Preliminary radiosynthesis study of the novel ^{131}I-labelled 1,2,3-dithiazole-based radioligand for imaging of L-amino acid transporters (LAT1)

M. Pocięgiel, J. Pijarowska-Kruszyna, A. Jaroń, P. Garnuszek, R. Mikołajczak

National Centre for Nuclear Research, Radioisotope Centre POLATOM, Otwock, Poland

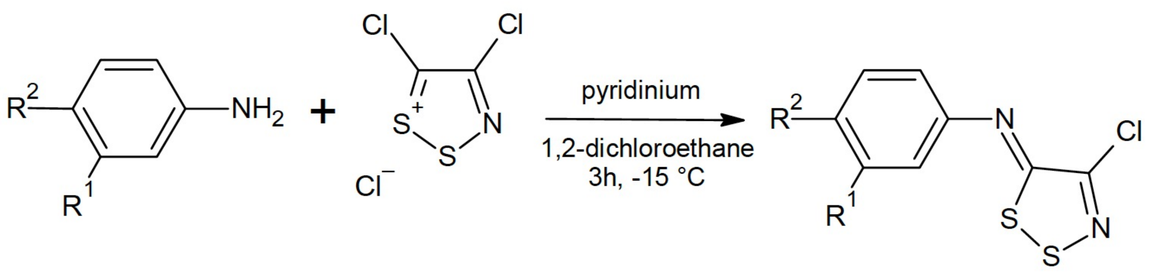
AIM

The L-type amino acid transporter (LAT1) is considered as pharmacological target for cancer. This system is highly expressed in many types of cancer and contributes to tumor growth and survival [1,2]. Recently, the new inhibitors of the human LAT1 transporter based on the 1,2,3-dithiazole scaffold were reported [3]. In this study, fluorine and iodine-containing compounds with high inhibition of LAT1 (>90%): 4-chloro-N-(3-fluorophenyl)-5H-1,2,3-dithiazol-5-imine (3DFA), 4-chloro-N-(4-fluorophenyl)-5H-1,2,3-dithiazol-5-imine (4DFA), 4-chloro-N-(3-iodophenyl)-5H-1,2,3-dithiazol-5-imine (3DIA) and 4-chloro-N-(4-iodophenyl)-5H-1,2,3-dithiazol-5-imine (4DIA) were selected as potential radioligand. The specific aim of this work was to developed an efficient and convenient method for radiosynthesis of ^{131}I-agents. We hypothesize that ^{131}I-compounds could be synthesized at high yield as a result of Cu(II) catalyzed aromatic nucleophilic substitution (S_NAr) reaction using iodine-131 from the appropriate precursors based on boronic acid pinacol esters (3ABAPE and 4ABAPE).

MATERIALS AND METHODS

SYNTHESIS OF PRECURSOR

The synthetic approach was adopted based upon the published procedures [3,4], with some modifications. The essential feature of this path was the reaction of a primary aromatic amine with 4,5-dichloro-1,2,3-dithiazolium chloride (Appel's salt). The target precursors 4-chloro-*N*-(3-pinacolboronatephenyl)-*5H*-1,2,3-dithiazol-5-imine (3ABAPE) and 4-chloro-*N*-(4-pinacolboronatephenyl)-*5H*-1,2,3-dithiazol-5-imine (4ABAPE) were obtained by condensation of Appel's salt with 3- or 4-aminophenylboronic acid pinacol esters.



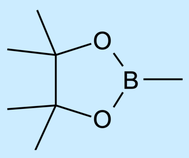
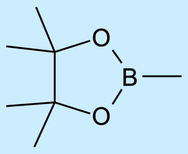
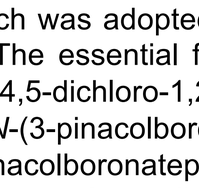
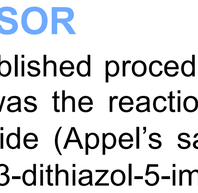
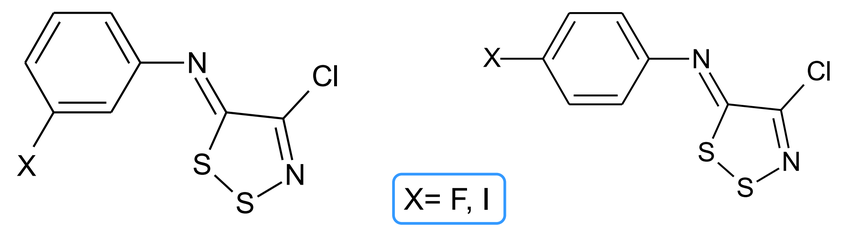
Substrate		Product		Yield [%]
R¹	**R²**	**R¹**	**R²**	
	H –		H –	70
		4-chloro-*N*-(3-pinacolboronatephenyl)-*5H*-1,2,3-dithiazol-5-imine (**3ABAPE**)		
H –		H –		68
		4-chloro-*N*-(4-pinacolboronatephenyl)-*5H*-1,2,3-dithiazol-5-imine (**4ABAPE**)		

Table 1. Synthesis of boronic acid pinacol esters as a precursors.

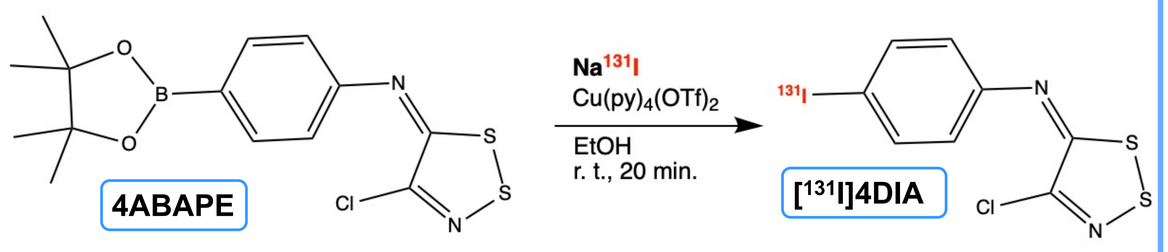
SYNTHESIS OF REFERENCE STANDARD (RS)

The non-radioactive derivatives as reference standards (3DFA, 4DFA, 3DIA, 4DIA) were synthesized in an analogous manner using 3- or 4-fluoroaniline or 3- or 4-iodoaniline as a substrate. The crude products were purified by flash chromatography and crystallized from pentane [3]. Overall production yield for synthesis standards were: 3DFA (90%), 4DFA (94%), 3DIA (97%), 4DIA (99%).



IODINE-131 RADIOSYNTHESIS

Copper mediated radiosynthesis using the obtained precursor 4ABAPE and iodine-131 was performed under mild conditions. The crude radioligand 4-chloro-N-(4-([^{131}I]iodophenyl)-5H-1,2,3-dithiazol-5-imine **([^{131}I]4DIA)** was initially purified using SiO_2 cartridge. Both analytical radio-TLC and HPLC analyses demonstrated high radiochemical purity (RCP) of more than 98% (n=3). The typical HPLC chromatograms of 131-iodine radioligand are shown in Scheme 2. The identity of [^{131}I]4DIA (Rt=7.48 min) was confirmed by co-injection with non-radioactive standard 4DIA (Rt=7.35 min). The results of RCP during radiosynthesis optimalization allow to start *in vitro* step.



Scheme 1. Radiosynthesis reactions of [^{131}I]4DIA.

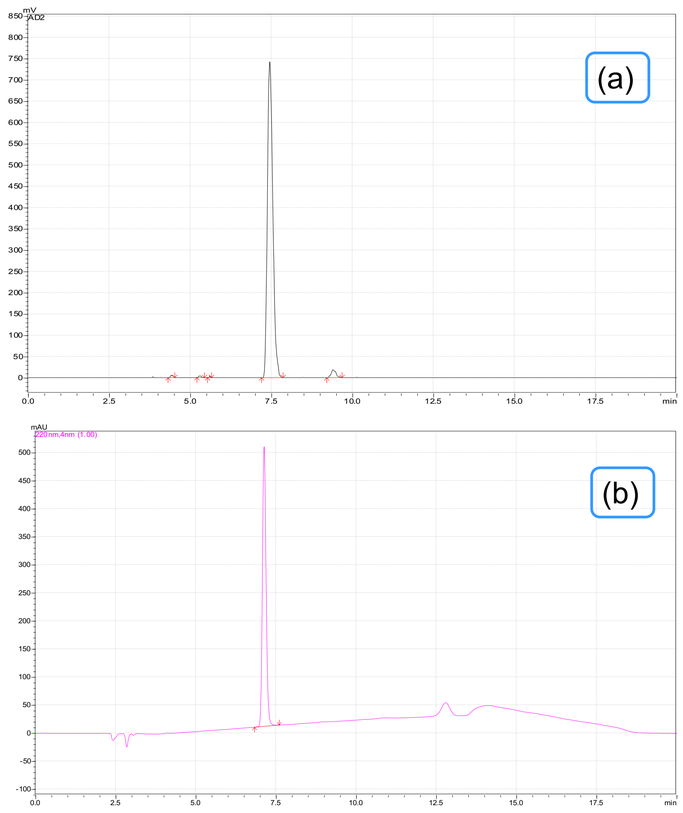
LIPOPHILICITY OF REFERENCE STANDARDS

- Lipophilicity of 3DFA and 4DFA was evaluated by using two methods. The octanol–water partition coefficient (logP) were quantified using the classic shake-flask method. After equilibration and phase separation, the aqueous and octanol phases of all samples were analyzed on HPLC. The logP values were calculated based on the evaluated concentration in the water and octanol phase.

- In addition, the $\log k_w$ was determined by HPLC method, by changing the content/gradient of methanol as the organic phase of the HPLC eluent, thus verifying and extending the information on compounds lipophilicity. Calculation of $\log k_w$ based on Synder-Soczewinski equation.

Compound	log P*	log k_w**
3DFA	2.66	3.84
4DFA	2.47	3.78

Table 2. Lipophilicity of reference standard: *determination by classic extraction method, **determination by HPLC MeOH method.



Scheme 2. HPLC chromatorgams of: (a) [^{131}I]4DIA radiodetector, (b) 4DIA UV/VIS detector.

Radio-HPLC

Column:
Luna C18 (2), 250x4.6 mm
Eluents:
A: 0.1% TFA in H_2O
B: 0.1% TFA in ACN
Gradient:

time (min.)	% B
0	80
10	95
15	80

λ = 220 nm
Flow rate: 1.0 mL/min
Temperature: 30 °C

Radio-TLC

Plates: silica gel 60
Eluents: dichlorometane

RESULTS AND CONCLUSIONS

Precursors for a potential radiopharmaceutical were obtained with high efficiency. Their identity was confirmed using NMR and MS spectroscopy. All synthetized compounds were chemically stable over time (stable for 22 months at 2-8 ºC). These results confirm their suitability for wider use in radiosynthesis. The logP parameter for lipophilicity with two methods: for 3DFA and 4DFA were 2.66 and 3.84, while for 4DFA they were 2.47 and 3.78, respectively. As for the lipophilicity, it was reported that compounds with 1 < logP < 3 are characterized by moderate lipophilicity and may be partially bioaccumulated [4]. The promising results of ^{131}I-iodination provide opportunities to further study develop this radiosynthesis which opens the path to *in vitro* research. Radiosynthesis occurs under mild conditions, with a short time and with high yield. The developed purification method gives high purity results confirmed by instrumental analysis.

BIBLIOGRAPHIC REFERENCES

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[3] M. Pociegiel et al., *WKNKO-1*, (2020).
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