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Radioiodination and Biological Assessment of Olsalazine, as a Highly Selective Radiotracer for Ulcerative Colitis Imaging in Mice

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ABSTRACT

Ulcerative colitis (UC) is a chronic, regressive natural disease. The use of conventional diagnostic procedures such as magnetic resonance imaging, ultrasonography, and X-rays during the dormant and early stages of the disease does not aid in diagnosing the disease. As a result, a novel design, such as labelled compounds, were used to image ulcerative colitis disease. In this study, olsalazine was labeled with [$^{125/131}$I] and the labelling parameters were adjusted to obtain a high radiochemical yield (98.5%). In addition, the olsalazine radiotracer gave 96.0% purity in rate serum for up to twelve hours before it started to degrade at twenty-four hours, and it was stable also, in saline for up to twenty-four hours. Molecular docking was used to assess a complex's affinity for its biological target, and the PPARγ receptor. The biological assessment was also performed in mice models of both standard and ulcerative colitis. The results demonstrated that [$^{125/131}$I] iodoolsalazine had a high uptake of 79.5% (ID/g) at 120 minutes post-injection and is still high to 77% at 24 hours. So, the labelled compound, [$^{125/131}$I] iodoolsalazine, could be considered a new potential selective radiotracer for preclinical diagnostic research of ulcerative colitis.

INTRODUCTION

Ulcerative colitis (UC) is an inflammatory bowel disease that causes ulcers and inflammation in the digestive tract and damage to the lining of the colon, large intestine, and rectum. Symptoms typically worsen gradually rather than suddenly [1]. Furthermore, it can be exhausting and can occasionally result in life-threatening elaborations. Although there is no effective treatment for this immune disease (ulcerative colitis), there are some medications that can reduce the symptom of this immune disease. Ulcerative colitis occurs when the immune system malfunctions [2]. It typically attacks conquerors in the body, much like the flu. As intruders in the body, the immune system recognizes good gut bacteria in the intestines and cells lining the colon, which cause the symptoms of this disease [3].

The most commonly ulcerative colitis symptoms are bloody diarrhea, cramping abdominal pain, a sudden urge to poop, and various other signs [4]. Ulcerative colitis can be diagnosed using various techniques such as magnetic resonance imaging, radiography, and ultrasonography. However, they are useless for early, dormant stages of diagnosis. 5-aminosalicylic acid was the first medication to treat and keep ulcerative colitis in remission [5]. Furthermore, due to their strong affinity for specific receptors as PPAR blockers (blockers of peroxisome proliferator-activated receptors), 5-aminosalicilic acid and other thiazolidinediones were used as anti-inflammatory drugs in treating inflammatory bowel disease [6-28].

Iodine-125 radioisotope has a small energy of about 35 keV and the half-life time is about 60 days which, was suitable to complete the study of all the factors affecting the labeling reaction. While iodine-131 radioisotope has a high energy of about 364 keV as a gamma ray emitter, and has a half-life time of about 8 days, due to its high energy, it is preferable to be used in biological assessment studies[29-32]. Previous studies used labeled compounds for imaging ulcerative colitis such as radiotracers [^{131}I]iodosulfasalazine and [^{131}I]iodobalsalazide which gave 75.5 % and 73 % at

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twenty-four hours post-injection. So, we look forward to finding a new radiotracer compound that is localized in the target organ with a higher percentage than the compounds that were studied, up to 24 h. [83,84].

In this study, olsalazine was labeled with radioactive iodine [$^{125/131}$ I], and the factors affecting the labeling process were assessed to obtain the highest labeling yield of [$^{125/131}$I]iodoolsalazine. In addition, the biological assessment of the radiotracer olsalazine was also performed in mice models of both standard and ulcerative colitis for up to 24 hours.

MATERIALS AND METHODS

1-Materials

All chemicals and buffer solutions were of analytical grade and purchased from Merck Co., USA. Olsalazine was bought from the American Sigma-Aldrich Chemical Company. TLC plates were provided by Merck. In addition, Sodium [^{125}I] iodide (185 MBq/50 µL) diluted in 0.04 M NaOH, was purchased from the Institute of Isotopes, Budapest, Hungary. Sodium [^{131}I] iodide (3.8 GBq/ml) diluted in 0.05 M NaOH, was given as a gift from the Radioactive isotopes Factory (RPF), Atomic Energy Authority, Inshas, Egypt.

2-Equipments

Nuclear Enterprises Ltd.'s Scalar Ratemeter SR7 (USA) was used to measure radioactivity with a sodium iodide detector counter. Automated paper electrophoresis equipment, model EC-3000 p-series (USA), including power supply and chamber units were used. Additionally, we utilized HPLC (RP-18 column) connected with pumps, injector needle, and ultraviolet spectrometer detector acting at a wavelength of 345 nm. Elemental analyses and mass spectra (^{13}CNMR and ^{1}HNMR) were performed at the National Research Center, Cairo, Egypt.

3-Animals

In the biological assessment, Swiss Albino mice were used (35-45g). Food and water supplies were isolated for four hours and followed by receiving a 20 mg dose of the antibiotic streptomycin. Then, twenty hours later, unlimited food and water were provided. Finally, four hours before injecting experimental mice with Salmonella Typhimurium (50 µL) Phosphate-Buffered Saline solution containing approximately 1×10^{8} CFU of bacteria, water, and food were prohibited [29-30]. Following that, food and water were given out as soon as possible after two hours following the infection. The Labeled Compound Department, Atomic Energy Authority's biodistribution study methodology received approval from the Animal Ethical and Care Committee (Ethical authorized EAEA/2019/188).

EXPERIMENTAL

1-Radiolabeling of olsalazine

Two-necked glass flask with a rubber septum was used as a reflux condenser and a temperature-controlled water bath to dry the solution after adding about 20 µL of [^{125}I] NaI solution to the reaction mixture. Olsalazine substrate (200 µg) was added to the reaction flask, followed by 100 µl of Chloramine-T solution (1 mg/mL ethanol). An magnetic stirrer was used to stir the mixture for 30 minutes at 37 °C. 100 µl of sodium metabisulphite solution (90 mg/mL H_2O) was added to stop the reaction, which quenched the iodide oxidation reaction, resulting in the absence of iodine Fig. 1(A, B) [30-31].

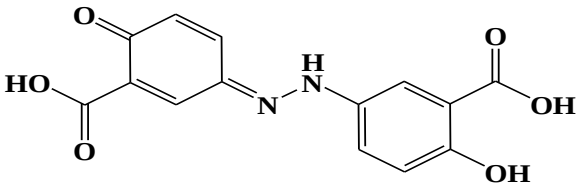


Fig. (1) A: Olsalazine

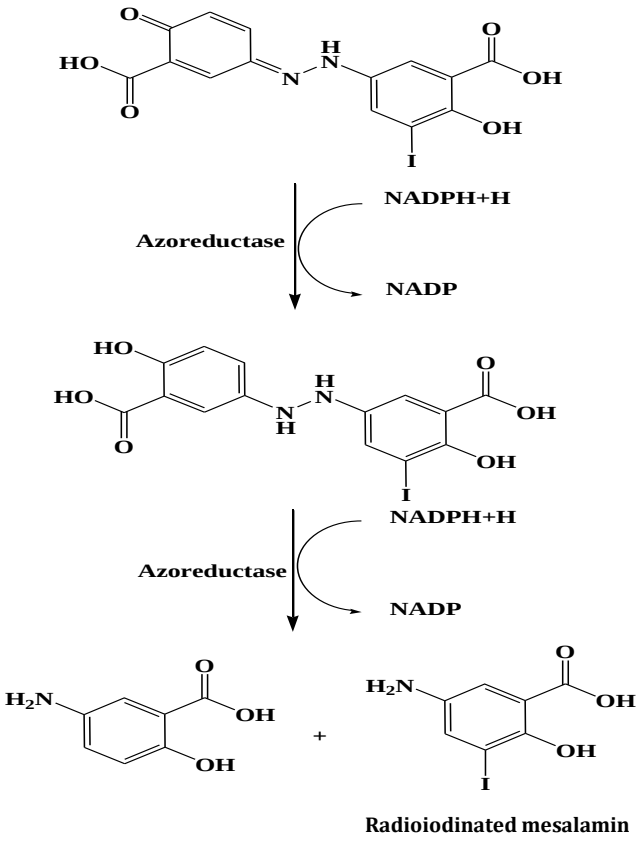


Fig. (1)B: Metabolic pathway of [^{125}I] iodoolsalazine

2-Radiochemical purity yield determination

They were determined using PC, PE, and HPLC

2.1-Paper chromatography (PC)

The radiochemical yield to [^{125}I] iodoolsalazine was investigated using thin-layer chromatography with aluminum silica gel sheets. A reaction mixture of 5 µL (1.50 MBq) was spotted at 2cm from the bottom edge of a 12cm long strip, and it was left on the strip until it developed. The plate was ascending in a cylinder using the solvent development, containing (9:1) ethanol: chloroform solution. After developing the solvent, each section (one cm) was tested for radioactivity detection by gamma counter. The radiochemical yield was also determined using the electrophoresis method to confirm the first technique's results.

2.2-Paper electrophoresis (PE)

We used Whatman paper (46cm length and 2cm width) was used, then two µL of the reaction mixture were added on the paper sheet at a distance of twelve cm from the negative electrode side, and the two chambers were filled with an electrolyte saline solution. The apparatus was operated at 300 V for three hours. After finishing the processing time; the paper was dried and cut into one cm-wide strip. Each strip was then counted using a gamma counter.

2.3- HPLC analysis

HPLC was used to confirm the radiochemical purity of [^{125}I] iodoolsalazine. RP-column was used to analyze about 10 µL of [^{125}I] iodoolsalazine. The mobile phase was formed of methanol and the buffer solution (pH 7.2) which was composed of (NaH_2PO_4 : Et_4NOH) (45:55). For 15 minutes, a flow rate of 1 ml/min.was used. The purity of [^{125}I] iodoolsalazine was determined by HPLC after complete purification to be 98% [31-37].

3- Physicochemical estimation

3.1- Biological Assessment

The radiotracer [^{131}I] iodoolsalazine (100 µL, 0.75 MBq) was given intravenously into caudal venous at 7 hours post-injection. In the two mice models, standard and microbial, six groups of mice (5 animals each, for a total of thirty mice) were used. The mice were sedated with chloroform before being decapitated five minutes, 0.5, 1, 2, 3, 6, and 24 hours after the injection. The radiotracer dosing was assessed as a percentage of the administered dosage per gram tissue throughout the body. The ANOVA test was used to calculate the P value of results described as the SD mean, and the suggested significance level is $P < 0.05$.

3.2-The in-vivo stability

This process was done by appending 100 µL of [^{125}I] iodoolsalazine, to 1900 µL of a recently prepared rat serum. It happened at 37°C temperature for 24 hours. In addition, 100 µL of [^{125}I] iodoolsalazine was checked with 1900 µL of saline. About 50 µL of [^{125}I] iodoolsalazine solution was injected into HPLC at different times, utilizing γ-detectors [38-47].

3.3-Blocking study

Several doses of the substrate (Non-radioactive olsalazine), were given in the range of zero to 1000 µg and then administered intravenously for two hours before the administration of [^{131}I]iodoolsalazine. The percent uptake of the colon (target organ, ulcerative) was predestined at 2 hours post-injection of [^{131}I]iodoolsalazine (n = 5) [48-61].

3.4-Synthesis iodoolsalazine as a non-radioactive

Fig. 1 shows [^{127}I] iodoolsalazine as a non-radioactive. Olsalazine (0.3 g) was dissolved in a small amount of ethyl alcohol before adding chloramine-T (0.23 g) and sodium iodide (0.17 g) diluted in 5 ml of distilled water and stirring continuously for thirty minutes. The reaction was monitored using TLC for 45 minutes at 37 °C. A amount of 0.95 g of sodium metabisulphite was used to degrade the excess iodine and stop the reaction,. [^{127}I] iodoolsalazine was extracted and purified using 2: 1 v/v from (silica hexane: ethyl acetate) after numerous washing procedures, including re-crystallization with diethyl ether, followed by multiple washing procedures, including re-crystallization with DEE alcohol. The solvent was then evaporated under vacuum, yielding 0.37 g of yellow crystal containing 85% ([^{127}I] iodoolsalazine). The product yield was analyzed using (13C NMR), 1H NMR, HPLC, and elemental analysis, as described in the literature [62, 63].

3.5-Characterization of (cold [^{127}I] iodoolsalazine)

In characterizing synthesized cold [^{127}I] iodoolsalazine, ^{1}H NMR, ^{13}CNMR, spectral data, elemental investigations, and HPLC were used. The following analyses verified the molecular structure of the non-radioactive iodoolsalazine ([^{127}I] iodoolsalazine), whose empirical formula is [$C_{14}H_9IN_2O_6$], [MP:176-178°C]: A molecule peak, [M+H]+, was visible in the mass spectra at (m/z 428.55). Calculated elemental investigation was (C, 42.21; H, 2.10; and N, 6.54%). Data

revealed that (C, 42.23; H, 2.11; and N, 6.56%). Its ^{13}C NMR δ ppm (DMSO-d^6) revealed peaks at: 133.6,119,120, 172,124.6,126,181, 137,131.3, 136.7, **128,**124,138,179.11. Furthermore, ^{13}C NMR δ ppm (d^6-DMSO) of cold [^{127}I] iodoolsalazine revealed peaks at: 131.2,118,122, 173,125.2,127,179,135,132.4, 133.9, **85.9,**122,135,178.12. When hydrogen was replaced with iodine, the C-3 resonance of olsalazine's 13C NMR shifted from 128 to 85.9, indicating an increase in the electron density [64]. Peaks at: 7.67-811 (m,3H, aromatic ring), 8.25 (s, 1H, aromatic ring), 8.29 (s, 1H, aromatic ring), 7.90 (s, 1H, NH), 11.33 (s, 2OH, 2COOH), and 5.66 were visible in its 1H NMR (d6-DMSO, 400 MHz) (s, 1H, OH of phenolic-OH). This information supports the hypothesized structure of [^{127}I] iodoolsalazine, successfully produced by radiosynthesis as shown in Fig.1.

3.6- In silico Study

To carry out the docking investigations, the structure preparation methodology was utilized in Molecular Operating Environment, Protein Data Bank archive website (PDB; http://www.rcsb.Org), and the crystal structures of Azoreductase [PDB code: 3keg] and PPAR [PDB code: 4xta] were utilized (MOE).

RESULTS AND DISCUSSION

1- Reaction optimization

The factors influencing the labelling yield (LY) procedure, such as the pH of the reaction mixture, quantity of substrate, the quantity of oxidizing agent, and time of the reaction, were completely optimized at 300 µg of the substrate, 100 µg of an oxidizing agent, pH 8, and 30 minutes at 37 °C temperature. The maximum radiochemical yield of [^{125}I] iodoolsalazine was 98.5%. Each parameter was developed individually to keep all of the factors at rest constant (Figs. 2A-2D). Figure 2A depicts the effect of different concentrations of a substrate on the labelling yield (LY), indicating that the maximum LY of [^{125}I] iodoolsalazine was (98.5%) at 300 µg of olsalazine and 10 µl Na[^{125}I] (3.7 MBq), while the other factors were held constant [64-72]. The pH of the reaction mixture is an important factor influencing labelling yield (Fig. 2B). By studying the effect of pH in the range 2-15, it was discovered that pH 8 is the optimum value for achieving the highest labelling yield of [^{125}I] iodoolsalazine radiotracer [84]. Furthermore, the effect of the reaction time and oxidizing agent (Ch-T) amount was investigated to obtain the highest labelling yield (98.5%) at 30 minutes and 100 µg, respectively (Fig. 2, C and D) [73-77]). Finally, the results showed that [^{125}I] iodoolsalazine was stable in serum for up to 12 hours, yielding 96.0% purity, before dropping to 90.0% after 24 hours but remaining stable in saline for up to 24 hours.

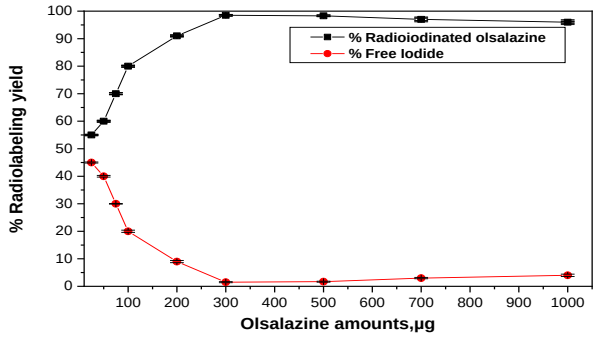


Fig. (2) A: Variation in [^{125}I]iodoolsalazine radiochemical yield as a function of various olsalazine amount; reaction conditions: 10 µL (~3.7 MBq) Na[^{125}I], (x µg) olsalazine, 100 µg of Ch-T, pH 8; at R.T for 30 min.

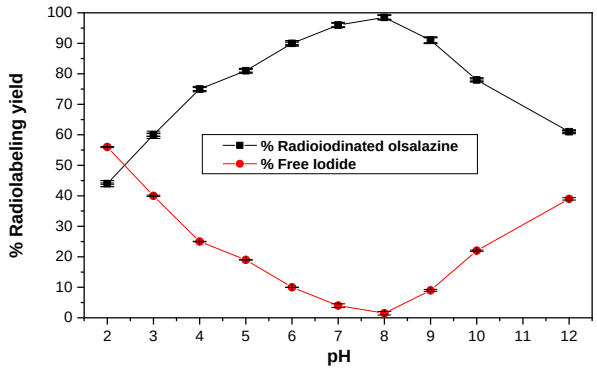


Fig. (2) B :Variation in [^{125}I] iodoolsalazine radiochemical yield as a function of pH; reaction conditions: 10 µL (~3.7 MBq) Na[^{125}I], 300 µg of olsalazine,100µg of Ch-T, at different pH; at R.T for 30 min.

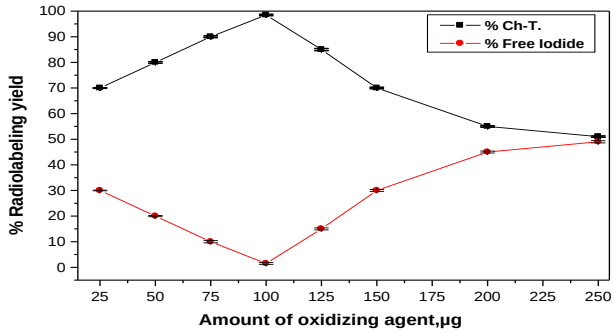


Fig. (2) C: Variation in [^{125}I] iodoolsalazine radiochemical yield as a function of various oxidizing agent; reaction conditions: 10 µL (~3.7 MBq) Na[^{125}I], 300 µg olsalazine, (x µg) of Ch-T, at pH 8; at R.T for 30 min.

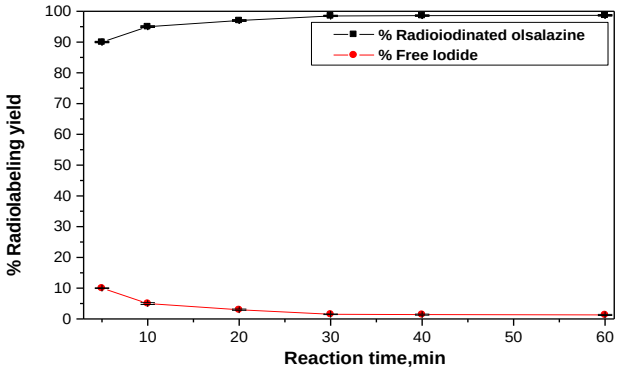


Fig. (2)D: Variation in [^{125}I] iodoolsalazine radiochemical yield as a function of reaction time; reaction conditions: 10 µL (~3.7 MBq) Na[^{125}I], 300 µg of olsalazine,100 µg of Ch-T, at pH 8 ; at R.T for various intervals of time

2- Evaluation of [^{125}I] iodoolsalazine purity

It was determined using paper chromatography, PE, and HPLC. The species, such as free iodide and radiochemical yield of a labeled compound, [^{125}I] iodoolsalazine %, can be detected according to [64-67]. The synthesized [^{125}I] iodoolsalazine with an excellent radiochemical yield of >98%. Results of PE revealed that the [^{125}I] iodoolsalazine occurs at three centimeters from the starting side, while the free iodide occurs at 13 cm from the starting side near to anode. High-performance radio chromatography was also used to confirm the purity of [^{125}I] iodoolsalazine and, its free iodide during a flow rate of one mL.min^{-1}. Firstly, the R_t value of [^{125}I] iodoolsalazine was 11.66 minutes (high purity ≥98%) and the R_t value of the free iodide was 4.5 minutes (Fig. 3A). However, for olsalazine, it was Rt = 11.20 minutes (Fig. 2B). The data revealed that a radiochemical purity of [^{125}I]iodoolsalazine was 98%. In addition, Fig. 3C indicated that HPLC analysis of cold non-radioactive iodoolsalazine ([^{127}I]iodoolsalazine) gave identical R_t value of radio-iodoolsalazine at 11.2 minutes, typically like Fig. 3B.

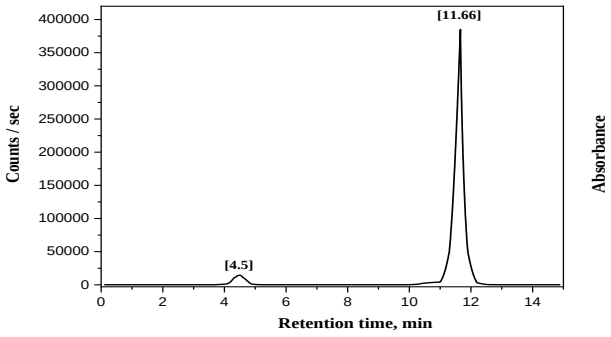


Fig. (3)A: HPLC separation for free iodide (4.5 min), and [^{125}I] iodoolsalazine (11.66 min)

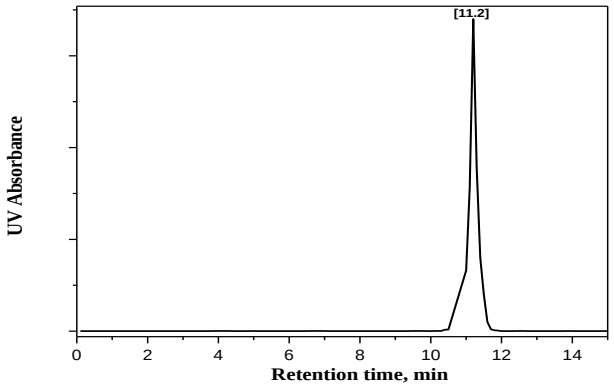


Fig. (3)B: HPLC- Ultra Violet of olsalazine

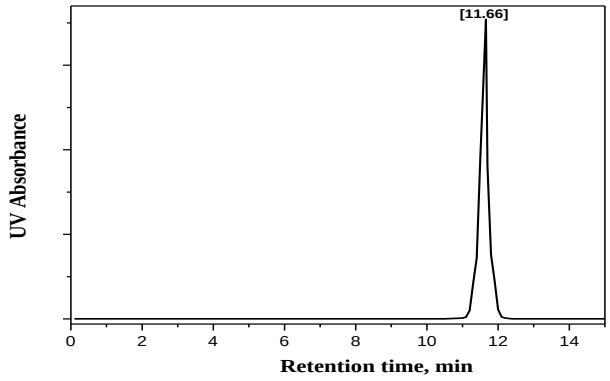


Fig. (3)C: HPLC-Ultra Violet of cold non-radioactive [^{127}I] iodoolsalazine at Rt 11.66 min

3-Blocking study of (PPARγ) antagonists

250-1000 µg of olsalazine was used to pre-dos mice as unlabeled olsalazine for 120 minutes before the injection of [^{131}I] iodoolsalazine radiotracer. This reduced colon (ulcerative) absorption from 79.55% to 9.5% ID/g organ at 120 minutes after injection. It occurred because of its combination with (PPARγ) antagonists in the colon. This experiment concludes that this radiotracer, [^{131}I] iodoolsalazine, can be used effectively in imaging (PPARγ) antagonists(Fig. 4).

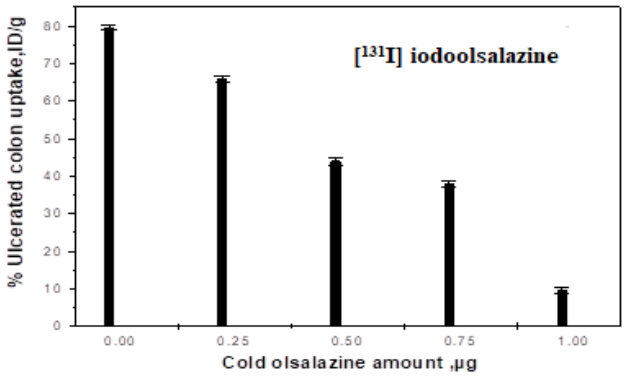
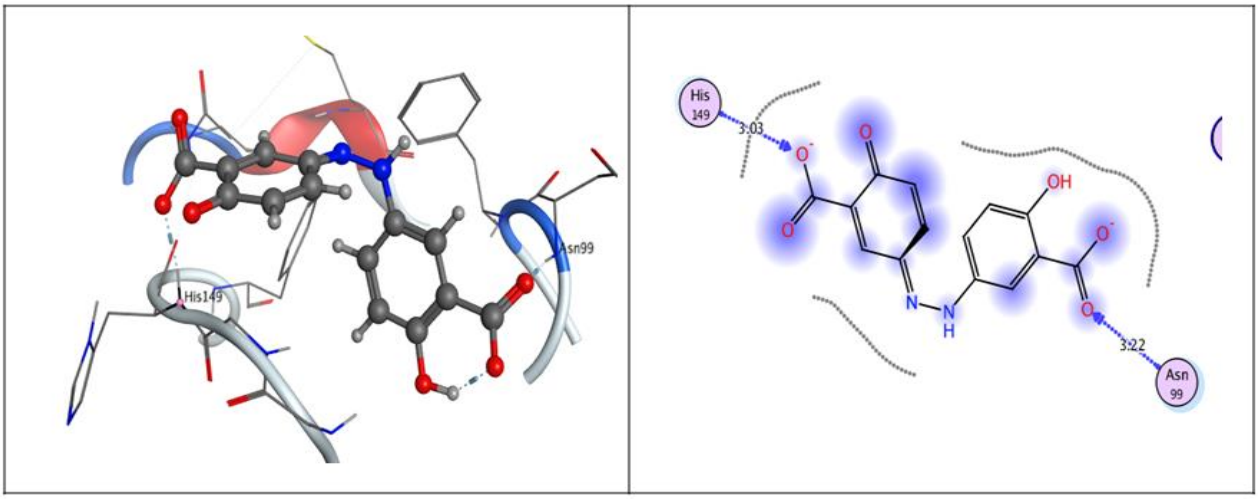


Fig. (4): [^{131}I] iodoolsalazine prevent UC uptake in ulcer-bearing mice at 2 hours after injection

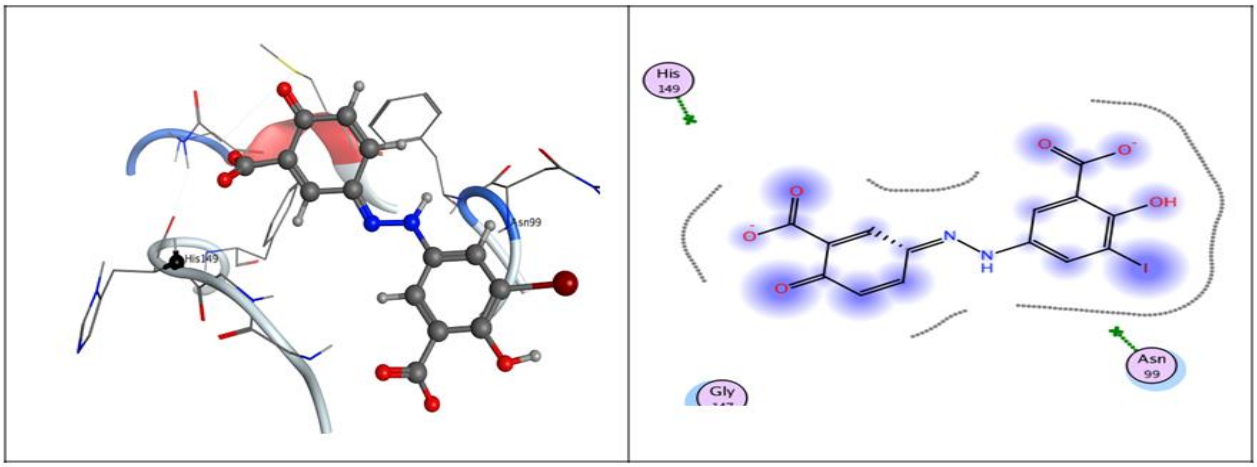
4- Investigations using Molecular Modelling

Investigations using molecular modeling of olsalazine on azoreductase [PDB code: 3keg] displayed a docking score of -15.4661 kcal/mol and had 2 hydrogen bonds with 2 different amino acids, the first with H is 149 of distance (3.03Å) and the second with A sn 99 of distance (3.22Å). [^{125}I] Iodoolsalazine radiotracer showed a docking score of -9.8087 kcal/mol attached to the major pocket with hydrophilic connection, as presented in Figs. (5, 6, 7). Additionally, the findings of the docking investigation on the metabolites 5-ASA and the iodinated 5-ASA on PPARγ [PDB code: 4xta] were as follows: 5-ASA had a docking score of -8.144 kcal/mol and a hydrogen bond with Arg 289 that was 1.53 nm away, as seen in Fig. 8. According to Fig. (9), the radiotracer [^{125}I] iodomeslamine formed two hydrogen bonds with two separate amino acids, one with Arg 234 at a distance of (1.6) and the other with Leu 229 at a distance of (2.95). 9. In conclusion, heavy free iodide did not affect the ability of olsalazine or its metabolites to bind to their targets.



I II

Fig. (5) I: Olsalazine's top-ranked 3D pose demonstrating interactions at the azoreductase location
II: Olsalazine's top-ranked 2D pose demonstrating interactions at the azoreductase location



I II

Fig. (6) I: The best 3D pose of [^{125}I] iodoolsalazine demonstrating interactions in the azoreductase location
II: The best 2D pose of [^{125}I] iodoolsalazine demonstrating interactions in the azoreductase location

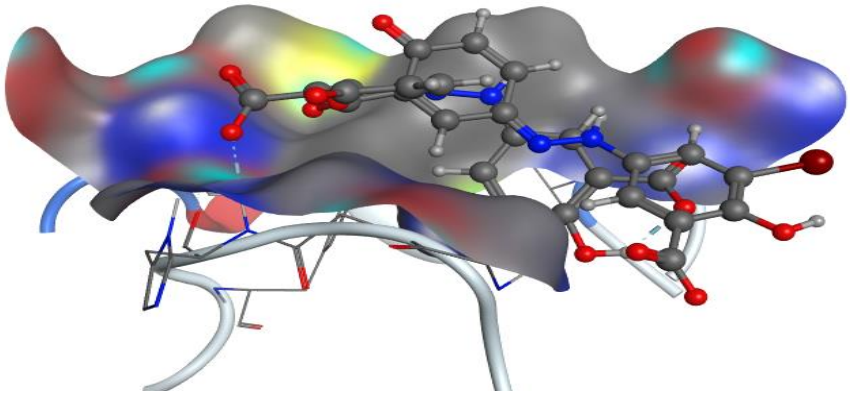


Fig. (7): The interactions between the top-ranked 3D poses of olsalazine and [^{125}I] iodoolsalazine in azoreductase are overlaid

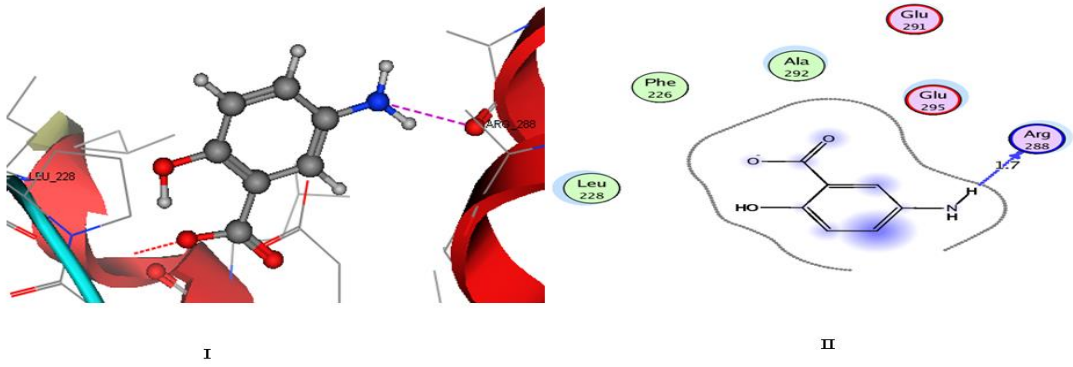


Fig. (8) I: PPAR site interactions are seen in the top-ranked 3D posture of the 5 amino salicylic acid
II: PPAR site interactions are seen in the top-ranked 2D posture of the 5 amino salicylic acid

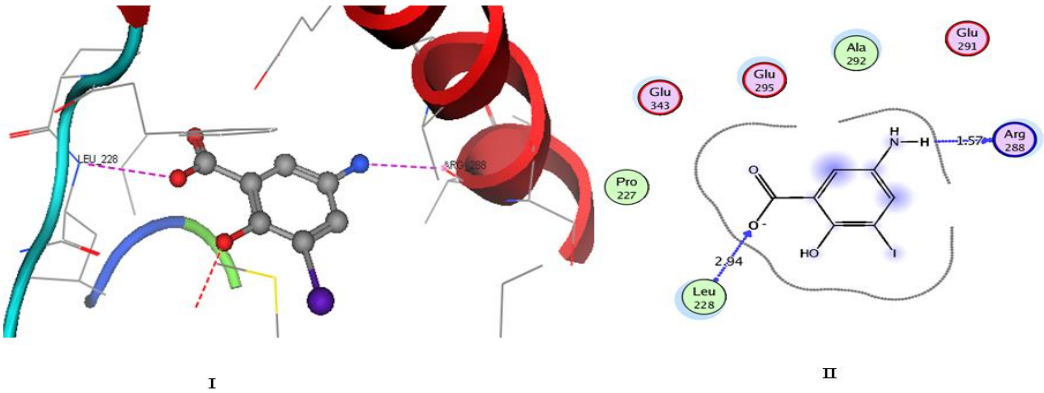


Fig. (9) I: Iodinated 5-amino salicylic acid's top-ranked 3D posture demonstrating interactions at the PPAR location
II: Iodinated 5-amino salicylic acid's top-ranked 2D posture demonstrating interactions at the PPAR location

5- Biological Assessment

A biological assessment of [^{131}I] iodoolsalazine in several body organs and fluids of normal mice is shown in Table (1). All results were expressed as [%ID/g organ ±S.D] [85-100]. The labelled compound, [^{131}I] iodoolsalazine, is quickly dispersed in most organs at five mins. after injection. At three hours post-injection (p.i.), the kidney's uptake was 5.54%, and at 24 hours p.i., it had decreased to 1.21%. Additionally, the liver uptake at three hours after injection was 32.33% and reached 6.11% at 24 hours (p.i.). Therefore, the hepatobiliary route system is responsible for the majority of the radiotracer [^{131}I] iodoolsalazine's excretion. [78-99]. It was found that the target organ (ulcerated colon) was about 38.16 % at 120 minutes post-injection, and it also remained available to give 35.14% at twenty-four hours p.i. It included more

than just the listed compounds of [^{131}I] iodosulfasalazine and [^{131}I] iodobalsalazide [100-110].

A biological assessment of [^{131}I] iodoolsalazine in several body organs and fluids in ulcerative colitis mice is presented in Table (2). The findings showed that Tables (1) and (2) are identical, except for Table (2)'s greater uptake of [^{131}I] iodoolsalazine in the target organ (ulcerative colitis). The uptake of the [^{131}I] iodoolsalazine-labeled compounds in the target organ was 79.55% at 120 minutes post-injection and remained high at 77.00% at 24 hours post-injection. which is higher than the two radiotracers [^{131}I] iodosulfasalazine and [^{131}I] iodobalsalazide gave 75.5 and 73 % at 24 hours post-injection. according to [111-126]. Therefore, it is clear that (PPAR) antagonist expression levels rise at the onset of the disease, and the radiotracer is anticipated to interact with that receptor, which is strongly expressed in the target organ, ulcerative colitis.

The results of the current investigation show that the labelled compounds, [^{131}I] iodoolsalazine has a higher percent injected dose per gram organ value than the two radiotracers [^{131}I] iodosulfasalazine and [^{131}I] iodobalsalazide recently prepared at 24 hours post-injection. Additionally, the labelled compounds, [^{131}I] iodoolsalazine, remained available till 24 hours post-injection to give 77 %, which does not study over this long time, 24 hours.

CONCLUSION

The labelled compound, [^{131}I] iodoolsalazine, may be successfully synthesized with high radiolabeling purity (98.5%) and higher stability in saline and serum. The labelled compound, [^{131}I] iodoolsalazine, has a high uptake in the target organ (ulcerative colitis) with a high uptake of 79.55% at 120 minutes post-injection and still high to 77% at 24 hours. This injected dose value is also higher than that of newly identified drugs like [^{131}I] iodosulfasalazine and [^{131}I] iodobalsalazide till 24 hours post-injection. So, the labelled compound, [^{131}I] iodoolsalazine, could be considered a new potential selective radiotracer for preclinical diagnostic research of ulcerative colitis.

A DISPUTE OF INTEREST

There was no conflict of interest disclosed by the authors.

INFORMATION ABOUT DATA AVAILABILITY

At the Egyptian Atomic Energy Authority, we gathered all the data.

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Table (1) Biodistribution of [^{131}I] iodoolsalazine radiotracer in normal mice at various times [group (A)]

Organs & body fluids	% I.D./g at various times post injection							
	5 min	30 min	60 min	120 min	3hrs	6hrs	12 hrs	24 hrs
Blood	15.66±1.22	13.25±0.29	3.95±0.39	2.2 ± 0.17	1.2± 0.18	1.11 ± 0.14	0.98 ± 0.09	0.91±0.04
Bone	1.32±0.24	1.20±0.27	1.15±0.18	0.98±0.16	0.89±0.11	0.88± 0.10	0.79± 0.08	0.68± 0.03
Muscle	3.7 ± 0.29	2.8 ± 0.20	1.8 ± 0.20	1.3 ± 0.12	1.1±0.09	0.96± 0.08	0.91± 0.07	0.90± 0.06
Brain	1.11± 0.09	1.1 ± 0.30	0.98±0.00	0.95±0.00	0.80±0.20	0.71± 0.08	0.69± 0.07	0.67± 0.05
Lungs	1.32±0.24	1.12± 0.12	1.0 ± 0.13	0.98±0.11	0.95±0.08	0.89± 0.07	0.78± 0.07	0.69± 0.06
Heart	1.15±0.17	1.13 ±0.09	0.98±0.06	0.96±0.05	0.89±0.05	0.78± 0.04	0.67± 0.04	0.61± 0.04
Liver	5.12±0.17	10.9±1.14	17.6±1.19	28.30±1.20	32.33±1.15	23.15±1.30	11.17±0.79	6.11±0.19
Kidneys	2.23 ± 0.13	2.9 ±0.16	3.12±0.15	4.77 ± 0.09	5.54±0.90	3.12± 0.19	1.88± 0.14	1.21± 0.08
Thyroid	1.14± 0.12	1.12± 0.25	0.99± 0.08	0.98 ± 0.07	0.92±0.06	0.89 ±0.06	0.86 ± 0.05	0.79± 0.05
intestine	5.35±0.90	6.33 ± 0.25	7.67± 0.19	13.33±0.85	11.33±0.76	9.19± 0.80	7.15± 0.60	3.19± 0.34
Colon	13± 1.36	18.55±1.27	20.33±1.26	38.16±1.33	38.11±0.98	37.1±1.15	36±0.78	35.14±1.30
Stomach	1.14±0.33	1.11 ± 0.24	0.97±0.03	0.96±0.02	0.90±0.02	0.89± 0.02	0.86± 0.02	0.80± 0.02

Mean±SEM (n=5)

Table (2) Biodistribution of [^{131}I] iodoolsalazine radiotracer in ulcerative colitis mice at various times [group (B)]

Organs & body fluids	% I.D./g at various times post injection							
	5 min	30 min	60 min	120 min	3hrs	6hrs	12 hrs	24 hrs
Blood	15.65±1.43	14.12±0.98	3.77±0.65	2.4 ± 0.12	1.4± 0.11	1.14 ± 0.19	0.98 ± 0.09	0.95±0.01
Bone	1.43±0.66	1.29±0.31	1.18±0.16	1.11±0.14	1.00±0.00	0.98± 0.01	0.90± 0.01	0.80±0.01
Muscle	3.6 ± 0.20	2.5 ± 0.25	1.9 ± 0.28	1.6 ± 0.11	1.15±0.11	0.93± 0.02	0.92± 0.02	0.91±0.02
Brain	1.13± 0.07	1.2 ± 0.40	0.99±0.02	0.97±0.01	0.90±0.20	0.81± 0.01	0.80± 0.01	0.79±0.01
Lungs	1.25±0.33	1.14± 0.17	1.22±0.65	0.93±0.11	0.92±0.01	0.91± 0.01	0.89± 0.02	0.77±0.01
Heart	1.19±0.16	1.12 ±0.11	1.11±0.08	0.99±0.04	0.98±0.01	0.89± 0.03	0.86± 0.01	0.76±0.01
Liver	5.44±0.19	11.33±1.19	18.11±1.12	29.32±1.44	31.37±1.13	24.19±1.10	12.13±0.78	5.88±0.14
Kidneys	2.25 ± 0.11	2.88 ±0.17	3.19±0.12	4.98 ± 0.11	5.33±0.95	3.18± 0.12	1.76± 0.12	1.33±0.22
Thyroid	1.17± 0.10	1.14± 0.21	1.11± 0.90	0.90 ± 0.1	0.90±0.01	0.87 ±0.01	0.83 ± 0.00	0.80±0.01
intestine	5.44±0.96	6.28 ± 0.20	7.35± 0.13	12.46±0.98	10.27±0.90	9.88± 0.90	5.19± 0.98	3.12±0.61
Ulcerated colon	28± 1.19	34 ± 1.77	58 ± 1.18	79.55±1.66	78.7±0.99	77.9±1.76	77.3 ± 0.90	77± 1.44
Stomach	1.13±0.39	1.12 ± 0.11	0.99±0.09	0.98±0.00	0.97±0.00	0.90± 0.00	0.89± 0.00	0.88±0.00

Mean±SEM (n=5)

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