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Research Paper

Optimization of labeled ^{125}I- pyrimidine derivative and its biological evaluation

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Abstract

This study describe the organic synthesis of 2-iodobenzamido (2-N-nitrobenzen-5, 6, 7, 8 tetrahydrobenzothieno [2, 3-d]) pyrimidine -4-(3H) one as an example for some pyrimidine derivative used a new series of as potential cancer chemotherapeutic agents. The precursor derivative is α-(2-iodobenzamido)-β - (4-nitrophenyl)-N- [3-ethoxy-carbonyl-4, 5, 6, 7-tetrahydrobenzothiophen-2-l] acrylic acid amide which react with hydrazine hydrate. The purification process was done via crystallization using solvent ethanol. The overall yield 78% the structure of the synthesized compound was confirmed by correct analytical and spectral data .Also, The synthesized compound was labeled with radioactive iodine -125 via nucleophilic substitution reaction ,in the presence cuprous chloride, the labeling process was carried out at 95oC for 60 min. the radiochemical yield was determined by using thin layer chromatography and the yield is equal to 80%.Preliminary in-vivo study was examined in normal mice were performed after intravenous injection through the tail vein and the data show the labeling compound was cleared quickly from most body organs. The radioiodinated compound showed high brain uptake .The results in this study suggest that radioiodinated pyrimidine derivative may be useful as cancer chemotherapeutic agents.

Keywords: *Pyrimidine Derivatives / Iodine -125/ Tissues Distribution.*

1. Introduction

Pyrroles and Pyrimidine derivatives are an important class of heterocyclic compounds having different biological activities(1). Therefore, many methods for the synthesis of substituted Pyrroles have been described in the literature (1, 2). Design and synthesis of thieno [2, 3-d] pyrimidine as potential cancer chemotherapeutic agents have been extensively studied (4-10).Recently, some new pyridine, pyrimidine and their derivatives have been synthesized and used as analgesic, anticonvulsant and anti-parkinsonian agents (11-14). Catalysts are used to improve the radiochemical yield and decrease the reaction time in isotopic exchange reaction especially in radioiodination reactions with the short lived ^{123}I isotope. Copper compounds have been successfully employed for promoting nucleophilic substitution reactions in non-activated aromatic compounds which lake electron withdrawing substituents. Copper (I) salts have proven to be useful for the catalysis of isotopic exchange reactions in the synthesis of radioiodinated N-isopropyliodoamphetamine, m-iodobenzylguanidine, o-iodohipuric acid and the receptor binding ligands spiperone and ketanserine (15).In this work, we have investigated the factors affecting radiolabeled compound such as pH of the reaction mixture, substrate concentration، reaction time, cuprous chloride concentration and temperature to obtain the labeled compound in high radiochemical yield and purity. Preliminary, in vivo study of ^{125}I pyrimidine derivative in normal mice was done to elucidate the biological behavior of this labeled compound

2. Experimental

2.1. Materials

All melting points were uncorrected. The IR spectra were recorded on pyeunicam sp-11100 Spectrophotometer. Mass spectra were performed by a shimadzu GC-MS-QP 100 Ex (shimadzu, Japan) Elemental analysis were carried out the Microanalytical Research Center, Faculty of Science, and Cairo University. All other chemicals were purchased from Merck Co. Radioactive iodine-125 was purchased from Institute of Isotopes Co., Ltd. (IZOTOP), Budapest, Hungary.

2.2. Chemical syntheses

N-aminothieno (2-N-nitrobenzen-5, 6, 7, 8 tetrahydrobenzothieno [2, 3-]) pyrimidine-4-(3H) one
A mixture of α-(2-iodobenzamido)-β-(4-nitrophenyl)-N-[3-ethoxy-carbonyl-4, 5, 6, 7-tetrahydrobenzothiophen-2-l] acrylic acid amide (0.01 mol.) and hydrazine hydrate (0.012 mol.) in n-butanol (20ml) refluxed for 2h. The reaction mixture was cooled and recrystallized from proper solvents.
The Structure of 2-iodobenzamido N-aminothieno(2-N-nitrobenzen-5,6,7,8 tetra hydro benzothieno [2,3-d])pyrimidine-4-(3H)one (pyrimidine derivative) was based on Correct elemental analysis, IR spectrum of 50b revealed bands at 3307 & 3251 cm−1 [NH2 /NH] and broad band at 1644 (C=O) and 1H-NMR spectrum of [(pyrimidine derivative; DMSO-d6] afforded signals at δ1.9 & 2.8 [m, 8H, tetrahydrobenzo], 4.78 (broad, 2H, NH2),7.15-8.13[m,9H, Ar-H^{+}CH=] and 8.16 [s, 1H, NH].

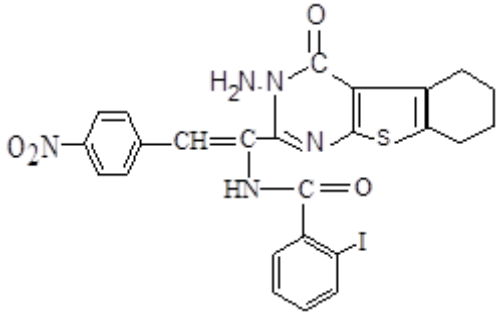


Fig. 1: Structural Formula of Pyrimidine Derivative

Table 1: Characteristics Data for the N-Aminothieno (2-N-Nitrobenzen-5, 6, 7, 8 tetrahydrobenzothieno [2, 3-]) Pyrimidine-4-(3H) One

Comp No	M.P [^{o}C]	Yield%	Mol.Formula (M.wt)	Elemental analysis Calcd./ found				
				C%	H%	N%	I%	S%
ANTBP	150-151	78	$C_{25}H_{16}O_4N_5IS$ (320.344)	49.24 49.20	2.62 2.70	11.5411.60	20.8320.90	5.25 5.30

2.3. Method of labeling

The desired amount of Na^{125}I (1mCi) was transferred to a V-shaped bottom 1 mL reaction vial followed by certain concentration of pyrimidine derivative, cuprous chloride and certain volume of glacial acetic acid then mixed well. The vial was sealed and heated in an oil bath to the desired temperature and reaction time. The vial was cooled and the mixture was neutralized with 0.1 M NaOH. The final solution was subjected to chromatographic analysis to determine the radiochemical yields % and the radiochemical purity of the final product.

2.4. TLC analysis

The radiochemical yield and purity of ^{125}I pyrimidine derivative was determined by TLC as follows: samples were taken by a microliter syringe from the reaction mixture and placed on the starting line of TLC (Merck Silica Gel-60) which was previously impregnated with $Na_2S_2O_3$ (20 mg/mL) to inhibit the oxidation of radioiodide to a volatile form, then chromatographed for about 60 min (corresponding to 10 cm migration of the solvent). Freshly prepared mixture of ethyl acetate : ethanol (1:1, v/v) [21] was used as developing system (Rf = 0.9 for ^{125}I- and Rf = 0.05 for ^{125}I pyrimidine derivative The strips were removed, dried and cut into 0.5 cm segments and assayed for radioactivity using SR.7 gamma counter. The radiochemical yield % was calculated as follows.

$$\text{Radiochemical Yield (\%)} = \frac{\text{Activity of the product}}{\text{Total activity}} \text{ X } 100$$

2.5. Synthesis of ^{125}I pyrimidine derivative

About 10 µL of Na^{125}I (1mCi) was transferred to a V-shaped bottom 1 mL reaction vial followed by 100 µL aqueous solution containing 4 mg pyrimidine derivative and 100 µL glacial acetic acid and mixed well then 0.3 mg Cu_2Cl_2 was

dissolved in 0.1 N HCl was added.. The vial was sealed and heated in an oil bath to 95^{o}C for 60 min. The vial was cooled and the mixture was neutralized with 600µL of 0.1 M NaOH. The unreacted iodide is then removed by filtration of the solution through a silver chloride impregnated filter.

2.6. Bio distribution studies

This experiment was done by diluting the neutral solution of the labeled (^{125}I pyrimidine derivative with 3ml of saline for injection and filtered through 0.22µm Millipore filter into a sterile sealed vial.100µl(100-150MBq) was injected in the tail vein of Albino white mice weighing approximately 25g each (3 groups each of 3 mice). The mice were maintained on normal diet in metabolic cage. The mice were sacrificed at 30min and 2h post- injection. Samples of fresh blood, bone and muscle were collected in pre-weighted vials and counted. The different organs were removed, counted and compared to a standard solution of the ^{125} pyrimidine derivative.The average percent values of the administrated dose/organ were calculated.

3. Results and discussion

In the nucleophilic substitution, the attacking reagent (the nucleophile) brings an electron pair to the substrate and the leaving group comes away with an electron pair. So that, all nucleophiles are Lewis bases in SN_2, the C–Y bond is formed as the C–X bond is broken, where the groups X must leave as the group Y come. The usual order of halide nucleophilicity is $I^- > Br^- > Cl^- > F^-$ (this order is solvent dependent) (16-18). On the other hand, the reaction is not simple, but depends on the structure of the starting materials (the substrates), the conditions under which the reaction is carried out, and the presence of catalyst. The picture of the reaction becomes more interesting when aromatic halides are the substrates as in our case (19). However, the mechanism is not necessarily the same for all other reactions. Also, the neighboring groups assist the reaction by forming an intermediate with radio iodide while the presence of excess base tends to inhibit the reaction due to the formation of hypo iodide ion (i.e. OI^-) (20-22) On the basis of the previous details ^{125}I pyrimidine derivative depends on the following parameters.

3.1. Effect of substrate concentration

Increasing the concentration of the starting material pyrimidine derivative will usually increase the total incorporation of radioiodine. Fig.(2) illustrates the influence of pyrimidine derivative concentration on the radiochemical yield % and a balance must be found between yield and specific activity, to some extent over which the increase in the substrate concentration does not affect the radiochemical yield %. The increase in the radiochemical yield according to the increase in the substrate concentration is due to the increase in the interaction between the substrate and the radioactive iodide.

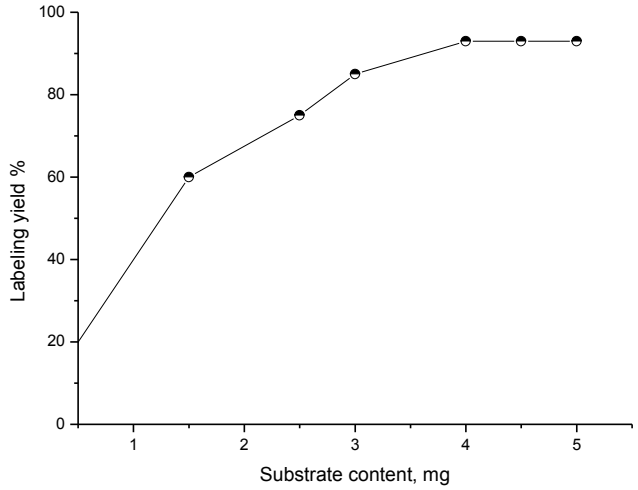


Fig. 2: Variation of the Radiochemical Yield % of ^{125}I Pyrimidine Derivative with Substrate Pyrimidine Derivative Contents (Mg) (X Mg Pyrimidine Derivative, 10µl Na ^{125}I, T = 20 Min, Temp. 100^{o}c, PH = 7]

3.2. Effect of reaction pH

The initial results obtained revealed that the radiochemical yield % increases gradually to 94 at pH 7 then, decreases dramatically on increasing the pH values. This is due to the high solubility of the substrate in glacial acetic acid while

the high pH values decrease the radiochemical yield because it inhibits the exchange reaction. Figure (3) illustrates that the optimum pH for the isotopic exchange is 94%.

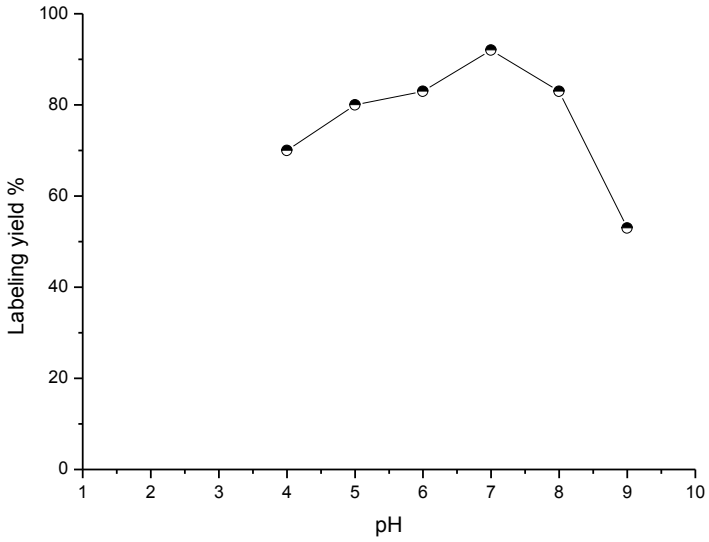


Fig. 3: Variation of the Radiochemical Yield % of^{125}i Pyrimidine Derivative with PH under the Same Reaction Conditions

3.3. Effect of reaction time

The reaction was carried out at various time intervals at temperatures of 95^{o}C. The reaction proceeds well by increasing the time of exchange up to equilibrium (20–60 min) with a radiochemical yield% over 94% at 95 ^{o}C as shown on Fig. (4)

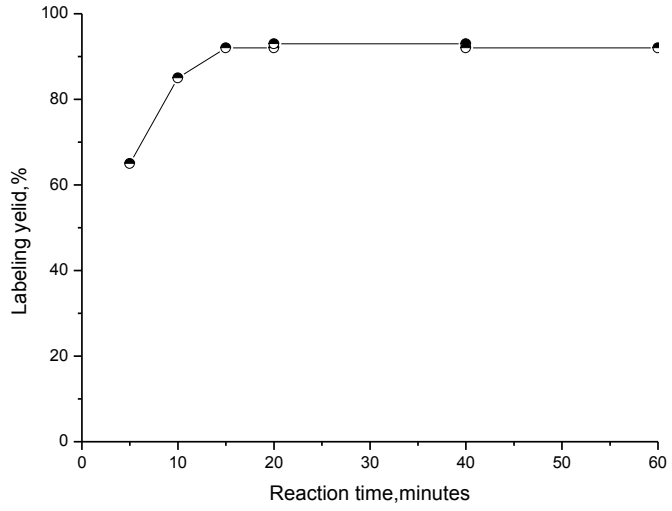


Fig. 4: Variation of the Radiochemical Yield of ^{125}I Pyrimidine Derivative as a Function of Reaction Time under the Same Reaction Conditions.

3.4. Effect of amount of catalyst (Cu_2Cl_2)

The radiochemical yield increase by increase the catalyst amount due to the increase in the interaction between Cu_2Cl_2, NaI and substrate to certain limits .After which the radiochemical yield decrease with increase the Cu_2Cl_2 amount this can be explained by the disproportionation of Cu^{+} ions to Cu metal and Cu^{++} and this disproportionation can be avoided by using reducing agent such as Ascorbic acid.

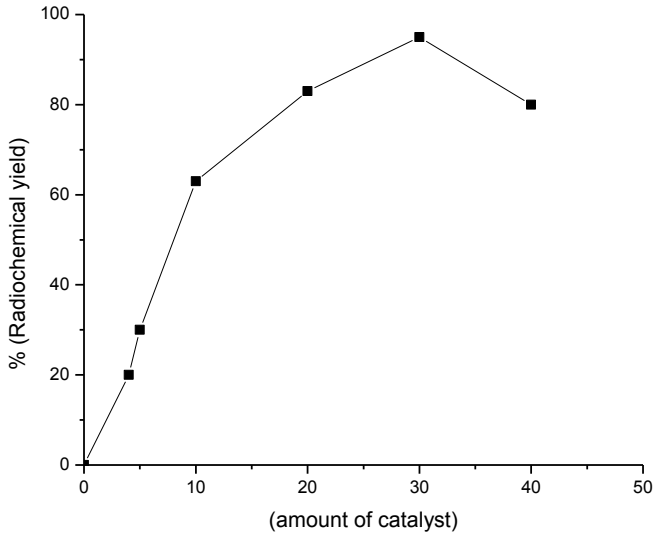


Fig. 5: Variation of the Radiochemical Yield of ^{125}I Pyrimidine Derivative with the Amount of Catalyst [4 Mg (IBATP + X µg Cu_2Cl_2+ 5µl $Na^{125}I$, Temp.95^{o}c Reaction Time = 20 Min]

3.5. Biological study

Due to the similarity of reported pyrimidine as potential cancer chemotherapeutic agents [4]. Pyrimidine derivative showed a higher and faster peak of brain uptake and a faster washout from the brain in the normal mice. The ^{125}I pyrimidine derivative can be used as imaging or therapeutic agent. To follow up the biological distribution of the ^{125}I pyrimidine derivative, it was injected in normal albino mice via the tail vein, and the organs uptake was determined at different time intervals. The results of this study was summarized in table 3,the ^{125}I- pyrimidine derivative tracer shows early high uptake in stomach and heart equal to 6.2 % ± 0.2 and 6.9% ± 1.3 at 5min post injection respectively

Table 3: Bio distribution of Radioactivity after Intravenous Administration of ^{125} I Pyrimidine Derivative in Mice

Organ or body fluied	Time after injection				
	5min	10min	20min	30min	60min
Blood	3.9 ± 0.1	3.1 ± 0.1	2.9 ± 0.1	1.9 ± 0.1	1.2 ± 0.1
Liver	11.3±1.4	10.3±1.4	9.3±1.4	8.6±1.4	8.3±1.4
Kidney	9.3±0.8	8.2±0.8	6.3±0.8	4.3±0.8	2.3± 0.8
Intestestine	1.8±0.3	9.8±0.3	11.4±0.3	13.5±0.3	16.8±0.3
Spleen	3.5±0.9	4.4±0.9	6.5±0.9	9.7±0.9	13.5±0.9
Lung	14.1±2	13.1±2	11.1±2	10.1±2	9.1±2
Stomach	6.2±0.2	5.2±0.2	4.2±0.2	3.2±0.2	2.2±0.2
Heart	6.9±1.3	5.9±1.3	4.5±1.3	3.9±1.3	3.9±1.3
Brain	3.5±0.4	5.5±0.4	4.2±0.4	2.1±0.4	1.6±0.4

Table 4: Bio distribution of Iodine-125 in Normal Mice (Vial Content: 100)

Organ or body fluied	Time after injection	
	5min	30min
Blood	1.9 ± 0.1	0.6±0.4
Liver	8.3±1.4	6.6±0.5
Kidney	9.3±0.8	3.5±0.2
Intestestine	1.8±0.3	1.2±2.4
Spleen	3.5±0.9	1.1±0.2
Lung	10.1±2	1.1±0.2
Stomach	1.2±0.2	2.1±0.5
Heart	5.9±1.3	1 ±0.5
Brain	3.5±0.4	1 ±0.5

4. Conclusions

^{125}I pyrimidine derivative was synthesized via nucleophilic substitution reaction, in the presence cuprous chloride, the labeling process was carried out at 95^{o}C for 60 min. the radiochemical yield was determined by using thin layer chromatography and the yield is equal to 80%.Preliminary in-vivo study was examined in normal mice were performed after intravenous injection through the tail vein and the data show the labeling compound was cleared quickly from most body organs and showed high brain uptake .The results in this study suggest that radioiodinated pyrimidine derivative may be useful as cancer chemotherapeutic agents.

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