



## Facile one-pot strategy for brain imaging using radiolabeled [<sup>99m</sup>Tc]-tricarboxyl histamine complex in mice.



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### Abstract

This investigation aims to label the histamine ligand, 2-(1H-imidazol-4-yl) ethanamine, using a [<sup>99m</sup>Tc]-tricarboxyl core method for brain imaging. The optimization of all variables factors influencing the radiochemical reaction is carried out to produce the labeled compound with a high radiochemical yield of 98.5%. The radiolabeled compound was stable for up to 6 hours, with a logarithm of the partition coefficient value at  $1.49 \pm 0.04$ . This value suggests the lipophilic characteristics of the [<sup>99m</sup>Tc]-tricarboxyl histamine compound, indicating its potential to cross the blood-brain barrier effectively. The bioevaluation of the radiotracer [<sup>99m</sup>Tc]-tricarboxyl-histamine complex exhibited a notable brain uptake of 8.5% ID/organ at 5 minutes post-injection and, eliminated through the urinary system within 2 hours post-injection. This study explores the promising developments in brain imaging facilitated by the [<sup>99m</sup>Tc]-tricarboxyl histamine complex.

**Keywords:** Histamine ligand; radiolabeled; [<sup>99m</sup>Tc]-tricarboxyl core; bioevaluation; brain imaging.

### 1. Introduction

Prior studies have assessed many labeled compounds as brain imaging tracers. Nevertheless, a significant drawback for several radiotracers is that the brain does not absorb enough of the labeled molecule to allow for long-term tracking of the tracer. We tried using a neurotransmitter to circumvent these limitations [1-6]. Histamine a key organic nitrogenous compound, is recognized for its dual role as a neurotransmitter and modulator of the immune response. This highlights the importance of histamine in physiological processes in which it may be bound [7-12]. Nuclear medicine has made significant progress with the introduction of [<sup>99m</sup>Tc] N complexes [13-16]; the final radiotracer's chemical and biological properties are influenced by the unique +2 oxidation state of the [<sup>99m</sup>Tc] nitrido core. Even using different [<sup>99m</sup>Tc]nitrido complexes for brain imaging, problems including limited brain uptake and a small brain/blood ratio still exist [17-19].

Many radiotracers, such as those mentioned in references [20-26], have undergone thorough evaluation to assess their efficacy as pharmaceuticals for brain imaging purposes. Nevertheless, in brain imaging state continues to face a pivotal challenge, primarily stemming from the insufficient concentration of the tracer within the brain, resulting in low uptake levels. This limitation is further exacerbated by the tracer's inherently short duration of activity within the brain, impeding the accurate and sustained visualization of brain structures and functions. So, new radiopharmaceuticals were used to overcome this problem. The current research is focused on labeling histamine with the radioactive, <sup>99m</sup>Tc, to create the [<sup>99m</sup>Tc] tricarboxyl histamine complex. Various factors influencing the radiolabeling efficiency process were examined by techniques of separation paper chromatography, electrophoresis, and high-performance liquid chromatography (HPLC). The accumulation

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of this complex in the brains of mice was tested at different time intervals post-injection [27-31]. Figure 1A shows the chemical structure of histamine, Figure 1B, 1 C shown the proposed structure [ $^{99m}\text{Tc}$ ] tricarbonyl histamine complex.

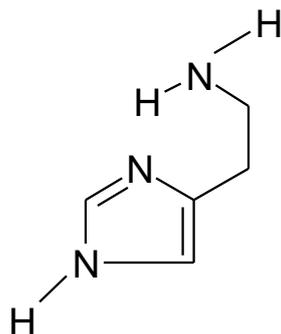


Fig.1A. The chemical structure of histamine

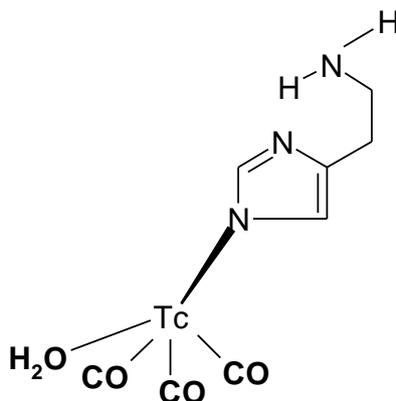


Fig.1B The proposed structure of  $^{99m}\text{Tc}$ -tricarbonylhistamine

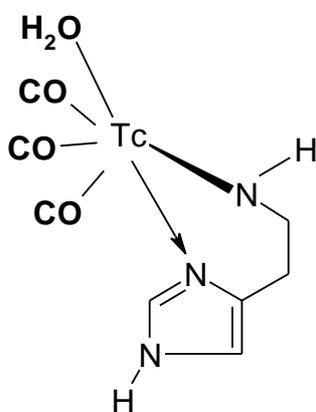


Fig.1C. The proposed structure of [ $^{99m}\text{Tc}$ ]tricarbonyl histamine

## Experimental

### Materials and methods

The provider of histamine was Sigma-Aldrich Chemical Company, situated in St. Louis, Missouri, USA. The other chemicals of reactive grade were acquired from Merck, based in Whitehouse Station, New Jersey, USA, known for its high-quality chemical products. The utilization of purified deoxygenated bi-distilled D.D.H<sub>2</sub>O was crucial in the experimental processes. Technetium-99m was eluted as  $^{99m}\text{TcO}_4^-$  from  $^{99}\text{Mo}/^{99m}\text{Tc}$  generator (activity :1Ci, Radioisotope Production Factory, Egyptian Atomic Energy Authority). Aluminum sheets coated with silica gel 60-F254 (20 x 25 cm) were procured from Merck for thin-

layer chromatography (TLC). The filter paper was identified as Whatman TM grade International, a reputable supplier of Ltd., located in Maidstone, Kent, United Kingdom, and known for its exceptional filtration products. All chemicals used in the experiment were of analytical grade, eliminating the necessity for further purification steps and simplifying the experimental procedures for efficiency and precision.

### Apparatus

A NaI scintillation  $\gamma$ -counter model Scalar Ratemeter SR7 (Nuclear Enterprises Ltd., USA) was utilized to measure radioactivity. The paper electrophoresis (PE) was obtained from E.C. Corporation in Albany, OR, USA. The research was carried out in an HPLC system (Shimadzu, Kyoto, Japan) which consisted of a Rheodyne injector, an LC-9A controller pump, and a SpD-6A detector. The elution in the gradient system involved H<sub>2</sub>O (solvent A) and acetonitrile with 0.1% trifluoroacetic acid (solvent B) (0-28 min, 90% A-10% A; 28-30 min, 10% A; 30-32 min, 10% A- 90% A). Separation was carried out using a Li Chrosorb RP-C18 column (250x9.4 mm) packed with 5  $\mu$ m particles. The compounds were calibrated for UV light detection at 256 nm. The temperature of the column was kept at 25°C, at a flow rate of 1.0 mL/min. Fractions with volumes ranging from 1.0 mL to 25 mL were individually collected and recorded with a  $\gamma$ -ray scintillation counter.

### Animals

Twenty to thirty-gram Male Swiss Albino mice were utilized in the bioevaluation studies. The mice were sourced from the Biological Application Department at the Nuclear Research Centre of the Egyptian Atomic Energy. They were housed in a controlled room with a temperature of 24  $\pm$  5°C, a 12-hour light-dark cycle, a relative humidity of 60  $\pm$  4%, and provided with water and rodent chow throughout the study period. The study was conducted with the protocols established by the Animal Ethics Committee of the Egyptian Atomic Energy Authority (Ethical Approval REC-NCRRT/1A/24).

### Synthesis of Precursor [<sup>99m</sup>Tc(CO)<sub>3</sub>(H<sub>2</sub>O)<sub>3</sub>]<sup>+</sup>

Alberto et al. [15] proposed a modified synthon production method. Following established protocols, a solution containing 4 mg of Na<sub>2</sub>CO<sub>3</sub>, 5.5 mg of NaBH<sub>4</sub>, and 15 mg of Na/K tartrate in 0.5 ml D.D H<sub>2</sub>O. After subjecting the solution to treatment with carbon monoxide gas purge for 5 minutes about 1.0 ml of <sup>99m</sup>TcO<sub>4</sub><sup>-</sup> (37 MBq) was introduced. Following this, the solution underwent heating at a temperature of 100°C for 30 minutes. Once the prepared solution had cooled in an ice bath for 15 minutes, 300  $\mu$ L of phosphate buffer (0.5 M, pH 7.5): 1M HCl (1:3 v/v) was added to adjust its pH to 8. The synthon was carried out using HPLC analysis [32-33].

### Synthesis of [<sup>99m</sup>Tc]-tricarboxyl-histamine

A volume of 0.5 mL of the precursor [<sup>99m</sup>Tc (CO)<sub>3</sub>(H<sub>2</sub>O)<sub>3</sub>]<sup>+</sup> was introduced into 0.1 mL of the histamine ligand aqueous solution (250  $\mu$ g). The pH of the reaction mixture was adjusted to 7, followed by heating at 100°C for 30 minutes. A single histamine molecule binding in a 1:1 ratio to the metal oxocation through didentate chelation can create the [<sup>99m</sup>Tc]-tricarboxyl-histamine complex. Utilization of HPLC was conducted for the purification of the [<sup>99m</sup>Tc]-tricarboxyl-histamine complex.

### Purification of [<sup>99m</sup>Tc]-tricarboxyl- histamine complex

In a system where acetonitrile was used as the mobile phase, thin layers were treated until they reached one-fourth of their original size. The layers gradually developed were removed, left to dry in the air, and finally sliced into strips measuring 1 cm wide. These strips were subsequently quantified using a  $\gamma$ -counter. To confirm the radiochemical conversion, paper electrophoresis was conducted by the recommendations made by Sanad and collaborators [34-40]. The process involved depositing 2-5  $\mu$ L of the reaction mixture onto a Whatman #1 sheet (measuring 2.5 cm in width and 45 cm in length), positioned 10 cm away from the cathode terminal. Electrophoresis was carried out using normal saline solution (0.9%) as the electrolyte, with the system running for 3 hours at 300 volts. Upon completion, the strip was dried, and divided into 1 cm for each fragment for further analysis with a  $\gamma$ -counter. Additionally, further purification by directly injecting a quantity of 20 microliters from the reaction mixture, and HPLC analysis to predict the complex.

### Physicochemical evaluation

#### Determination of lipophilicity

The partition coefficients of the [<sup>99m</sup>Tc]-tricarboxyl-histamine complex in octanol/water were measured under pH 7.4 conditions, and their dispersion in n-octanol and phosphate-buffered saline (PBS) was investigated. After vigorous mixing for 5 minutes, a mixture of 800  $\mu$ L of PBS (pH 7.4) and 1 mL of n-octanol was combined with 200  $\mu$ L of the sample and incubated at 25°C for 30 min. The organic and aqueous layers were separated by centrifuging the mixture at 5000 rpm for 5 minutes. Subsequently, using a  $\gamma$ -counter, the measurement of each layer (100  $\mu$ L) was conducted. The log Po/w values can be expressed from five repetitions of the partition coefficient value [41-45].

#### Stability of [<sup>99m</sup>Tc]-tricarboxyl-histamine complex

The stability of [<sup>99m</sup>Tc]-tricarboxyl-histamine complex was examined in two media, rat serum, and PBS. About 0.2 mL of purified [<sup>99m</sup>Tc]-tricarboxyl-histamine complex was mixed with 1.8 mL of serum, and this mixture was incubated at 37°C until use. Similarly, in phosphate-buffered saline (pH 7.5), the result of the [<sup>99m</sup>Tc]-tricarboxyl-histamine stability was

assessed [5  $\mu\text{L}$ ,  $3.20 \times 10^{-3}\text{GBq}$ ] using the TLC technique. The complex was then counted at different time intervals by gamma counter [46-49].

### Blocking study

Various quantities of unlabelled histamine (cold) were utilized within the 0-1000  $\mu\text{g}$  range. The histamine was administered to the mice 30 minutes before the administration of the radiotracer, and the brain uptake percentage was calculated 30 minutes after the injection of the radiotracer, [ $^{99\text{m}}\text{Tc}$ ]-tricarbonyl-histamine complex (n = 5).

### Molecular modeling

Docking studies were conducted utilizing the Molecular Operating Environment protocols version 2014.10. The structural analysis involved the examination of the histamine H1 receptor bound to histamine (HIS) complex, with the crystal structure obtained from the Protein Data Bank managed by the Research Collaboration for Structural Bioinformatics available on the RCSB website.

This process of molecular docking enables the exploration of potential interactions between the receptor and ligand, offering valuable insights into the binding mechanisms at a molecular level, which can aid in drug design and development efforts in the field of pharmacology [50-53].

### Bioevaluation studies

Bioevaluation of [ $^{99\text{m}}\text{Tc}$ ]-tricarbonyl-histamine complex was evaluated in male Swiss albino mice weighing (20-30) g. About 0.2 mL (3.6 MBq) of [ $^{99\text{m}}\text{Tc}$ ]-tricarbonyl-histamine complex was intravenously injected into six groups of five normal mice each, for a total of thirty mice, all six weeks old.

The organ distribution of the [ $^{99\text{m}}\text{Tc}$ ]-tricarbonyl-histamine complex was measured (by time point) by sacrificing the animals at different time intervals: 5, 10, 15, 30, 60, and 120 min. [54-60]. Throughout the experiment, adjustments were made for physical decay and background radiation.

### Statistical Analysis

All data were analysed using a one-way variance test. The results are reported as the mean  $\pm$  SEM and statistical significance was set at  $p < 0.05$ .

### Results and discussion

#### Optimization of reaction

The impact of different substrate (histamine) concentrations on radiochemical yield is displayed in Table 1. With 250  $\mu\text{g}$  of substrate and a free [ $^{99\text{m}}\text{Tc}$ ]-tricarbonyl core (7.5 MBq) the radiochemical conversion of the [ $^{99\text{m}}\text{Tc}$ ]-tricarbonyl-histamine complex was 98.5%.

Additionally, Table 2 demonstrates the impact of different pH values on the radiochemical conversion of the [ $^{99\text{m}}\text{Tc}$ ]-tricarbonyl-histamine complex was 98% at pH 7. A study of the impact of reaction time on the radiochemical conversion as shown in Table 3 indicates that the highest conversion to [ $^{99\text{m}}\text{Tc}$ ]-tricarbonyl-histamine complex reached 98% at 30 min. The *in vitro* stability of the [ $^{99\text{m}}\text{Tc}$ ]-tricarbonyl histamine complex was assessed in saline and rat serum for up to 24 hours as shown in Tables 4, and 5.

The radiotracer for [ $^{99\text{m}}\text{Tc}$ ]-tricarbonyl-histamine complex was stable in saline and reached 97.5% after 24 h. On the other hand, the purity of [ $^{99\text{m}}\text{Tc}$ ]-tricarbonyl-histamine complex in serum, reached 92.0% after 12 h. then dropped to 89% after 24 h.

**Table (1): The impact of different substrate (histamine) concentrations on the radiochemical yield of [ $^{99\text{m}}\text{Tc}$ ]-tricarbonyl histamine complex**

Histamine ( $\mu\text{g}$ )	[ $^{99\text{m}}\text{Tc}$ ]-tricarbonyl histamine complex	[ $^{99\text{m}}\text{Tc}$ ]tricarbonyl core
100	82 $\pm$ 0.14	18 $\pm$ 0.10
150	87 $\pm$ 0.11	13 $\pm$ 0.09
200	95 $\pm$ 0.12	5 $\pm$ 0.09
250	98.5 $\pm$ 0.16	1.5 $\pm$ 0.07
300	98 $\pm$ 0.14	2.0 $\pm$ 0.06
350	97.2 $\pm$ 0.18	2.8 $\pm$ 0.05
400	97 $\pm$ 0.16	3.0 $\pm$ 0.07

Values represent the mean  $\pm$  SEM, n = 3

**Table (2): The impact of different pH values on the radiochemical yield of [<sup>99m</sup>Tc]-tricarbonyl histamine complex**

pH	[ <sup>99m</sup> Tc]-tricarbonyl histamine complex	[ <sup>99m</sup> Tc]tricarbonyl core
5	65.0 ± 0.13	35.0 ± 0.16
6	80.0 ± 0.12	20.0 ± 0.14
7	98.0 ± 0.12	2.0 ± 0.09
8	91.5 ± 0.18	8.5 ± 0.08
9	82.0 ± 0.12	18.0 ± 0.9
12	68.0 ± 0.14	32.0 ± 0.18

Values represent the mean ± SEM, n = 3

**Table (3): The impact of reaction time on the radiochemical yield of [<sup>99m</sup>Tc]-tricarbonyl histamine complex**

Reaction time (min.)	[ <sup>99m</sup> Tc]-tricarbonyl histamine complex	[ <sup>99m</sup> Tc]tricarbonyl core
5	82.0 ± 0.17	18.0 ± 0.12
15	94.0 ± 0.11	6.0 ± 0.16
30	98.0 ± 0.11	2.0 ± 0.07
45	97.0 ± 0.14	3.0 ± 0.06
60	97.1 ± 0.16	2.9 ± 0.08

Values represent the mean ± SEM, n = 3

**Table (4): In-vitro stability of [<sup>99m</sup>Tc]-tricarbonyl histamine complex in saline**

Time (h)	[ <sup>99m</sup> Tc]-tricarbonyl histamine complex	[ <sup>99m</sup> Tc]tricarbonyl core
3	98.5 % ± 1.04	1.5 ± 0.03
6	98.0 ± 0.59	2.0 ± 0.65
12	97.9 ± 0.44	2.1 ± 0.19
18	97.8 ± 0.97	2.2 ± 0.18
24	97.5 ± 0.66	2.5 ± 0.09

Values represent the mean ± SEM, n = 3

**Table (5): In-vitro stability of [<sup>99m</sup>Tc]-tricarbonyl histamine complex in serum**

Time (h)	[ <sup>99m</sup> Tc]-tricarbonyl histamine complex	[ <sup>99m</sup> Tc]tricarbonyl core
3	98.4 ± 0.95	1.6 ± 0.33
6	97.0 ± 0.65	3.0 ± 0.27
12	92.0 ± 1.22	8.0 ± 0.19
18	90.6 ± 0.27	9.4 ± 0.84
24	89.0 ± 0.39	11.0 ± 0.99

Values represent the mean ± SEM, n = 3

### Purification of [<sup>99m</sup>Tc]-tricarbonyl- histamine complex

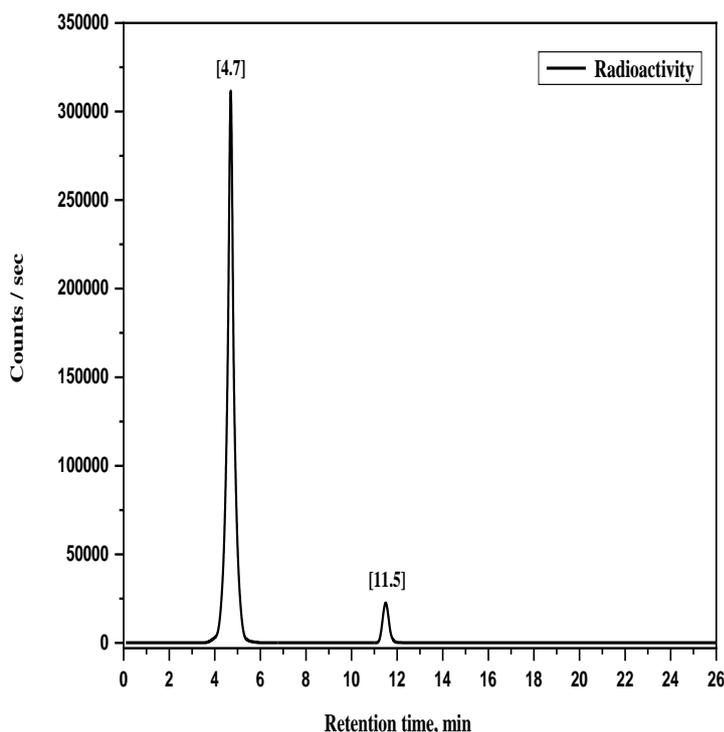
The electrophoresis pattern of [<sup>99m</sup>Tc]-tricarbonyl-histamine complex indicated that labeled compound was observed traveling roughly 1.6 cm towards the anode with a percentage reached 98% while, the free pertechnetate moved in the direction of the anode around 10.9 cm. These results confirm the thin-layer chromatography (TLC) results [61-70].

The reaction mixture was also utilized by High-Performance Liquid Chromatography (HPLC) for the assessment of radiochemical purity yield as illustrated in Figure 2A, which displayed distinct peaks corresponding to both free [<sup>99m</sup>Tc]-tricarbonyl and [<sup>99m</sup>Tc]-pertechnetate, with retention times of 4.7 and 11.5 minutes, respectively. A remarkable radiochemical yield of 98% was obtained through the effective synthesis of the [<sup>99m</sup>Tc]-tricarbonyl precursor.

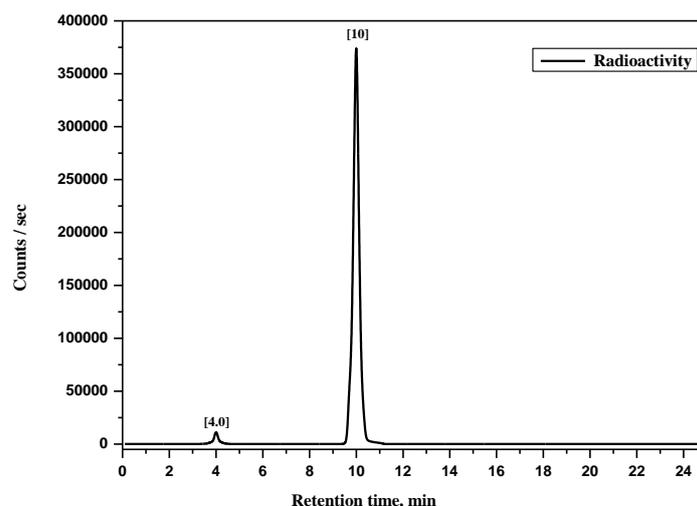
The tricarbonyl-triaqua nucleus was meticulously isolated from the mixture to eradicate any residual pertechnetate before the interaction with histamine. Furthermore, separation by HPLC revealed a high purity of 98% for the [<sup>99m</sup>Tc]-tricarbonyl histamine complex. The retention time (Rt) values for the free [<sup>99m</sup>Tc]-tricarbonyl and [<sup>99m</sup>Tc]-tricarbonyl histamine complex were recorded as 4.0 and 10.0 minutes, respectively, as depicted in Figure 2B.

This comprehensive evaluation through HPLC highlighted the efficiency of the synthesis process in producing the desired [<sup>99m</sup>Tc]-tricarbonyl compound. The meticulous separation of the tricarbonyl-triaqua nucleus underscored the importance of purification steps in achieving high radiochemical purity, as demonstrated by the HPLC analysis results.

The distinct peaks observed in the chromatogram indicated the successful formation of the [<sup>99m</sup>Tc]-tricarbonyl histamine complex, further confirming the effectiveness of the synthesis methodology employed in this study [71-74].



**Fig.2A. The HPLC radiochromatogram of [<sup>99m</sup>Tc]tricarbonyl precursor, at Rt = 4.7 min and free [<sup>99m</sup>Tc]pertechnetate at Rt = 11.5 min.**



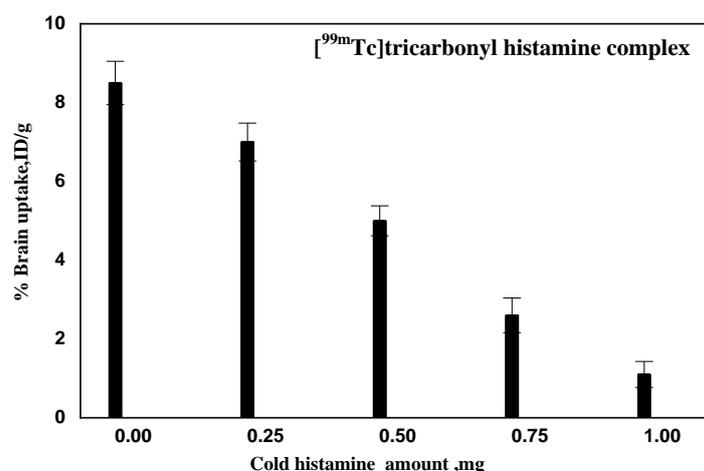
**Fig.2B. High performance liquid chromatography (HPLC) analysis. The  $R_t$  values of free [ $^{99m}\text{Tc}$ ]tricarbonyl and [ $^{99m}\text{Tc}$ ]tricarbonyl histamine complex were 4.0 and 10 min, respectively.**

#### The effect of partition coefficient factor

The impact of the partition coefficient factor can be observed through the analysis of the radiotracer derived from the [ $^{99m}\text{Tc}$ ]-tricarbonyl-histamine complex, which has been documented to exhibit a logarithm of the partition coefficient measuring at  $1.49 \pm 0.04$ . This value indicates the lipophilic nature of the [ $^{99m}\text{Tc}$ ]-tricarbonyl histamine compound, suggesting its ability to penetrate the blood-brain barrier effectively. The significance of understanding the partition coefficient factor lies in its implications for the pharmacokinetics and pharmacodynamics of such compounds, particularly in their distribution within biological systems [75-86].

#### Blocking study of [ $^{99m}\text{Tc}$ ]-tricarbonyl-histamine complex, receptor

Various amounts of unlabeled histamine (cold) were employed in the range of 0.0-1000 $\mu\text{g}$ . The administration of cold histamine to the mice took place 30 minutes before the administration of the radiotracer, with the brain uptake percentage being calculated 5 minutes after the injection of the radiotracer, [ $^{99m}\text{Tc}$ ]-tricarbonyl-histamine complex. Consequently, there was a decrease in brain uptake from 8.5 to 1.11% ID/g organ at 5 minutes post-injection as shown in Figure 3. This reduction can be attributed to the specific binding of the hot labeled compound, [ $^{99m}\text{Tc}$ ]-tricarbonyl-histamine, with its receptors in the brain. This research indicates that [ $^{99m}\text{Tc}$ ]-tricarbonyl-histamine has the potential for successful utilization in the imaging of the brain [87-90].



**Fig. 3 [ $^{99m}\text{Tc}$ ]tricarbonyl histamine complex inhibition brain uptakes in normal male Swiss Albino mice at 5 min p.i. (% ID/g organ  $\pm$  SEM, n = 5).**

### Molecular Modeling Studies

Molecular docking was conducted on the complex structures 1 and 2 of the [ $^{99m}\text{Tc}$ ]-tricarbonyl-histamine, in conjunction with the histamine H1 receptor (PDB code: 7DFL), which were subsequently compared to the ligand co-crystallized with histamine that was previously documented to have an interaction with it as indicated in references [91-94]. The results of the docking analysis revealed that the [ $^{99m}\text{Tc}$ ] tricarbonyl histamine complex structure-1 displayed a docking score of -9.7288 kcal/mol, forming one hydrogen bond with Tyr431 at a distance of 2.69 Å. On the other hand, the [ $^{99m}\text{Tc}$ ] tricarbonyl histamine complex structure-2 exhibited a docking score of -9.5608 kcal/mol and formed one hydrogen bond with Tyr431 at a distance of 2.56 Å (Refer to Figure 4 for visualization of the docking results). In contrast, histamine itself demonstrated a docking score of -11.0478 kcal/mol, showcasing interactions with three hydrogen bonds: Asn107 at a distance of 2.35 Å, Asn198 at a distance of 2.67 Å, and Tyr431 at a distance of 2.63 Å as shown in Figure 5. Consequently, it can be deduced that both Histamine and the [ $^{99m}\text{Tc}$ ] tricarbonyl Histamine complex structures 1 and 2 share a binding motif when interacting with the histamine H1 receptor. This information provides valuable insights into the structural aspects of these complexes and their interactions with the receptor, shedding light on potential pharmacological implications.

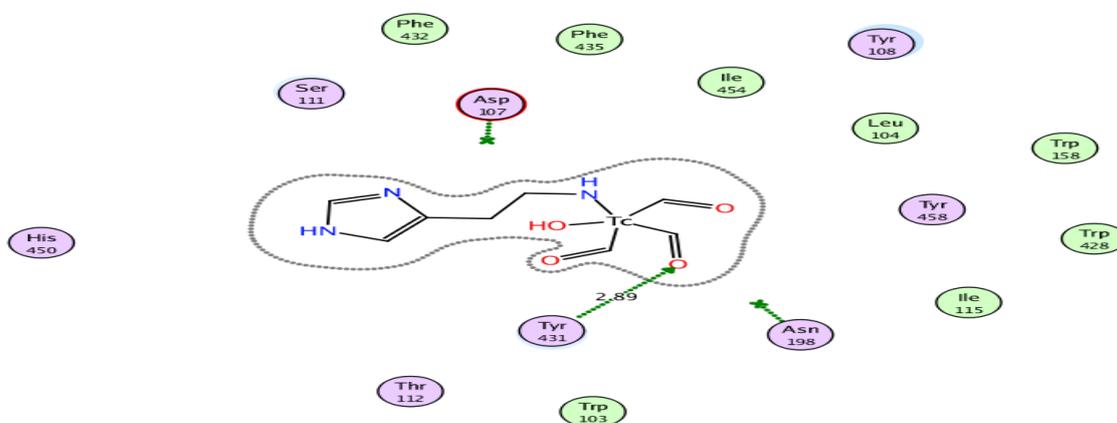


Fig. 4A: Top ranked 2D-pose of [ $^{99m}\text{Tc}$ ] tricarbonyl histamine structure-1 showing interactions in H1R active site.

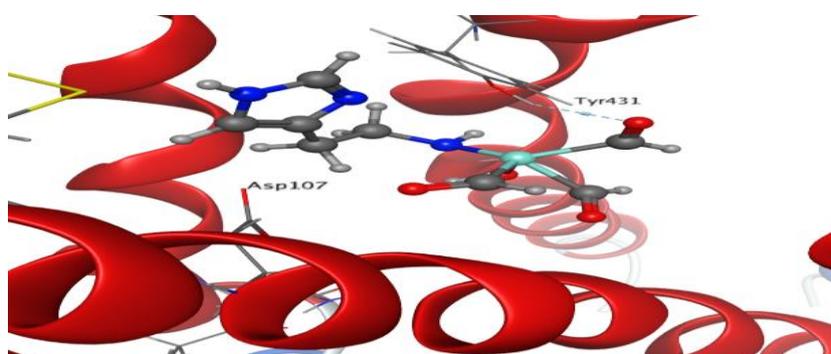


Fig. 4B: Top ranked 3D-pose of [ $^{99m}\text{Tc}$ ] tricarbonyl histamine structure-1 showing interactions in H1R active site

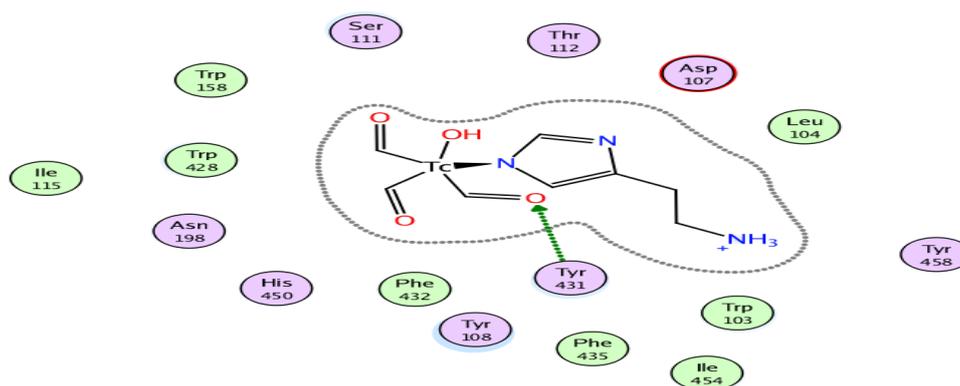


Fig. 4C: Top ranked 2D-pose of [ $^{99m}\text{Tc}$ ] tricarbonyl histamine structure-2 showing interactions in H1R active

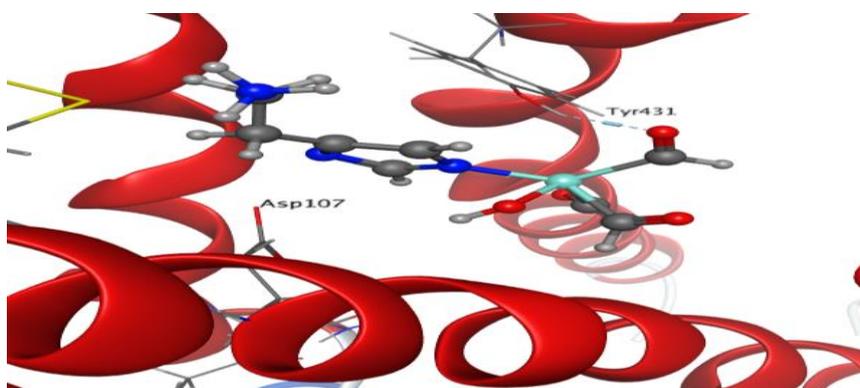


Fig. 4D: Top ranked 3D-pose of [ $^{99m}\text{Tc}$ ] tricarbonyl histamine structure-2 showing interactions in H1R active site

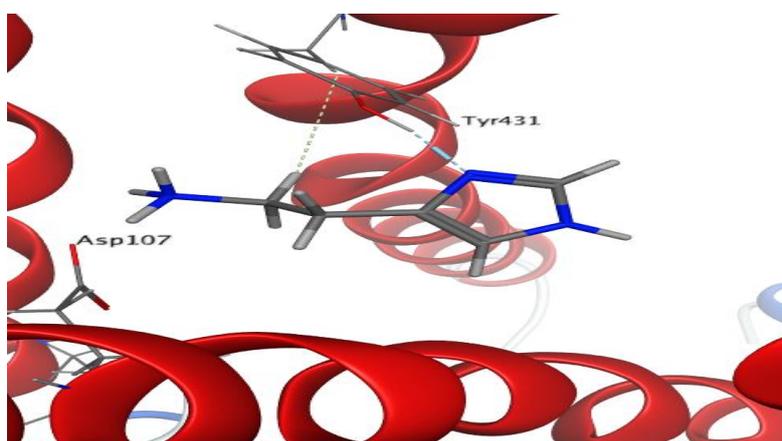
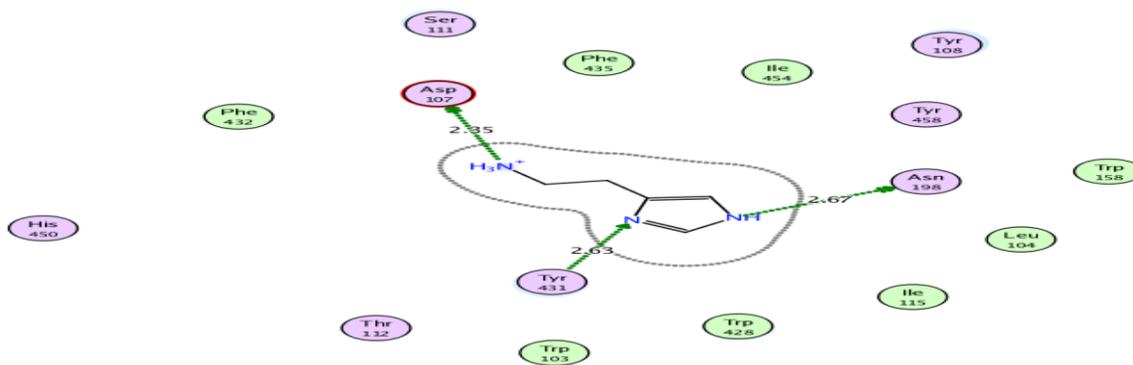


Fig. 5A: 3D Self-docking of histamine showing interactions in H1R active site (PDB code 7DFL).

#### Bioevaluation study

Table 6 illustrates the bioevaluation of the tricarbonyl histamine complex with radiotracer [ $^{99m}\text{Tc}$ ] in various body fluids and organs. The radioactivity levels in terms of the average percent injected dose per g organ tissue (%ID/g organ  $\pm$  SD). The data presented indicates that the initial blood uptake peak of the radiotracer [ $^{99m}\text{Tc}$ ]-tricarbonyl histamine complex was recorded at  $11.99 \pm 0.55$  within 5 minutes post-injection, followed by a gradual decrease in clearance to  $0.99 \pm 0.00\%$  at 120 minutes post-injection. Furthermore, the absorption levels in the brain, identified as the target organ, were notably at  $8.5 \pm 0.00\%$  ID/organ at the 5-minute mark post-injection.



**Fig. 5B: 2D Self- docking of histamine showing interactions in H1R active site (PDB code 7DFL).**

This suggests a rapid accumulation of the radiotracer [ $^{99m}\text{Tc}$ ]-tricarboxyl histamine complex in the brain, indicating specific binding to receptors within the brain, with a gradual decline to  $1.66 \pm 0.001$  at the 120-minute mark post-injection. The primary route of elimination for the tracer is through urine excretion, as supported by existing literature [95-103]. The efficiency of the [ $^{99m}\text{Tc}$ ]-tricarboxyl histamine complex surpasses that of the [ $^{99m}\text{Tc}$ ]-histamine complex, which exhibited a lower percentage at 5 minutes post-injection, signifying its potential superiority. Radiotracers commonly used in clinical practice, such as [ $^{99m}\text{Tc}$ ]-ethyl cysteinyl dimer ([ $^{99m}\text{Tc}$ ]-ECD) and [ $^{99m}\text{Tc}$ ]-hexamethylpropyleneamineoxime ([ $^{99m}\text{Tc}$ ]-HMPAO), have shown a strong affinity for brain tissues [86-87]. Therefore, the promising results of the [ $^{99m}\text{Tc}$ ]-tricarboxyl histamine complex suggest its potential utility in successful brain SPECT imaging procedures. The study findings reveal that the radiotracer [ $^{99m}\text{Tc}$ ]-tricarboxyl histamine complex exhibits a notably higher % ID/g organ  $\pm$  SD value compared to these established radiotracers, underscoring its potential as a valuable tool in nuclear medicine imaging.

**Table .6 Biodistribution of [ $^{99m}\text{Tc}$ ]-tricarboxyl histamine complex in normal mice at different time intervals**

Organs & body fluids	% I.D./organ at different times post injection (Mean $\pm$ SEM, n = 5)					
	5 min	10 min	15 min	30 min	60 min	120 min
Blood	11.99 $\pm$ 0.15	10.17 $\pm$ 0.15	4.11 $\pm$ 0.19	2.8 $\pm$ 0.18	1.77 $\pm$ 0.01	0.99 $\pm$ 0.00
Kidneys	3.67 $\pm$ 0.19	5.99 $\pm$ 0.17	9.12 $\pm$ 0.13	15.37 $\pm$ 0.15	9.22 $\pm$ 0.18	4.21 $\pm$ 0.16
Liver	2.77 $\pm$ 0.19	3.69 $\pm$ 0.27	4.78 $\pm$ 0.19	3.11 $\pm$ 0.01	2.95 $\pm$ 0.01	1.91 $\pm$ 0.02
Spleen	1.18 $\pm$ 0.01	1.17 $\pm$ 0.01	1.15 $\pm$ 0.02	1.09 $\pm$ 0.03	1.08 $\pm$ 0.02	0.88 $\pm$ 0.01
Stomach	0.99 $\pm$ 0.00	0.96 $\pm$ 0.00	0.95 $\pm$ 0.00	0.92 $\pm$ 0.00	0.91 $\pm$ 0.00	0.90 $\pm$ 0.00
Intestine	1.98 $\pm$ 0.19	2.54 $\pm$ 0.17	3.88 $\pm$ 0.02	1.79 $\pm$ 0.05	1.22 $\pm$ 0.02	1.67 $\pm$ 0.01
Heart	1.13 $\pm$ 0.01	1.12 $\pm$ 0.02	1.95 $\pm$ 0.02	1.90 $\pm$ 0.01	1.09 $\pm$ 0.02	0.88 $\pm$ 0.03
Lungs	1.18 $\pm$ 0.04	1.17 $\pm$ 0.05	1.08 $\pm$ 0.04	1.06 $\pm$ 0.03	1.04 $\pm$ 0.02	1.03 $\pm$ 0.01
Brain	8.5 $\pm$ 0.00	7.15 $\pm$ 0.00	5.11 $\pm$ 0.00	4.16 $\pm$ 0.00	3.22 $\pm$ 0.00	1.66 $\pm$ 0.01

Values represent the mean  $\pm$  SEM, n = 5

### Conclusion

The radiotracer [ $^{99m}\text{Tc}$ ]-tricarboxyl-histamine complex can be synthesized using optimized factors that yield a high radiochemical yield (98%). Biodistribution studies have shown that this complex demonstrates a significant brain absorption of 8.5% ID/g organ just 5 minutes post-injection. In comparison to previously described radiotracers like [ $^{99m}\text{Tc}$ ]-ethyl cysteinyl dimer ([ $^{99m}\text{Tc}$ ]-ECD) and [ $^{99m}\text{Tc}$ ]-hexamethylpropyleneamineoxime ([ $^{99m}\text{Tc}$ ]-HMPAO). The outcomes of this research indicate that [ $^{99m}\text{Tc}$ ]-tricarboxyl-histamine has the potential for successful utilization in the imaging of the brain

### Conflict of Interest

There is no conflict of interest associated with this publication.

### Data Availability Statement

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