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A BORONATED BENZAMIDE AS MELANOMA-SEEKING AGENT

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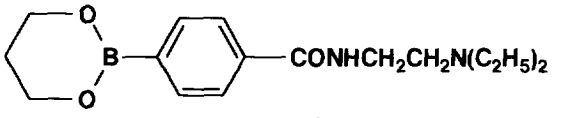
Abstract : A boronated benzamide which accumulates in melanin-rich tissues in mice is presented.

A prerequisite for boron neutron capture therapy (BNCT) is the selective incorporation of boron-10 in malignant cells.[1,2] A boronated analogue of L-tyrosine, coined 4-borono-L-phenylalanine (BPA), has been tested *in vivo* by Mishima's group in Japan,[3] and the BNCT cures obtained for melanoma brought this compound under clinical evaluation in Europe and Usa. Meanwhile, there has been a general search for new vectors presenting a selective delivery of boron to cancerous tissues and compounds such as boronated antibodies, thiouracil derivatives or other biomolecules have been considered. [2]

Recent work established N-alkyl substituted iodobenzamides as suitable radiopharmaceuticals for the scintigraphic detection of melanoma; [4-8] the synthesis of a boronated analogue based on the tumor-seeking properties of benzamides has been undertaken in our laboratories and we now disclose the preparation and biological evaluation of a boronated benzamide, **1**.[9] which has shown a specific uptake in melanin-rich tissues.



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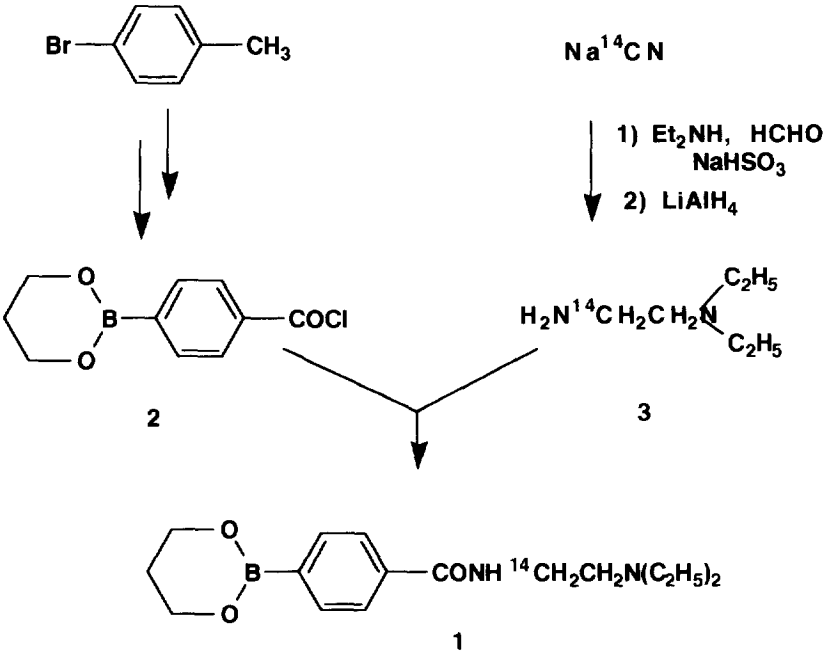
The synthesis of ^{14}C-**1** was realized by coupling the dioxaborinane **2**, previously prepared [10] from p-toluoylboronic acid,[11] with N,N-diethyl-1,2-diaminoethane, ^{14}C-**3**; the latter has been obtained by a Mannich reaction of commercially available ^{14}C-sodium cyanide with diethylamine and formaldehyde,[12] followed by reduction. **3** was then condensed [9] with **2** to afford ^{14}C-labelled **1** (specific activity = 275 MBq / mmol).



The biological evaluation was performed after *i.v* caudal injection of ^{14}C-**1**, HCl in B 16 xenografted mice and its biodistribution [13] is reported in the accompanying table. It can be seen that a significant uptake (*ca.* 3 % of the injected dose after 3 hours) of **1** is observed in both melanin-rich uvea and B 16 melanomic cells, which remain the sole boronated tissues after 24 hours, except for excretatory organs. It should be noted that blood clearance occurs rapidly and that, on the other hand, **1** does not cross significantly the blood-brain barrier ; also of interest is the low acute toxicity of **1** : LD_{50} = 1.86 mmole / kg in OF1 mice.

These data reveal that boronated benzamides can be considered as potential boron-carriers in tumors such as melanoma.

Table : Biodistribution of ^{14}C-1 in B16 melanoma bearing mice (% injected dose/g ; mean ± SD) after i.v. administration (2.6 μmol/mice).

TIME AFTER INJECTION	1 H	3 H	24 H
Melanoma	3.26 ± 0.34	2.89 ± 0.91	0.52 ± 0.22
Brain	0.19 ± 0.05	0.18 ± 0.04	
Liver	5.11 ± 1.08	4.76 ± 0.79	0.22 ± 0.02
Muscle	2.95 ± 0.44	1.96 ± 0.58	0.04
Lungs	1.91 ± 0.44	1.24 ± 0.23	
Spleen	2.61 ± 0.22	1.53 ± 0.27	
Kidney	4.88 ± 0.95	3.38 ± 0.19	0.13 ± 0.01
Blood	2.53 ± 1.34	0.97 ± 0.12	
Uvea		2.78 ± 0.31	0.84 ± 0.39

The semi-convergent scheme which has been adopted for the preparation of **1** is flexible enough for modifications: preparation of ^{10}B-enriched material (the natural abundance of 10-boron being 19.8 %) can be realized when necessary and, on the other hand, this scheme makes possible variations of substituents on either the boronic acid or amino moieties. Indeed, if benzamides lipophilicity is thought to be important , an increase of the lipophily in **1** (log P = -1) might afford additional tumor-seeking properties since other benzamides of much higher lipophilicity, such as p-iodobenzamide derivatives[4-8] (log P = 1) have yielded tumor-imaging agents.[14]

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9. To a solution of 2-(4-carboxyphenyl)-1,3,2-dioxaborinane [10] (1.8 g - 8.74 mmol) in dry chloroform (10 mL) were added under stirring thionyl chloride (5 mL) and N,N-dimethylformamide (200 µL) and the mixture was heated at 80 °C for 90 min. After cooling and concentration under vacuum, dry chloroform (10 mL) was added followed, under stirring, by N,N-diethyl-1,2-diaminoethane (1.066 g -9.17 mmol - 1.05 eq.) in chloroform (10 mL) then triethylamine (2.5 mL - 17.9 mmol - 2.05 equiv.). After 1 hr, the volatiles were removed and base (pH 11) then acid (pH 2) extraction with CH_2Cl_2 was performed. The remaining aqueous layer was adjusted to pH 7, concentrated to dryness and the residue taken-up in CH_3OH. After filtration, volatiles were removed and the residue now taken up in AcOEt. After drying and evaporation, **1** was obtained by recristallisation (2 crops) : 2.08 g (78 %). M.p. : 184-6 °C (EtOH/Et_2O); ^{1}H-nmr (300 MHz-$CDCl_3$) : 8.75 (b, 1H, NH); 8.0 and 7.8 (AA'-XX' system, $J_{app.}$= 7, 4H , aromatics); 4.15 (t, J= 5, OCH_2); 3.9 (q, J= 6, 2H, $NHC\underline{H}_2$); 3.25 (t, J=6, 2H, $C\underline{H}_2N(C_2H_5)_2$; 3.15 (q, J=6, 4H, $NC\underline{H}_2CH_3$); 2.05 (quintet, J=5, 2H, $OCH_2C\underline{H}_2$); 1.35 (t, J=6, 6H, CH_3).^{13}C-nmr (75 MHz-CD_3OD) : 171.1 (CO); 136.0 ($\underline{C}$-CO); 134.8 and 127.4 (CH's); 63.2 (CH_2O); 52.7; 47.8 and 36.3 ($\underline{C}H_2N$); 28.5 ($\underline{C}H_2CH_2O$); 9.3 (CH_3); due to quadrupolar relaxation, the carbon attached to boron is not detected.

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