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Research Article

Synthesis of 1-[4-(m-tolyl)amino-6-quinazolinyl]-3-[^{14}C]-methyl triazene: a radiolabeled probe for the combi-targeting concept

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Summary

The synthesis of 1-[4-(*m*-tolyl)amino-6-quinazolinyl]-3-[^{14}C]-methyl triazene (SMA41) is described. This triazene was designed to be hydrolyzed under physiological conditions to N^4-*m*-tolyl-quinazoline-4,6-diamine (SMA52), a moderate inhibitor of the epidermal growth factor receptor (EGFR) and the DNA alkylating species [^{14}C]-methyldiazonium. A radiolabeled probe was needed to test the hypothesis that *in situ* hydrolysis of SMA41 may induce alkylation of the ATP binding site of EGFR. ^{14}C-SMA41 was obtained with a radiochemical yield of 21% and a specific activity of 54.6 mCi/mmol, as determined by HPLC quantitation and scintillation counting. Radio-TLC analyses showed 98% radiochemical purity. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: ^{14}C-Labeled SMA41; EGFR tyrosine kinase inhibitor; alkyltriazene; synthesis

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Introduction

Recently, we reported a novel tumor targeting strategy termed the 'Combi-targeting Concept'.[1–4] This novel approach was based on the fundamental premise that molecules designed to simultaneously damage DNA and block the tyrosine kinase activity of oncoreceptors involved in cell signaling may show superior antiproliferative effects when compared with classical single-targeted antitumor drugs. In addition, we surmised that if the molecules are designed to heterolyze to a secondary inhibitor of the same target, more sustained antitumor effects may be induced. To verify these postulates, we designed SMA41 to target both genomic DNA and EGFR, a transmembrane tyrosine kinase whose overexpression is associated with aggressive tumor progression and poor prognosis.[5–7] SMA41 has been shown to: (a) block EGF-induced EGFR autophosphorylation on its own, (b) degrade to SMA52, another inhibitor of EGFR, (c) significantly damage DNA, and (d) induce 8-fold greater antiproliferative activity against EGFR-expressing tumor cells than its single-targeted DNA-damaging counterpart temozolomide.[2] In order to verify whether SMA41 not only damages DNA but also alkylates the EGF receptor, we recently attempted to ^{14}C-label the methyl group of the triazene moiety. As outlined in Figure 1, decomposition of SMA41 (3) would lead to the incorporation of ^{14}C into the released methyldiazonium ion. Here we report the synthesis and analysis of the first example of radiolabeled molecular probe of the 'Combi-targeting' strategy.

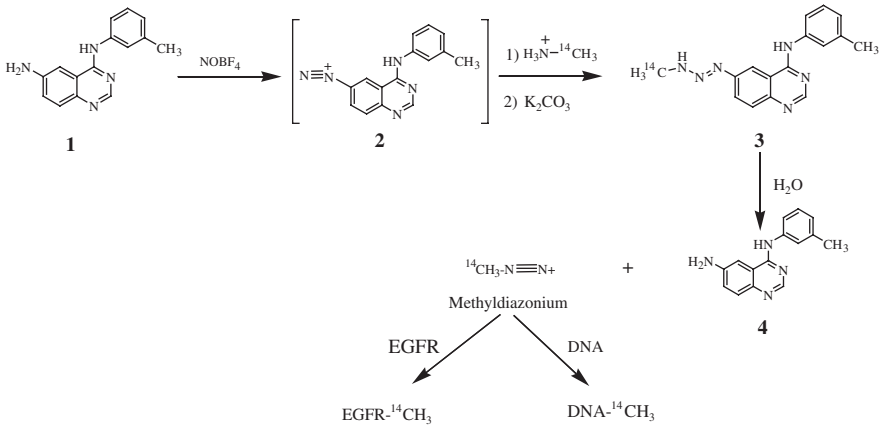

Figure 1. Synthesis of ^{14}C-labeled SMA41 (3)

Results and Discussion

The synthesis of both labeled and unlabeled SMA41 proceeded as outlined in Figure 1. Aminoquinazoline **1,** obtained as previously described,[8] was treated in acetonitrile with $NOBF_4$ at 0°C to provide the diazonium salt **2**. Treatment of **2** with aqueous methylamine in situ followed by raising the pH of the resulting mixture with potassium carbonate gave the desired quinazolinyl triazene **3** (SMA41) in good yield. Previous analysis demonstrated that in a cell culture medium, SMA41 can be converted to quinazoline diamine **4** in an 80% yield with a half-life of approximately 34 min as determined by UV analysis.[3]

The synthesis of the ^{14}C-labeled SMA41 proceeded in a similar fashion. To circumvent the difficulties associated with the handling of a suspension of $NOBF_4$ pellets in acetonitrile at micro-scale, the diazotization was performed at a 100 mg scale and a fraction of this solution (1.1 ml) was injected into an ampoule containing solid $^{14}CH_3NH_3^+ Cl^-$. SMA41 was obtained by subsequent injection of 100 µl K_2CO_3 (1 g/ml) into the ampoule. TLC (60/40 ethylacetate/hexane) showed a spot with $R_f = 0.37$, identical to that observed for an unlabeled SMA41 standard. It is important to note that due to the instability of triazenes of the same class as SMA41 under acidic conditions,[9,10] no acid was used in the micro-scale radiosynthesis.

As depicted in Figure 2, further analysis of the TLC plate with a Packard Canberra InstantImager, revealed a 98% radiochemical purity (see peak 1) with a minor impurity (2%) (see peak 2). HPLC analysis showed a peak at 9.6 min with an R_t identical to that of standard SMA41. To minimize on-column degradation, the pH of the mobile phase was adjusted to 8. A specific activity of 54.6 mCi/mmol was obtained using liquid scintillation counting of 5 µl aliquots of a ^{14}C-labeled SMA41 solution.

The level of specific radioactivity was sufficient to allow satisfactory analysis using 100 µl of a 0.1 mg/ml solution of SMA41. For example, when the EGFR overexpressing carcinoma of the vulva cell line A431 was exposed to ^{14}C-labeled SMA41 for 2 h at 37°C, the bound radioactivity was distributed through DNA, RNA and protein with counts in the 5000–18 000 cpm range. Results from the macromolecular distribution studies will be reported elsewhere.

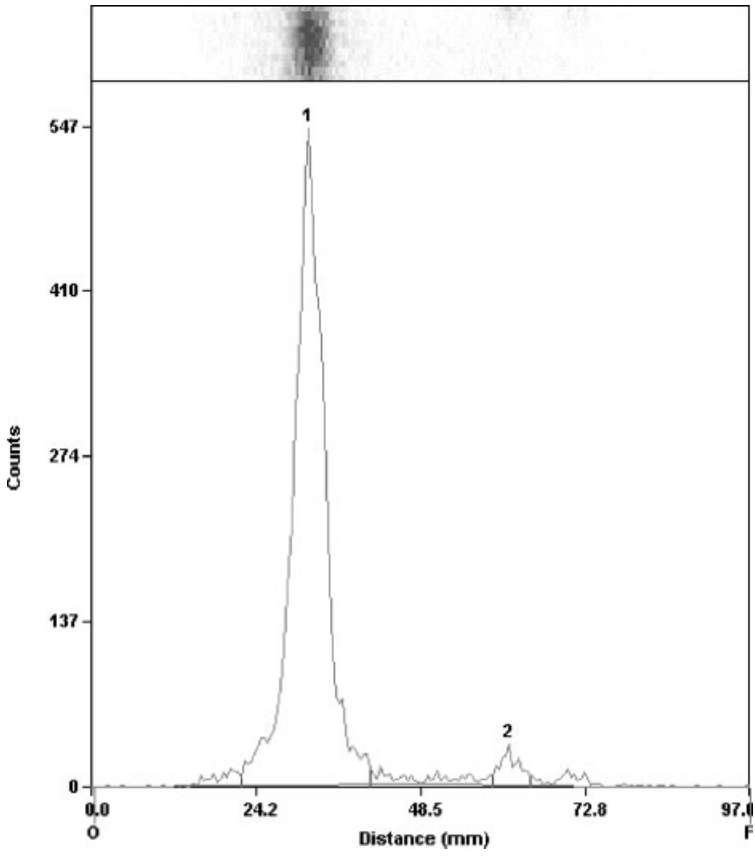

Figure 2. Radio-TLC of ^{14}C SMA41 eluted with a solution of 40% hexane in ethyl acetate

Experimental

2-Aminoquinazoline **1** was synthesized in our laboratory by known methods.[8] ^{14}C-methylamine hydrochloride (56 mCi/mmol) was purchased from Amersham (Piscataway, NJ). Acetonitrile and K_2CO_3 were purchased from Fisher Scientific (Nepean, ON, Canada) and $NOBF_4$ from Sigma-Aldrich (Oakville, ON, Canada).

Synthesis of Unlabeled SMA41

To a solution of 6-amino-4-[(*m*-tolyl)amino]quinazoline (1 g, 4 mmol) in acetonitrile (50 ml) 5 ml of acetic acid was added. The mixture was kept at 0°C for 1 h after which a suspension of $NOBF_4$ (2 equiv.) in

acetonitrile was added dropwise. A 40% aqueous methylamine solution (1 ml) was added and the resulting reddish solution neutralized by addition of saturated sodium carbonate until a two-phase mixture was formed. Ethyl acetate (200 ml) was added and the aqueous layer removed. The resulting pale brown ethyl acetate solution was dried over anhydrous potassium carbonate and evaporated under vacuum to give a brown oil which was re-dissolved in a minimum volume of the same solvent. Hexane was added by portion until a persistent pale brown precipitate was formed. The mixture was filtered and the resulting precipitate collected by filtration, and dried under vacuum to provide the monoalkyltriazene **3** (SMA41) as a pale brown powder (0.7, 60%): mp 75–80°C (dec.); ^{1}H NMR (DMSO) δ 10.67 (br q, 1 H, $NHCH_3$), 9.74 (s, 1 H, NH), 8.51 (s, 2 H, H2), 8.47 (s, 2 H, H5), 7.9 (d, 1 H, $J = 9$, H7), 7.70 (br s d, 3 H, H2', H6', H8, overlap), 7.24 (t, 1 H, $J = 7.5$ Hz, H5'), 6.90 (d, 1 H, $J = 7.5$ Hz, H4'), 3.07 (d, 3 H, $J = 4$, $HNCH_3$), 2.31 (s, 2 H, $ArCH_3$); ^{13}C NMR 158.4, 154.1, 149.9, 148.7, 140, 138.1, 129.4, 128.879, 125.3, 124.8, 123.7, 123.4, 120.0, 116.4, 115.3, 31.3, 21.9; FABMS M + 1 (I%) 293 (43), 250 (14), 234 (12).

Synthesis of ^{14}C-Labeled SMA41

Compound **1** (100 mg, 25 mmol) was dissolved in acetonitrile (33 ml) and cooled on ice to 0°C for 10 min. To this solution, a suspension of $NOBF_4$ (93.4 mg, 10.9 mmol) in acetonitrile (3 ml) was added dropwise. A fraction of the mixture (1.1 ml) was injected into previously cooled ^{14}C-methylamine hydrochloride (0.3 mg, 4.3 μmol, 250 μCi) and the mixture was made alkaline by subsequent addition of 100 μl of K_2CO_3 (1 g in 1 ml distilled water). The acetonitrile layer was removed, evaporated, and the resulting residue was redissolved in ether. This SMA41-containing solution was dried over anhydrous K_2CO_3, evaporated, and the resulting residue reconstituted in dry acetonitrile. The solution was stored at –20°C until further use. SMA41 was characterized by co-chromatography with an unlabeled sample of SMA41 on an aluminum oxide TLC plate and by HPLC. The yield was approximately 60% as estimated by HPLC quantitation. Caution! Triazenes degrade on silica gel plates and under acidic conditions.

Radiochemical purity

The radiochemical purity was determined by performing TLC on an aluminum oxide plate using a solution of 40% hexane in ethyl acetate.

The plate was subsequently scanned in a Packard Canberra InstantImager radioanalyzer. Percent purity was calculated by determining the percentage of radioactivity in the peak corresponding to SMA41 divided by the total radioactivity of the plate using the Packard InstantImager software package.

Specific Radioactivity

The specific radioactivity was measured by first determining the concentration of the ^{14}C-labeled SMA41 solution. This was obtained from a calibration curve established with a series of known concentrations of unlabeled SMA41. The specific radioactivity was determined by dividing radioactivity obtained from liquid scintillation counting of 5 µl aliquots of a ^{14}C-labeled SMA41 solution by the HPLC-determined concentration.

The HPLC conditions were as follows: C4 Deltapak column (300×3.9 mm, 15 µm), with a mobile phase of methanol/H_2O = 7:3, pH 8, at a flow rate of 0.5 ml/min, and detection at 254 nm. The injection volume was 5 µl.

Acknowledgements

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