compounds bearing saturated fluoroalkyl chains. In parallel, and keeping in mind that haloalkylamines can undergo intramolecular cyclisation [33,34], we synthesized more constrained derivatives containing fluoroallyl and fluoropropargyl synthons, together with pegylated analogues enlarging the distance between the fluorine atom and the tertiary amine. Finally, we explored the Huisgen 1,3-dipolar cycloaddition between azides and alkynes, known as "click" chemistry, which is recognized as a powerful synthetic methodology allowing rapid and convenient radiofluorinations [35].

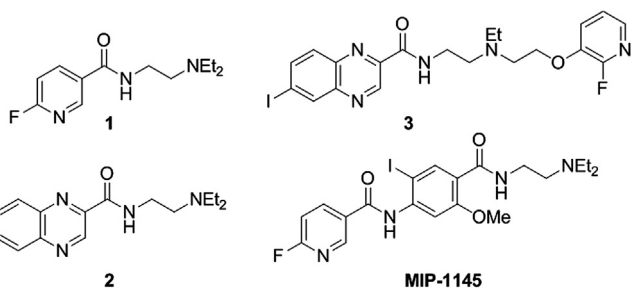

Fig. 1. Several benzamide derivatives developed for imaging and/or targeted radionuclide therapy of melanoma.

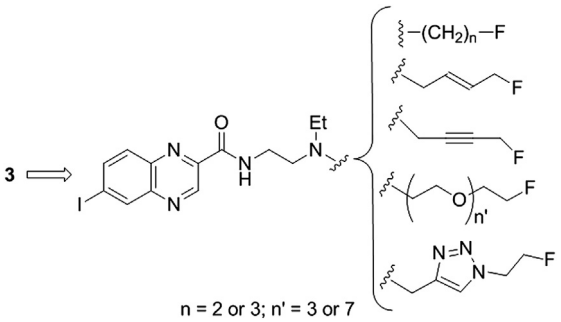

Fig. 2. Pharmacomodulation of the fluoro side chain of **3**.

The synthesis of compounds **8** and **14**, bearing fluoroethyl or fluoropropyl side chains respectively, is illustrated in Scheme 1. First, alcohol **4** [32] was converted into the fluorinated compound **5** using (diethylamino)sulphur trifluoride (DAST) at −50 °C. Deprotection of the phthalimide **5** was achieved by the action of hydrazine monohydrate to give unstable primary amine **6**, which was rapidly condensed with *p*-nitrophenyl 6-iodoquinoxaline-2-carboxylate (**7**) [36] to provide the desired amide **8**. A modified synthetic pathway was designed to produce the fluoropropyl derivative **14** in order to introduce the fluorine atom as late as possible in the synthesis. Phthalimide **9** [37] was alkylated with commercially available (3-bromopropoxy)-*tert*-butyldimethylsilane to afford compound **10**. In a similar manner as for **6**, phthalimide **10** was then deprotected to give primary amine **11** in quantitative yield and coupled to the activated ester **7** to provide amide **12**. Desilylation of **12** using tetrabutylammonium fluoride (TBAF) afforded alcohol **13**, which was finally converted into the desired fluorinated compound **14** following the protocol optimized for **5**. We note that due to its instability, tracer **14** has to be synthesized and purified just before use.

To synthesize amides **17** and **20** (Scheme 2) we investigated a direct alkylation of *N*-[2-(ethylamino)ethyl]-6-iodoquinoxaline (**15**) [36] using either (*E*)-4-fluoro-but-2-enyl toluene-4-sulfonate (**16**) [38] or 4-fluoro-but-2-ynyl toluene-4-sulfonate (**19**). The latter was produced *via* a two-step reaction sequence according to the procedure developed for compound **16** involving ditosylation [39] of commercially available 1,4-butynediol, and subsequent monofluorination [38] of **18** with TBAF in refluxing tetrahydrofuran. This strategy afforded amides **17** and **20** with moderate yields of 47% and 38% respectively. The (*E*)-configuration of final alkene **17** was extrapolated from the vicinal spin–spin coupling constant of 15.8 Hz between the two olefinic protons of the corresponding dihydrochloride salt **39**.

Pegylated derivatives **33** and **34** were synthesized by a common strategy outlined in Scheme 3. Commercially available tetra- and octaethylene glycols were first monoprotected using *tert*-butyldimethylsilyl chloride (TBDMSCl) according to a slightly modified protocol developed by Kan and co-workers [40]. Iodination of the remaining free alcohol function of **21** and **22**, in the presence of triphenylphosphine and imidazole, afforded derivatives **23** and **24**. Subsequent nucleophilic substitution using phthalimide **9** [37] provided intermediates **25** and **26**. Under similar reaction conditions (temperature and reaction time), we observed that two equivalents of potassium carbonate were required to obtain **26** in