N.C.A.Radioiodination of Idoxifene

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Summary: We describe a method for n.c.a. radioiodination of pyrrolidino tamoxifen (Idoxifene) based on the electrophilic destannylation of tributylstannyl pyrrolidino tamoxifen. The methods of separation using preparative tlc and HPLC give >95% radiochemical purity and >70% recovery.

Keywords: Tamoxifen, Idoxifene, estrogen receptors, radioiodination, electrophilic destannylation.

INTRODUCTION

Idoxifene, (E)-1-[4-[2-(N-pyrrolidino)ethoxy]-1-(4-iodophenyl)-2-phenyl-1-butene, is an analogue of the non-steroidal antiestrogen tamoxifen with an iodine atom in the 4-position and a pyrrolidino ethoxy side chain (Figure 1). The iodine atom at the 4-position enhances the binding affinity for the target estrogen receptor (ER) and also prevents metabolic 4-hydroxylation (1,2). Idoxifene is superior to tamoxifen for the treatment of breast cancer by virtue of the increased cytotoxicity (3), higher antagonism of

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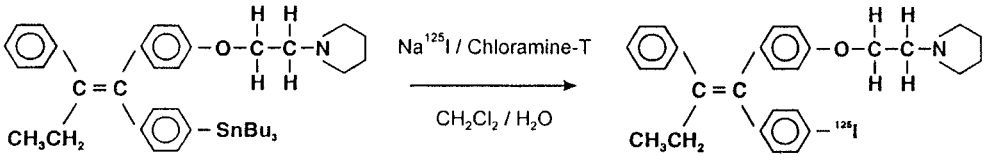

Precursor Idoxifene

calmodulin dependent processes (4) and lower partial agonist toxicity. The estrogen receptor to which idoxifene has high affinity binds directly to DNA, affecting both DNA synthesis and mitosis (5). It also binds to another biological site, which does not bind steroidal estrogens, known as the microsomal antiestrogen binding site (6). This additional binding may preclude the use of radioiodinated idoxifene for the targetted selective radiotherapy of estrogen receptor positive tissues. This aspect can be assessed by monitoring the precise biodistribution of the drug in humans. The presence of an iodine atom in idoxifene makes it an attractive drug for radiotracer studies, since the radioiodinated compound is structurally identical to the unlabelled compound and hence its biodistribution can be faithfully monitored. The availability of several radioisotopes of iodine can serve a variety of purposes. ^{123}I and ^{124}I labelled idoxifene can be used for SPECT and PET diagnostic imaging studies. The auger elecron emitting, longer half life (60 days) ^{125}I idoxifene can be used for preclinical evaluation.

Because of the low concentration of estrogen receptors in tumours, it is essential to use radioiodinated idoxifene of high specific radioactivity and high radiochemical purity. The tributyl stannyl pyrrolidino tamoxifen was considered to be a suitable precursor to introduce radioactive iodine by electrophilic destannylation using chloramine T as an oxidising agent(7). The procedure for the synthesis of 4-stannylated tamoxifen analogue (E)-1-[4-[2-(N-pyrrolidino)ethoxy]phenyl]-1-[4-(tributylstannyl)-2-phenyl-1-butene was published earlier(8).

MATERIALS AND METHODS

Tributyl stannyl pyrrolidino tamoxifen (precursor) was supplied by the Drug development section. Sodium metabisulphite($Na_2S_2O_5$), sodium fluoride(NaF), sodium chloride(NaCl) and ethanol(C_2H_5OH) were purchased from BDH plc.. Chloramine T(N-chloro-p-toluene-sulphonamide sodium salt), dichloromethane(CH_2Cl_2), diethyl ether[$(C_2H_5)_2O$], triethylamine $(C_2H_5)_3N$, hexane(C_6H_{14}), tetrahydrofuran(C_4H_8O)and acetonitrile(CH_3CN) of ANALAR grade were obtained from Aldrich Ltd.. All reagents were used as received. The no carrier added (n.c.a.) $Na^{125}I$ 100mCi/ml was supplied by ICN. All solutions were prepared using ultra pure water.

100µL (5µM) tributyl stannyl pyrrolidino tamoxifen (precursor) solution in ethanol was evaporated to dryness in the reacto-vial using compressed nitrogen and redissolved in 100µL dichloromethane. 50µL n.c.a. $Na^{125}I$ was added to it, followed by 10µL 5mM chloramine T solution. The reaction was allowed to proceed at room temperature for 30 minutes, with gentle stirring. The reaction was terminated by adding 10µL 2mM sodium fluoride solution and 10µL 10mM sodium metabisulphite solution, followed

by 200μL of saturated sodium chloride solution. The reaction product was extracted twice with dichloromethane and separated from the aqueous phase. The dichloromethane solution containing idoxifene (product) was washed twice with water, the aqueous phase was separated and discarded. The separation of precursor and the idoxifene was achieved on 500μm preparative PK6F silica gel plates supplied by Whatman International Ltd., using hexane:diethyl ether:triethyl amine (8:1:1) mixture as an eluent. The precursor and idoxifene were detected using UV at 254nm, at R_f 0.62 and 0.48 respectively. The silica layer containing idoxifene was scraped from the plate and washed twice with ethanol and filtered through a 0.2μm filter. The filtrate was evaporated to dryness using compressed nitrogen and stored at 4°C. The quality control of the final product was conducted using 250μm MK6F silica gel plate using above mentioned eluent and the tlc plates were monitored using Tracemaster 20, automatic tlc linear analyser supplied by Berthold(U.K.) Ltd. Radiochemical purity of >95% was obtained. Based on the total iodine, >70% of n.c.a. idoxifene was consistantly obtained. Similarly, using reverse phase tlc, MK $C_{18}F$ plates and a mixture of acetonitrile, tetrahydrofuran and triethylamine (8:2:0.1) as the eluent, precursor and idoxifene were detected at R_f 0.35 and 0.61 respectively. It was also possible to separate precursor and idoxifene using HPLC technique. This permitted larger amounts of radioiodine for labelling, reducing the manipulations involved in the procedure, avoiding the risk of aerial contamination and lowering the radiation exposure to the personnel. Using Alphasil 5μm normal silica 4.8mm x 250mm hplc column from HPLC Technology, equipped with Spectra physics SP 8000 system, SP 4290 integrator, Spectra 100 (UV and visible) variable wavelength detector monitoring at 254nm and Beckmann 170 radioisotope detector gave simultaneous detection of chemical and radiolabelled products

independently. The eluent consisted of hexane:diethyl ether and triethyl amine (8:1:0.1) at a flow rate of 1ml/min.. The precursor and idoxifene were detected at R_t 13.5 minutes and 18.8 minutes respectively. This was confirmed by comparing their elution times with their standard solutions.

RESULTS AND DISCUSSION

Preliminary work indicated that all solutions should be prepared fresh prior to use, as chloramine T solution loses its oxidising capacity on storage. It was realised that use of excess precursor was advisable to ensure maximum labelling. The use of high purity grade (ANALAR) reagents and ultra high purity grade water was essential to avoid formation of other competing anions arising from the impurities affecting the labelling adversely. The addition of sodium fluoride produces insoluble tri-n-butyltin fluoride and liberates the iodide or chloride into the aqueous phase(9). The washing of organic layer twice with water avoided subsequent formation of white precipitate which interefered with separation of idoxifene. It is worth noting that the performance of the silica column may deteriorate with use, because of triethylamine in the eluent. The labelling procedure and the separation must be performed in the fume cupboard designated for the radioactive work.

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