Egypt. J. Chem. **Vol. 68**, No. 3 pp. 37 - 50 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Facile one-pot strategy for brain imaging using radiolabeled [^{99m}Tc]-tricarbonyl histamine complex in mice.

M.H. Sanad[1], H. M. Eyssa[4], Safaa B. Challan[1,2*], A. B. Farag[6], Fatma Y. Abdou[3], Aiten M. Soliman[3], S. M. Abd-Elhaliem[1], Nermeen M. El Bakary[5]

[1]*Labeled Compound Department, Hot Laboratory Center, Egyptian Atomic Energy Authority, P.O. Box 13755, Cairo, Egypt*
[2]*Cyclotron Facility, Nuclear Research Center, Egyptian Atomic Energy Authority, P.O. Box 13755, Cairo, Egypt*
[3]*Drug Radiation Research Department, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, P.O. Box 11787, Cairo, Egypt*.
[4] *Radiation Chemistry Department, National Center for Radiation Research and Technology,Egyptian Atomic Energy Authority, P.O. Box 11787, Cairo, Egypt*.
[5]*Radiation Biology Department, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, P.O. Box 11787, Cairo, Egypt.*
[6]*Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ahram Canadian University, Giza, 12578 Egypt*

Abstract

This investigation aims to label the histamine ligand, 2-(1H-imidazol-4-yl) ethanamine, using a [^{99m}Tc]-tricarbonyl 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 ± 0.04. This value suggests the lipophilic characteristics of the [^{99m}Tc]-tricarbonyl histamine compound, indicating its potential to cross the blood-brain barrier effectively. The bioevaluation of the radiotracer [^{99m}Tc]-tricarbonyl-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 [^{99m}Tc]-tricarbonyl histamine complex.

Keywords: Histamine ligand; radiolabeled; [^{99m}Tc]-tricarbonyl 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 [^{99m}Tc] N complexes [13-16]; the final radiotracer's chemical and biological properties are influenced by the unique +2 oxidation state of the [^{99m}Tc] nitrido core. Even using different [^{99m}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, ^{99m}Tc, to create the [^{99m}Tc] tricarbonyl 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

*Corresponding author e-mail: safaabekheet@yahoo.com.; (Safaa B. Challan).
Received date 17 April 2024; Revised date 12 July 2024; Accepted date 21 July 2024
DOI: 10.21608/EJCHEM.2024.283339.9602
©2025 National Information and Documentation Center (NIDOC)

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}Tc] tricarbonyl histamine complex.

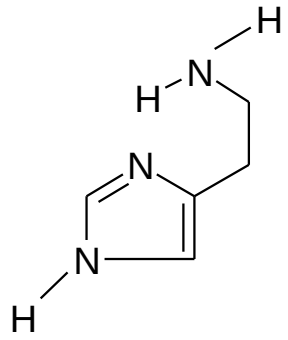

Fig.1A. The chemical structure of histamine

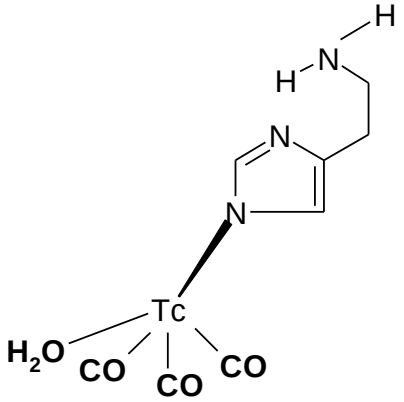

Fig.1B The proposed structure of ^{99m}Tc-tricarbonylhistamine

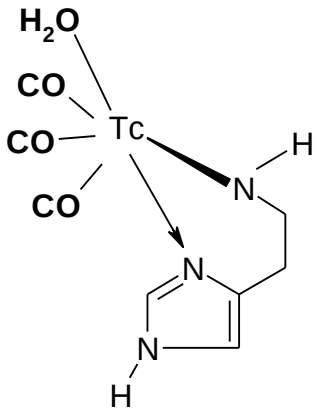

Fig.1C. The proposed structure of [^{99m}Tc]tricarbonyl histamine

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_2O was crucial in the experimental processes. Technetium-99m was eluted as $^{99m}TcO_4^-$ from $^{99}Mo/^{99m}Tc$ 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 gradeInternational, 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 γ-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_2O (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 (250x94.6 mm) packed with 5 µm 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 γ-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 ± 5°C, a 12-hour light-dark cycle, a relative humidity of 60 ± 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 $[^{99m}Tc(CO)_3(H_2O)_3]^+$

Alberto et al. [15] proposed a modified synthon production method. Following established protocols, a solution containing 4 mg of Na_2CO_3, 5.5 mg of $NaBH_4$, and 15 mg of Na/K tartrate in 0.5 ml D.D H_2O. After subjecting the solution to treatment with carbon monoxide gas purge for 5 minutes about 1.0 ml of $^{99m}TcO4$- (37 MBq) was introduced. Followingthis, 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 µL 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 [^{99m}Tc]-tricarbonyl-histamine

A volume of 0.5 mL of the precursor $[^{99m}Tc\ (CO)_3(H_2O)_3]^+$ was introduced into 0.1 mL of the histamine ligand aqueous solution (250 µg). 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 [^{99m}Tc]-tricarbonyl-histamine complex. Utilization of HPLC was conducted for the purification of the [^{99m}Tc]-tricarbonyl-histamine complex.

Purification of [^{99m}Tc]-tricarbonyl- 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 γ-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 µL 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 γ-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 [^{99m}Tc]-tricarbonyl-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 µL of PBS (pH 7.4) and 1 mL of n-octanol was combined with 200 µL of the sample andincubated 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 γ-counter, the measurement of each layer (100 µL) was conducted. The log Po/w values can be expressed from five repetitions of the partition coefficient value [41-45].

Stability of [^{99m}Tc]-tricarbonyl-histamine complex

The stability of [^{99m}Tc]-tricarbonyl-histamine complex was examined in two media, rat serum, and PBS. About 0.2 mL of purified [^{99m}Tc]-tricarbonyl-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 [^{99m}Tc]-tricarbonyl-histamine stability was

assessed [5 µL, 3.20 x 10^{-3}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 µg 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, [^{99m}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 [^{99m}Tc]-tricarbonyl-histamine complex was evaluated in male Swiss albino mice weighing (20-30) g. About 0.2 mL (3.6 MBq) of [^{99m}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 [^{99m}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 ± 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 µg of substrate and a free [^{99m}Tc]-tricarbonyl core (7.5 MBq) the radiochemical conversion of the [^{99m}Tc]-tricarbonyl-histamine complex was 98.5%.

Additionally, Table 2.demonstrates the impact of different pH values on the radiochemical conversion of the [^{99m}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 [^{99m}Tc]-tricarbonyl-histamine complex reached 98% at 30 min. The *in vitro* stability of the [^{99m}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 [^{99m}Tc]-tricarbonyl-histamine complex was stable in saline and reached 97.5% after 24 h. On the other hand, the purity of [^{99m}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 [^{99m}Tc]-tricarbonyl histamine complex

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

Values represent the mean ± SEM, n = 3

Table (2): The impact of different pH values on the radiochemical yield of [^{99m}Tc]-tricarbonyl histamine complex

pH	[^{99m}Tc]-tricarbonyl histamine complex	[^{99m}Tc]tricrbonyl 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 [^{99m}Tc]-tricarbonyl histamine complex

Reaction time (min.)	[^{99m}Tc]-tricarbonyl histamine complex	[^{99m}Tc]tricrbonyl 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 stabilityof [^{99m}Tc]-tricarbonyl histamine complex in saline

Time (h)	[^{99m}Tc]-tricarbonyl histamine complex	[^{99m}Tc]tricrbonyl 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 [^{99m}Tc]-tricarbonyl histamine complex in serum

Time (h)	[^{99m}Tc]-tricarbonyl histamine complex	[^{99m}Tc]tricrbonyl 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 [^{99m}Tc]-tricarbonyl- histamine complex

The electrophoresis pattern of [^{99m}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 [^{99m}Tc]-tricarbonyl and [^{99m}Tc]-pertechnetate, with retention times of 4.7 and 11.5 minutes, respectively. A remarkableradiochemical yield of 98% was obtained through the effective synthesis of the [^{99m}Tc]-tricarbonyl precursor.

The tricarbonyl-triaqua nucleus was meticulously isolated from the mixture to eradicate any residual pertechnetatebefore the interaction with histamine. Furthermore, separation by HPLC revealed a high purity of 98% for the [^{99m}Tc]-tricarbonyl histamine complex. The retention time (Rt) values for the free [^{99m}Tc]-tricarbonyl and [^{99m}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 [^{99m}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 [^{99m}Tc]-tricarbonyl histamine complex, further confirming the effectiveness of the synthesis methodology employed in this study [71-74].

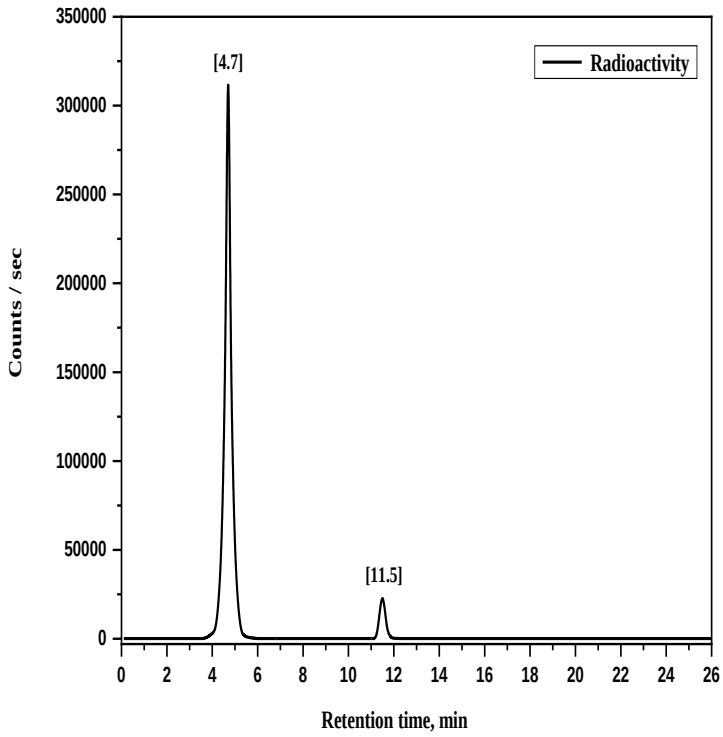

Fig.2A. The HPLC radiochromatogram of [^{99m}Tc]tricarbonyl precursor, atRt = 4.7 min and free [^{99m}Tc]pertechnetate at Rt = 11.5 min.

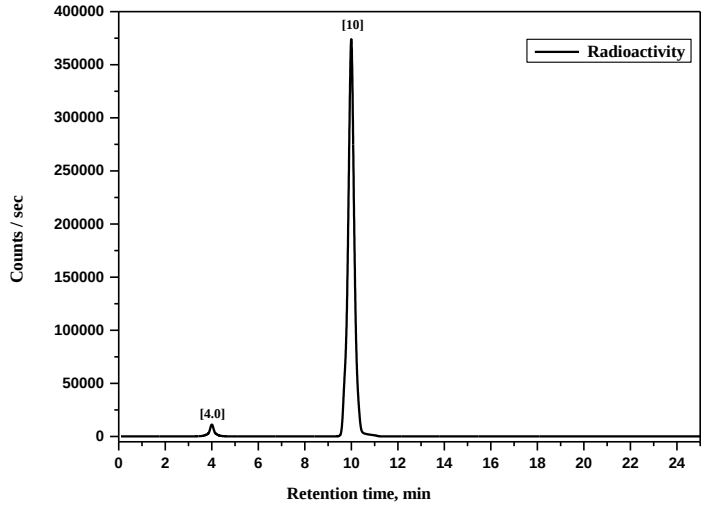

Fig.2B. High performance liquid chromatography (HPLC) analysis. The R_t values of free [^{99m}Tc]tricarbonyl and [^{99m}Tc]tricarbonyl histamine complexwere 4.0 and 10 min, respectively.

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}Tc]-tricarbonyl-histamine complex, which has been documented to exhibit a logarithm of the partition coefficient measuring at 1.49 ± 0.04. This value indicates the lipophilic nature of the [^{99m}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}Tc]-tricarbonyl-histamine complex, receptor

Various amounts of unlabeled histamine (cold) were employed in the range of 0.0-1000µg. 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}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}Tc]-tricarbonyl-histamine, with its receptors in the brain. This research indicates that [^{99m}Tc]-tricarbonyl-histamine has the potential for successful utilization in the imaging of the brain [87-90].

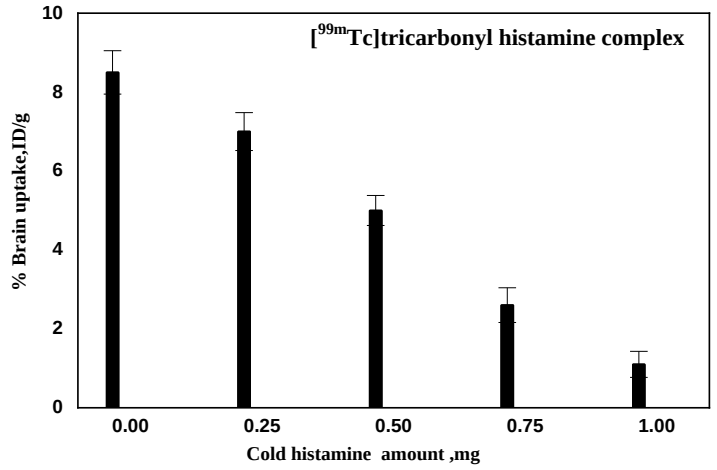

Fig. 3[^{99m}Tc]tricarbonyl histamine complex inhibition brain uptakes in normal male Swiss Albino mice at 5 min p.i. (% ID/g organ ± SEM, n = 5).

Molecular ModelingStudies

Molecular docking was conducted on the complex structures 1 and 2 of the [^{99m}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}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}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}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.

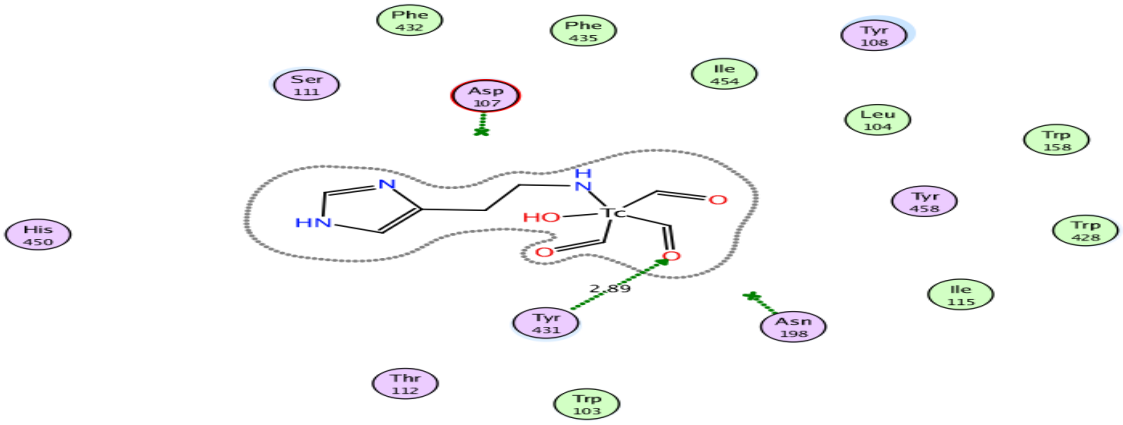

Fig. 4A: Top ranked 2D-pose of [^{99m}Tc] tricarbonyl histamine structure-1 showing interactions in H1R active site.

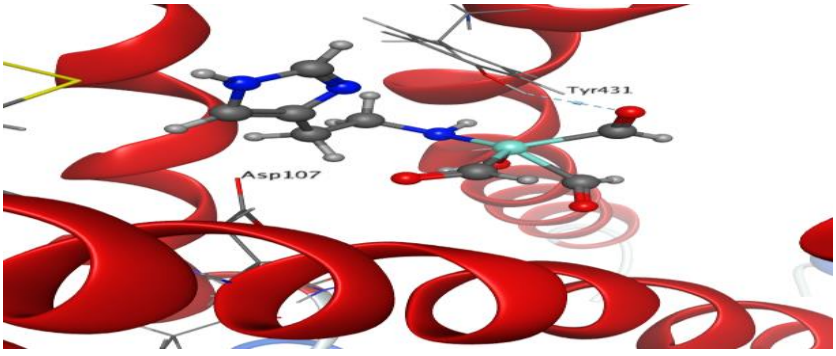

Fig. 4B: Top ranked 3D-pose of [^{99m}Tc] tricarbonyl histamine structure-1 showing interactions in H1R active site

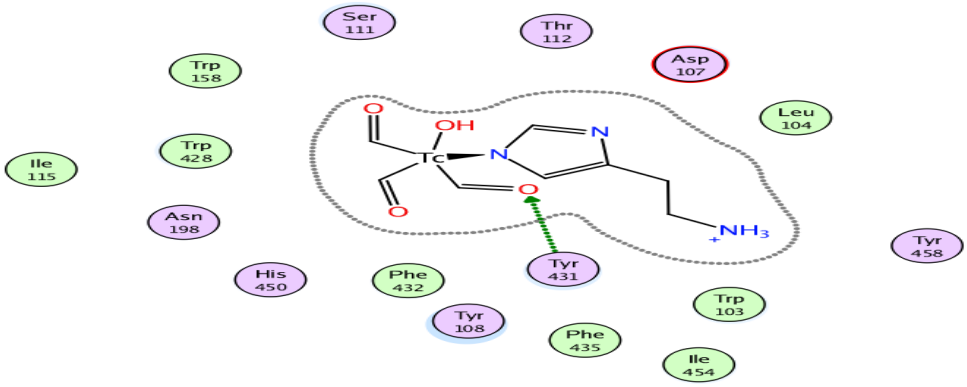

Fig. 4C: Top ranked 2D-pose of [^{99m}Tc] tricarbonyl histamine structure-2 showing interactions in H1R active

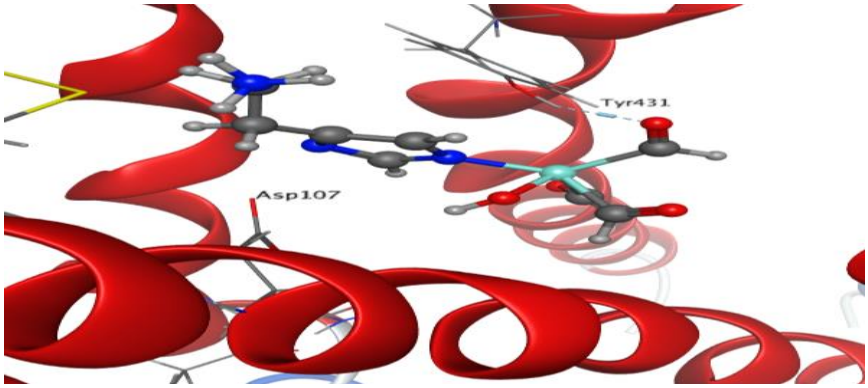

Fig. 4D: Top ranked 3D-pose of [^{99m}Tc] tricarbonyl histamine structure-2 showing interactions in H1R active site

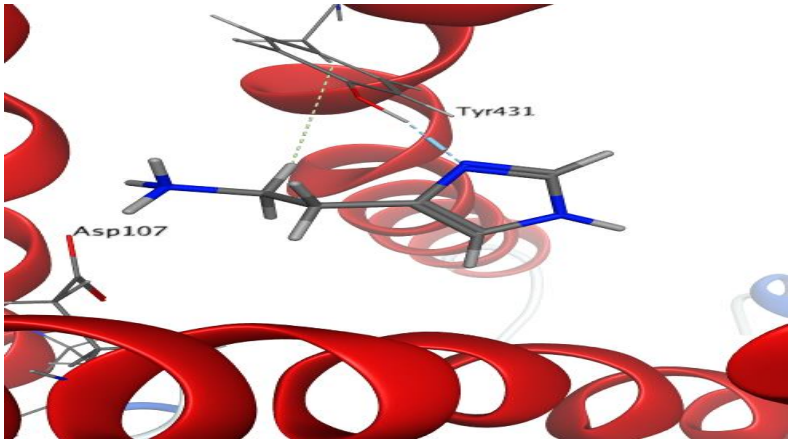

Fig. 5A: 3D Self-docking of histamine showing interactions in H1R active site (PDB code 7DFL).

Bioevaluation study

Table 6 illustrates the bioevaluation of the tricarbonyl histamine complex with radiotracer [^{99m}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 ± SD). The data presented indicates that the initial blood uptake peak of the radiotracer [^{99m}Tc]-tricarbonyl histamine complex was recorded at 11.99 ± 0.55 within 5 minutes post-injection, followed by a gradual decrease in clearance to 0.99 ± 0.00% at 120 minutes post-injection. Furthermore, the absorption levels in the brain, identified as the target organ, were notably at 8.5 ± 0.00%ID/organ at the 5-minute mark post-injection.

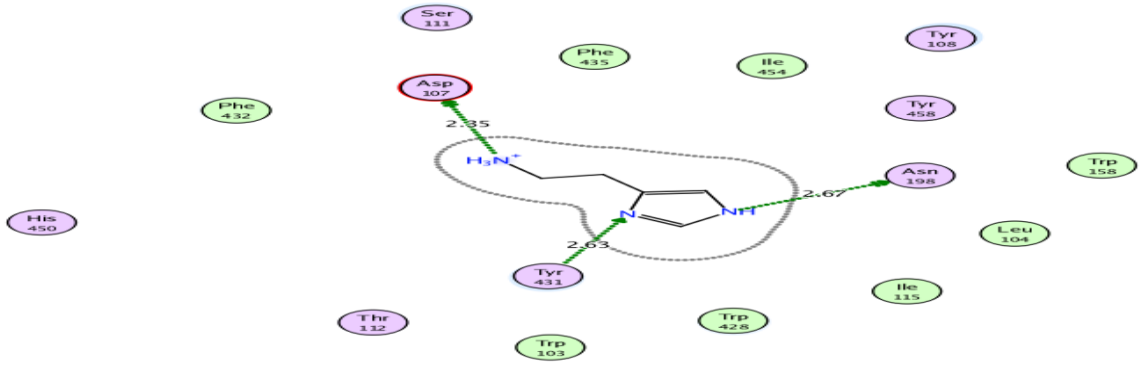

Fig. 5B: 2D Self- docking of histamine showing interactions in H1R active site (PDB code 7DFL).

This suggests a rapid accumulation of the radiotracer [^{99m}Tc]-tricarbonyl histamine complex in the brain, indicating specific binding to receptors within the brain, with a gradual decline to 1.66 ± 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}Tc]-tricarbonyl histamine complex surpasses that of the [^{99m}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}Tc]-ethyl cysteinate dimer ([^{99m}Tc]-ECD) and [^{99m}Tc]-hexamethylpropyleneamineoxime ([99mTc]-HMPAO), have shown a strong affinity for brain tissues [86-87]. Therefore, the promising results of the [^{99m}Tc]-tricarbonyl histamine complex suggest its potential utility in successful brain SPECT imaging procedures. The study findings reveal that the radiotracer [^{99m}Tc]-tricarbonyl histamine complex exhibits a notably higher % ID/g organ ± SD value compared to these established radiotracers, underscoring its potential as a valuable tool in nuclear medicine imaging.

Table .6 Biodistribution of [^{99m}Tc]-tricarbonyl histamine complex in normal mice at different time intervals

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

Values represent the mean ± SEM, n = 5

Conclusion

The radiotracer [^{99m}Tc]-tricarbonyl-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}Tc]-ethyl cysteinate dimer ([^{99m}Tc]-ECD) and [^{99m}Tc]-hexamethylpropyleneamineoxime ([^{99m}Tc]-HMPAO). The outcomes of this research indicate that [^{99m}Tc]-tricarbonyl-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

All the data were collected at the Egyptian Atomic Energy Authority.

Funding
No author obtained any financing for the realization of this research work or publication of the article by any funding institute or agency.

References

[1] Löscher W, Richter A (March 2000). "Piracetam and levetiracetam, two pyrrolidone derivatives, exert antidystonic activity in a hamster model of paroxysmal dystonia". European Journal of Pharmacology. 391 (3): 251–4. doi:10.1016/S0014-2999(00)00105-9. PMID 10729365.

[2] Cohen, Pieter A.; Zakharevich, Igor; Gerona, Roy (2019):"Presence of Piracetam in Cognitive Enhancement Dietary Supplements". JAMA Internal Medicine. 180 (3): 458–459. doi:10.1001/jamainternmed.2019.5507. PMC 6902196. PMID 31764936.

[3] Ahmed AH, Oswald RE (2010). "Piracetam defines a new binding site for allosteric modulators of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors". Journal of Medicinal Chemistry. 53 (5): 2197–203. doi:10.1021/jm901905j. PMC 2872987. PMID 20163115.

[4] Satpati D, Bapat K, Mukherjee A, Banerjee S, Kothari K, Venkatesh M. (2006): Preparation and bioevaluation of ^{99m}Tc-carbonyl complex of 5-hydroxy tryptamine derivative. Appl. Radiat. Isot. 64(8), 888-892 .

[5] Erfani M, Hassanzadeh L, Ebrahimi SES, Shafiei M. (2012):Synthesis and biological evaluation of ^{99m}Tc (CO) 3-OH-PP-CS2 for brain receptor imaging. Iran. J. Nucl. Medicine 20(1), 25.

[6] Walovitch RC, Hill TC, Garrity ST et al. (1989): Characterization of technetium-99m-L, L-ECD for brain perfusion imaging, Part 1: Pharmacology of technetium-99m ECD in nonhuman primates. J. Nucl. Med. 30(11), 1892-1901.

[7] Firstova YY, Abaimov DA, Kapitsa IG, Voronina TA, Kovalev GI (2011). "The effects of scopolamine and the nootropic drug phenotropil on rat brain neurotransmitter receptors during testing of the conditioned passive avoidance task". Neurochemical Journal. 28 (2): 130–141. doi:10.1134/S1819712411020048. S2CID 5845024.

[8] Tiurenkov IN, Bagmetov MN, Epishina VV (2007). "[Comparative evaluation of the neuroprotective activity of phenotropil and piracetam in laboratory animals with experimental cerebral ischemia]".Eksperimental' naiaiKlinicheskaiaFarmakologiia. 70 (2): 24–29. PMID 17523446.

[9] Zhang J, Wang X, Tian C. (2007): Synthesis of a bis-(N-butyl-dithiocarbamato)-nitrido^{99m}Tc complex: A potential new brain imaging agent.*J. Radioanal.Nucl.Chem.*273 15-17

[10] Zhang J, Wang X, Tian C. (2004): Synthesis and biodistribution of ^{99m}TcN (PDTC) 2 as a potential brain imaging agent.*J. Radioanal.Nucl.Chem.*262(2), 505-507.

[11] Lee B, Newberg A. (2005):: Neuroimaging in traumatic brain imaging.*NeuroRx*2(2), 372-383

[12] Ogasawara M, Yamauchi K, Satoh Y-I, et al. (2006): Recent advances in molecular pharmacology of the histamine systems: organic cation transporters as a histamine transporter and histamine metabolism. J. Pharmacol. Sci. 101(1), 24-30.

[13] Blandina P, Bacciottini L, Giovannini M, Mannaioni P. (1998): H3 receptor modulation of the release of neurotransmitters in vivo. In: Pharmacochemistry Library, (Eds). Elsevier 27-40.

[14] Bhadwal M, Satpati D, Singhal S, DevSarma H, Venkatesh M, Banerjee S. (2012) Preparation of ^{99m}Tc (CO) 3-carboxymethylthioethyl iminodiacetic acid and evaluation as a potential renal imaging agent. Curr.Radiopharm. 5(1), 65-70.

[15] Alberto R, Schibli R, Egli A, Schubiger AP, Abram U, Kaden TA. (1998): A novel organometallic aqua complex of technetium for the labeling of biomolecules: synthesis of [^{99m}Tc (OH2) 3 (CO) 3]+ from [99mTcO4]-in aqueous solution and its reaction with a bifunctionalligand.*J. Am. Chem. Soc.* 120(31), 7987-798.

[16] Sanad, M. H., Farouk, N., Fouzy, A. S. M. (2017): Radiocomplexation and bioevaluation of ^{99m}Tc nitrido-piracetam as a model for brain imaging. RadiochimicaActa.; 105(9): 729-737.

[17] Nuutinen S, Panula P. (2010): Histamine in neurotransmission and brain diseases.*Histamine in Inflammation* 95-107.(2006): Recent advances in molecular pharmacology of the histamine systems: organic cation transporters as a histamine transporter and histamine metabolism.*J. Pharmacol .Sci*. 101(1), 24-30.

[18] Blandina P, Bacciottini L, Giovannini M, Mannaioni P. (1998):H3 receptor modulation of the release of neurotransmitters in vivo. In: *Pharmacochemistry Library*, (Eds).Elsevier 27-40.

[19]Satpati D, Bapat K, Mukherjee A, Banerjee S, Kothari K, Venkatesh M.(2006): Preparation and bioevaluation of ^{99m}Tc-carbonyl complex of 5-hydroxy tryptaminederivative.*Appl. Radiat.Isot.*64(8), 888-892.

[20] Sanad, M. H., Rizvi, S. F. A., Farag, A. B. (2021): Synthesis, characterization, and bioevaluation of ^{99m}Tc nitrido-oxiracetam as a brain imaging model. Radiochimica Acta.;109(6): 477-483.

[21] Sanad M, Farag A, Rizvi S. (2021): In silico and in vivo study of radio-iodinated nefiracetam as a radiotracer for brain imaging in mice.*Radiochim. Acta*109(7), 575-582.

[22]Sanad M, Ibrahim AA. (2018): Preparation and biological evaluation of ^{99m}Tc N-histamine as a model for brain imaging: in silico study and preclinical evaluation.*Radiochim. Acta*106(3), 229-238.

[23] Amin A, Sanad M, Abd-Elhaliem S. (2013): Radiochemical and biological characterization of $^{99\ m}$Tc-piracetam for brain imaging. *Radiochemistry*.55: 624-62.

[24] Sanad, M., Farag, A., Husseiny, D. (2017): Radioiodination, molecular modeling and biological evaluation of aniracetam as a tracer for brain imaging. Egyptian Journal of Radiation Sciences and Applications, 30(2):131-143.

[25] Sanad M. H., Marzook E.A., O.A. El-Kawy (2017): Radiochemical and biological characterization of ^{99m}Tc-oxiracetam as a model for brain imaging. Radiochemistry, 59 (6):624-629.
[26] M. H. Sanad, F.A. Marzook, I.T. Ibrahim, S.M. Abd- Elhaliem, N.S. Farrag, (2023): Preparation and bioevaluation of radioiodinatedomberacetam as a radiotracer for brain imaging.Radiochemistry, 65, 114–121.
[27] M. H. Sanad, F. A. Marzook, S. B. Challan, H.M. Essam, B.F. Ayman (2023): Radioiodination, and Biological Assessment of Olsalazine, as a Highly Selective Radiotracer for Ulcerative Colitis Imaging in Mice. Arab. J. Nucl. Sci. &Applic., 56, 105-120.
[28] MH. Sanad, MH. Eysaa, FA. Marzook, AB. Farag, A. Elrefaei, AS.M. fouzy, SB. Challan (2023): Radiocomplexation, biological evaluation, and characterization of [^{99m}Tc]-5-[(3-carboxy-4-hydroxyphenyl)diazenyl]-2- hydroxybenzoic acid as a novel agent for imaging of ulcerative colitis in mice. Radiochemistry, 65:378-386.
[29]Marzook, E. A., Talaat, H. M., Challan, S. B. (2018): Comparative Biological Evaluation of ^{99m}Tc-Timonacic Acid Prepared Using Different Reducing Agents as a Complex for Hepatobiliary Imaging, journal of Radiochemistry. 60(3):309-315.
[30]Challan S. B., Massoud, A. (2017). Radiolabeling of graphene oxide by Tchnetium-99m for infection imaging in rats.Journal of Radioanalytical and Nuclear Chemistry, 314(3), 2189-2199.
[31]Sanad, M. H., Talaat, H. M., Fouzy, A. S. M. (2018): Radioiodination and biological evaluation of mesalamine as a tracer for ulcerative colitis imaging. Radiochimica Acta.;106(5):393-400
[32] M. H. Sanad, H. M. Eyssa, F. A. Marzook, et al., (2023): Radioiodinated Procainamide as radiotracer for Myocardial Perfusion Imaging in Mice. Pharm. Chem. J., 57(4), 543-549.
[33] MH Sanad, Nermien M Gomaa, Nermeen M El Bakary, FA Marzook, Sabry A Bassem. (2023): Radioiodination and Biological Evaluation of Novel Quinoline Derivative for Infective Inflammation Diagnosis.Pharm. Chem. J., 57(7), 1-11.
[34] Sanad M.H., Eyssa HM., FA Marzook, AB Farag, SFA Rizvi, SK Mandal (2021): Comparative bioevaluation and ^{99m}Tc-Sn (II) lansoprazole as a model for peptic ulcer localization. Radiochemistry.;63(5):642-650.
[35] S.B. Challan, S.I. Khater, A.M. Rashad (2022): Preparation, molecular modeling and in-vivo evaluation of ^{99m}Tc-Oseltamivir as a tumor diagnostic agent. Int. J. Radiat. Res., Vol. 20 No. 3, 635-642.
[36] M.A.Motaleb, M. El Tawoosy, S. B. Mohamed, I. H. Borei, H. M. Ghanem, and A. A. Massoud (2014) **:** ^{99m}Tc-Labeled Teicoplanin and its Biological Evaluation in Experimental Animals for Detection of Bacterial Infection. Journal of Radiochemistry.Vol 56, No (5), pp 544-549.
[37] Safaa Bekheet, M. El Tawoosy, A. A. Massoud, I. H. Borei, H. M. Ghanem, and M. A. Motaleb, (2014): ^{99m}Tc-Labeled Ceftazidime and Biological Evaluation in Experimental Animals for Detection of Bacterial Infection, American Journal of Biochemistry. 4(2): 15-24.
[38] Safaa B. Challanand A. A. Massoud, M. El Tawoosy, M. A. Motaleb, I. H. Borei and H. M. Ghanem (2018):^{99m}Tc-Labeled Erythromycin and Biological Evaluation in Mice for Detection of Bacterial Infection: Asian Journal of Physical and Chemical Sciences, 5(2):1-13.
[39] A.M.Amin, S.B.Mohamed, S.S.Moselhy and I.H.Boraei (2009): Technetium-99m Salbutamol: A single Photon Emission Imaging Agent for β_2 -Adrenoceptor and Regional Cerebral Blood Flow. Arab Journal of Nuclear Science and Application.Vol 42, No (2), pp 34-41.
[40] Ibrahim, I. T., Sanad, M. H. (2013): Radiolabeling and biological evaluation of losartan as a possible cardiac imaging agent J. Radiochemistry.;55:336-340.
[41] Sanad M. H, Ibrahim I.T. (2013): Radiodiagnosis of peptic ulcer with technetium-99m pantoprazole. Radiochemistry.;55:341-345.
[42] Sanad, M. H., Abd-Elhaliem, S. M., Abdou, F. Y., Soliman, A. M., &Farag, A. B. (2024). Radiolabeled Nefiracetam for Brain Imaging: Chromatographic Separation, Bio-Evaluation and Preclinical Assessment Studies. Pharmaceutical Chemistry Journal, 1-9.
[43] Sanad, M. H., Eyssa, H. M., Abd-Elhaliem, S. M., Farag, A. B., &Bassem, S. A. (2024). Radiocomplexation, Quality Control and Bioevaluation of [99mTc] tricarbonylRolipram for Brain Imaging in Mice. Pharmaceutical Chemistry Journal, 58(4), 652-660.
[44] Sanad, M. H., Eyssa, H. M., Marzook, F. A., Abd-Elhaliem, S. M., &Abdou, F. Y. (2024). Radiosynthesis, Preparation, and Biological Evaluation of [^{99m}TC] Tricarbonyl Pantoprazole for Stomach Ulcer Detection in Mice. Pharmaceutical Chemistry Journal, 1-7.
[45] Sanad, M. H., Amin, A. M. (2013): Optimization of labeling conditions and bioevalution of ^{99m}Tc-meloxicam for inflammation imaging. J. Radiochemistry.;55: 521-526.
[46] Sanad, M. H., Gomaa, N. M., El Bakary, N. M., Marzook, F. A., &Bassem, S. A. (2023). Radioiodination and Biological Evaluation of Novel Quinoline Derivative for Infective Inflammation Diagnosis. Pharmaceutical Chemistry Journal, 57(7), 1018-1028.
[47] Sanad, M. H., El-Tawoosy (2013): Labeling of ursodeoxycholic acid with technetium-99m for hepatobiliary imaging. J. Radioanal. Nucl. Chem.; 298:1105-1109.
[48] Sanad M. H., Emad H. B. (2014): Performance characteristics of biodistribution of ^{99m}Tc-cefprozil for in vivo infection imaging. J. Anal. Sci. Technol.; 5(1):1-9.
[49] M. H. Sanad, S.B. Challan, H.M. Essam, A. Massoud (2023): Assessment of Radiolabeled L-Carnitine for Hepatotoxicity Imaging in Rats. Radiochemistry, 65 (1), 101-113.

[50] A. Massoud, S. B. Challan, Nabila Maziad (2021): Characterization of poly vinyl pyrrolidone (PVP) with technetium-99m and its accumulation in mice. Journal of Macromolecular Science, Part A. Pure and Applied Chemistry. 2021,58(6)408-418.

[51] Challan, S. B.; Marzook, F. A.; Massoud, A. (2020): Synthesis of RadioiodinatedCarnosine for Hepatotoxicity Imaging Induced by Carbon Tetrachloride and Its Biological Assessment in Rats. Radiochim.Acta, 108, 397- 408.

[52] Sanad, M. H., Challan, S. B., Essam, H. M., Abdou, F. Y., &Farag, A. B. (2024). Design of a novel complex ^{99m}Tc-Nilutamide as a tracer for prostate cancer disorder detection in mice. RadiochimicaActa, https://doi.org/10.1515/ract-2024-0303

[53] Sanad M. H. Borai E (2015): Comparative biological evaluation between ^{99m}Tc tricabonyl and ^{99m}Tc-Sn (II) levosalbutamol as a β2-adrenoceptor agonist. Radiochim.Acta.; 103:879-891.

[54] Sanad M. H. Ibrahim I. T. (2015): Radiodiagnosis of Peptic Ulcer with Technetium-99m LabeledRabeprazole. Radiochemistry.; 57 (4): 425-430.

[55] Sanad, M. H., Abelrahman, M. A., Marzook, F. M. A. (2016): Radioiodination and biological evaluation of levalbuterol as a new selective radiotracer: a β2-adrenoceptor agonist. RadiochimicaActa, 104(5): 345-353.

[56] Borai E. H., Sanad M. H., Fouzy A. S. M. (2016): Optimized chromatographic separation and biological evaluation of ^{99m}Tc-clarithromycin for infective inflammation diagnosis. Radiochemistry.58:84-91.

[57] Sanad M. H, Saad M. M, Fouzy A. S. M., Marzook F., Ibrahim I.T. (2016): Radiochemical and biological evaluation of ^{99m}Tc-Labeling of phthalic acid using ^{99m}Tc-Tricabonyl and 99mTc-Sn (II) as a model for potential hazards imaging. J MolImag Dynamic, 6:1

[58] Sanad, M. H., Sakr, T. M., Abdel-Hamid, W. H.,Marzook, E. A. (2017): In silico study and biological evaluation of ^{99m}Tc-tricabonyl oxiracetam as a selective imaging probe for AMPA receptors. J.Radioanal.Nucl.Chem., 314(3):1505-1515.

[59] Sanad, M. H., El-Bayoumy, A. S. A., Ibrahim, A. A. (2017): Comparative biological evaluation between ^{99m}Tc (CO) 3 and ^{99m}Tc-Sn (II) complexes of novel quinoline derivative: a promising infection radiotracer. J. Radioanal. Nucl. Chem., 311(1):1-14.

[60] Sanad, M. H., Salama, D. H., Marzook, F. A. (2017): Radioiodinated famotidine as a new highly selective radiotracer for peptic ulcer disorder detection, diagnostic nuclear imaging and biodistribution. RadiochimicaActa, 105(5):389-398.

[61] Sanad, M. H., Farag, A. B., &Salama, D. H. (2018): Radioiodination and bioevaluation of rolipram as a tracer for brain imaging: in silico study, molecular modeling and gamma scintigraphy. J. Label Compd. Radiopharm., 61(6): 501-508.

[62] Sanad, M. H., Saleh, G. M., Marzook, F. A. (2017): Radioiodination and biological evaluation of nizatidine as a new highly selective radiotracer for peptic ulcer disorder detection. J. Label. Compd. Radiopharm.; 60(13): 600-607.

[63] Sanad, M. H., Challan, S. B., Marzook, F. A., Abd-Elhaliem, S. M., Marzook, E. A. (2021): Radioiodination and biological evaluation of cimetidine as a new highly selective radiotracer for peptic ulcer disorder detection. RadiochimicaActa.; 109(2): 109-117

[64] Sanad, M. H., Marzook, E. A., Challan, S. B. (2018): Radioiodination of olmesartanmedoxomil and biological evaluation of the product as a tracer for cardiac imaging. RadiochimicaActa.; 106(4):329-336.

[65] Sakr, T. M., Sanad, M. H., Abd-Alla, W. H., Salama, D. H., Saleh, G. M. (2018): Radioiodinatedesmolol as a highly selective radiotracer for myocardial perfusion imaging: In silico study and preclinical evaluation. Applied Radiation and Isotopes, 137: 41-49.

[66] Sanad M ,Challan S. B.(2017) Radioiodination and biological evaluation of rabeprazole as a peptic ulcer localization radiotracer. *Radiochemistry* 59: 307-312.

[67] Sanad, M. H., Rizvi, F. A., Kumar, R. R., Ibrahim, A. A. (2019): Synthesis and preliminary biological evaluation of ^{99m}Tc tricarbonylropinirole as a potential brain imaging agent. Radiochemistry.; 61(6):754-758.

[68] Sanad, M. H., Farag, A. B., FA Marzook., Mandal,S K., (2022): Radiocomplexation, Chromatographic Separation and Bioevaluation of [^{99m}Tc] Dithiocarbamate of Procainamide as Selective Labeled Compound for Myocardial Perfusion Imaging. Pharmaceutical Chemistry Journal.; 56(6): 777-784.

[69] Sanad, M. H., Rizvi, F. A., Kumar, R. R. (2020): Radiosynthesis and bioevaluation of ranitidine as highly selective radiotracer for peptic ulcer disorder detection. Radiochemistry.; 62(1):119-124.

[70] Sanad, M. H., Fouzy, A. S. M., Sobhy, H. M., Hathout, A. S., Hussain, O. A. (2018): Tracing the protective activity of Lactobacillus plantarum using technetium-99m-labeled zearalenone for organ toxicity. International Journal of Radiation Biology, 94(12): 1151-1158.

[71] Sanad, M. H., El-Tawoosy, M., Ibrahim, I. T. (2017): Preparation and biological evaluation of ^{99m}Tc-Timonacic acid as a new complex for hepatobiliary imaging. Radiochemistry.; 59(1): 92-97.

[72] Sanad, M. H., Sallam, K. M., Salama, D. H. (2018): ^{99m}Tc-Oxiracetam as a potential agent for diagnostic imaging of brain: labeling, characterization, and biological evaluation. Radiochemistry.;60(1): 58-63.

[73] Sanad, M., Saleh, G. M., Talaat, H. M., (2017): In silico study and preclinical evaluation of radioiodinatedprocaterol as a potential scintigraphic agent for lung imaging. Egyptian Journal of Radiation Sciences and Applications, 30(2):117-130.

[74] Sanad, M. H., Farag, A. B., SabryA B., FA Marzook (2022): Radioiodination of zearalenone and determination of Lactobacillus plantarum effect of on zearalenone organ distribution: In silico study and preclinical evaluation. Toxicology Reports., 9: 470-479.

[75] Sanad, M. H., Eyssa, H. M., Marzook, F. A., et al., (2021): Optimized chromatographic separation and bioevalution of radioiodinatedilaprazole as a new labeled compound for peptic ulcer localization in mice.Radiochemistry.; 63: 811-819.

[76] Sanad, M. H., Eyssa, H. M., Marzook, F. A., et al., (2021): Synthesis ,radiolabeling, and biological evaluation of ^{99m}Tc-Tricarbonyl mesalamine as a potential ulcerative colitis imaging agent.. Radiochemistry. 63 .842-835

[77] Sanad, M. H., FA Marzook., Farag, A. B., et al., (2022): Preparation, biological evaluation and radiolabeling of [^{99m}Tc]-technetium tricarbonyl procainamide as a tracer for heart imaging in mice. RadiochimicaActa.; 110(4): 267-277.

[78] Rizvi, S.F.A., Tania J., Wajeehah S., Sanad, M. H., Haixia Z (2022): Facile one-pot strategy for radiosynthesis of ^{99m}Tc-Doxycycline to diagnose staphylococcus aureus in infectious animal models. Applied Biochemistry and Biotechnology.;194, 2672-2683.
[79] Sanad, M. H., FA Marzook., Mandal,SK., Baidya M., (2022): Radiocomplexation and biological evaluation of [^{99m}Tc] tricarbonylrabeprazole as a radiotracer for peptic ulcer localization. Radiochemistry.; 64:211-218.
[80] Sanad, M. H., Eyssa., FA Marzook., Farag, A. B., (2021): Preparation and bioevaluation of [^{99m}Tc] tricarbonyl omeprazole for gastric ulcer localization in mice. Radiochemistry.; 64: 54-61.
[81] Yoshiharu K, Koji I, Jiro T. (1999): Technetium-99m complex of N-(2-pyridylmethyl) iminodiacetic acid as a new renal radiopharmaceutical. Annals of Nuclear Medicine.;13(2):127-132.
[82] Taylor A T, Lipowska M, and Marzilli L G. (2010): ^{99m}Tc (CO)3(NTA): A ^{99m}Tc Renal Tracer with Pharmacokinetic Properties Comparable to Those of 131I-OIH in Healthy Volunteers. J Nucl Med.;51(3):391-396.
[83] Klenc J, Lipowska M, Taylor A T, Marzilli LG. (2012): Synthesis and characterization of fac-Re (CO)3-aspartic-Nmonoacetic acid, a structural analogue of a potential new renal tracer, fac-99mTc (CO)3(ASMA). Eur J Inorg Chem.;(27):4334-434
[84] Sanad, M. H., Rizvi, S. F. A., Farag, A. B. (2022): Design of novel radiotracer ^{99m}Tc-N-tetrathiocarbamate as SPECT imaging agent: a preclinical study for GFR renal function. Chemical Papers.; 76(2):1253-1263.
[85] Sanad, M. H., Farag, A. B., Marzook, F. A., Mandal, S. K. (2022). Preparation, characterization, and bioevaluation of ^{99m}Tc-famotidine as a selective radiotracer for peptic ulcer disorder detection in mice. RadiochimicaActa.; 110(1): 67-74.
[86]Sanad M. (2014): Novel radiochemical and biological characterization of ^{99m}Tc-histamine as a model for brain imaging.*J. Anal.Sci Technol*.51.
[87] Walovitch RC, Hill TC, Garrity ST *et al*. (1989): Characterization of technetium-99m-L, L-ECD for brain perfusion imaging, Part 1: Pharmacology of technetium-99m ECD in nonhuman primates.*J. Nucl. Med.* 30(11), 1892-190.
[88] Neirinckx RD, Canning LR, Piper IM et al. (1987): Technetium-99m d, l-HMPAO: a new radiopharmaceutical for SPECT imaging of regional cerebral blood perfusion. J. Nucl. Med. 28(2), 191-202.
[89] Sanad, M. H., Ibrahim, A. A., Talaat, H. M. (2018): Synthesis, bioevaluation and gamma scintigraphy of Sup.^{99m}Tc-N-2-(furylmethyliminodiacetic acid) complex as a new renal radiopharmaceuticalJ. Radioanal.Nucl.Chem., 315(1):57-63.
[90] Sanad, M. H., Farag, A. B., Rizvi, S. F. A. (2021): In silico and in vivo study of radio-iodinated nefiracetam as a radiotracer for brain imaging in mice. RadiochimicaActa.; 109(7): 575-582.
[91] Molecular Operating Environment (MOE). (2008), Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada., H3A, 2R7.
[92] Naïm M, Bhat S, Rankin KN, et al. (2007): Solvated interaction energy (SIE) for scoring protein-ligand binding affinities. 1. Exploring the parameter space. J ChemInf Model.;47(1):122-133.
[93] Paul L. (2009): Protonate3D: assignment of ionization states and hydrogen coordinates to macromolecular structures. Proteins.;75:187-205.
[94] Xia, R., Wang, N., Xu, Z. *et al*. (2021): Cryo-EM structure of the human histamine H_1 receptor/G_q complex. *Nat Commun* 12, 2086.
[95] Sanad, M. H., Gizawy, M. A., Motaleb, M. A., Ibrahim, I. T., Saad, E. A. (2021): A comparative study of stannous chloride and sodium borohydride as reducing agents for the radiolabeling of 2,3,7,8,12,13,17,18-Octaethyl-21H,23H-Porphine with Technetium-99m for tumor imaging. Radiochemistry.; 63(4): 512-519.
[96] Sanad, M.H., (2007): Synthesis and labeling of some organic compounds with one of the most radioactive isotopes. Ph. D. Thesis, Chemistry Department, Faculty of Science, Ain-Shams University, Cairo, Egypt.
[97] Sanad, M. H., Sallam, K. M., Marzook F.A., (2017): Labeling and biological evaluation of^{99m}Tc-tricarbonyl-chenodiol for hepatobiliary imaging. Radiochemistry.; 59: 525–529.
[98] Sanad, M. H., Farag, A. B., Hussain, O. A., &Fuozy, A. S. M. (2024). Facile One-Pot Strategy for Radiocoplexation of [^{99m}tc]-Nitrido-Nebracetam for Brain Imaging: Biological Evaluation, Optimized Chromatographic Separation and Labeling Conditions. *Egyptian Journal of Chemistry*, *67*(13), 471-480.
[99] Sanad, M. H., Farag, A. B., Abdel-Rahman, G. N., &Fouzy, A. S. (2024). Facile One-Pot Strategy ForRadioprepration ofRadioiodinatedPhenylpiracetam As A New Highly Selective Radiotracer For Brainimaging. *Egyptian Journal of Chemistry*, *67*(13), 189-198.
[100] EI-Wetery, A.S.A., Fayz, M.A.A., Sanad, M.H., and EI-Hashash, M.A.M., (2007): Study on the Preparation of ^{99} ^{m}Tc-N (pyrimidine-2-yl-carbamoyl methyl) Iminodiacetic Acid as a New Complex for Hepatobiliary Imaging Agent. Arab Journal of Nuclear Sciences and Applications. 40, 109-118
[101] Lipowska M, Klenc J, Marzilli LG, Taylor AT. (2012): Preclinical evaluation of ^{99m}Tc (CO) 3-aspartic-N-monoacetic acid, a renal radiotracer with pharmacokinetic properties comparable to131 I-o-iodohippurate.*J. Nucl. Med.* 53(8), 1277-1283
[102] Lipowska M, Marzilli LG, Taylor AT. (2009):^{99m}Tc (CO) 3-nitrilotriacetic acid: a new renal radiopharmaceutical showing pharmacokinetic properties in rats comparable to those of 131I-OIH.*J. Nucl. Med.* 50(3), 454-460.
[103] Bhadwal M, Satpati D, Singhal S, DevSarma H, Venkatesh M, Banerjee S. (2012): Preparation of ^{99m}Tc (CO) 3-carboxymethylthioethyl iminodiacetic acid and evaluation as a potential renal imaging agent.*Curr. Radiopharm.*5(1), 65-70.