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Molecular Structure Determination and Stability Parameters Study of ^{99m}Tc-MDP (Technetium 99m Methylene Diphosphonate) Cold Kit and Analysis of Its Binding to Osteocalcin Receptor as a Bone Scan Agent

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ABSTRACT

This work evaluates the stability of tecnethium-99m methylene diphosphonate (^{99m}Tc-MDP) radiopharmaceutical and identifying its precise molecular structures and analyzing their binding to osteocalcin receptor. At first, different formulations of ^{99m}Tc-MDP cold kit were made in various conditions. Then, various molecular structures were evaluated and optimized using B3LYP/Lanl2DZ level of theory by Gaussian software at room temperature. The stability and reactivity properties of the optimized molecular structures were calculated using Frontier molecular orbitals (FMOs) theory. The binding of the molecular structures with the said receptor was analyzed using the molecular docking study. The investigation results indicated that the interactions between the molecular structures and osteocalcon receptor were related to the residues Leu 25, Asn 26, Asp 30, Cys 29, Tyr 42, Tyr 46 and Phe 38.

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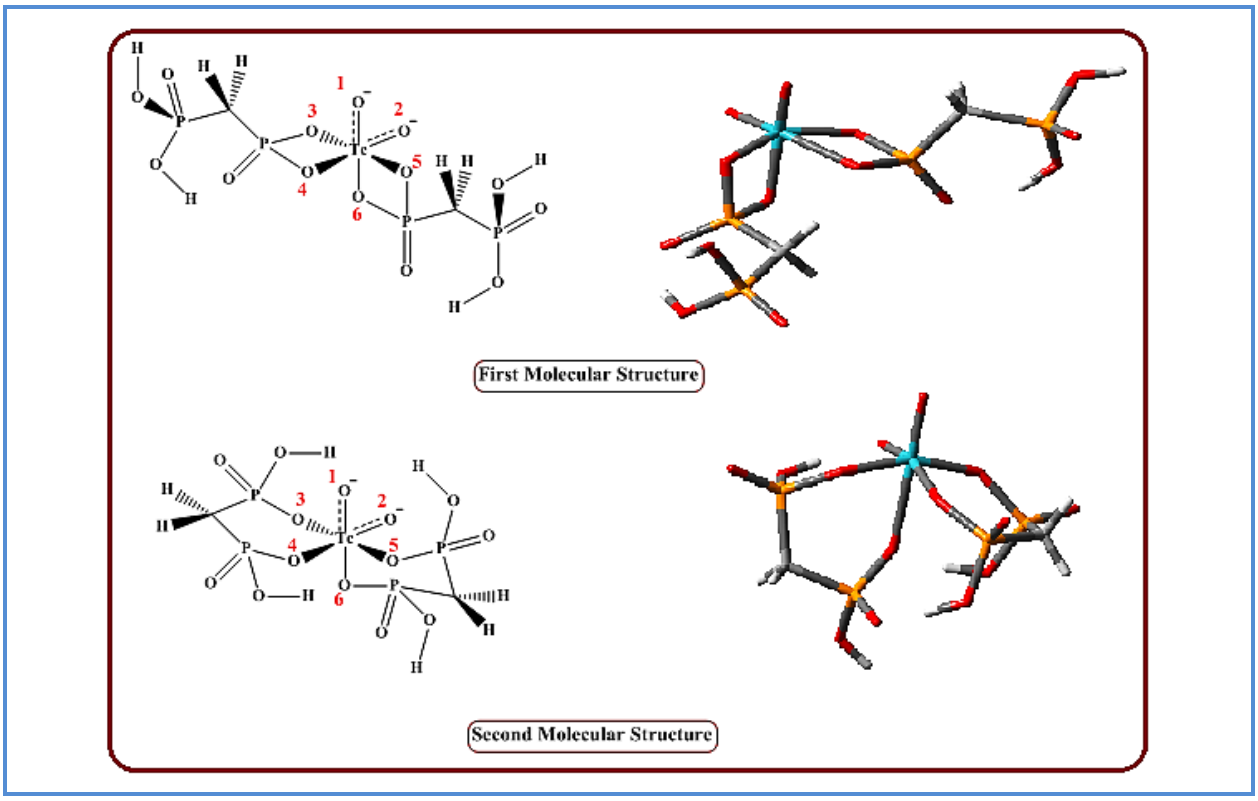
Graphical Abstract



Introduction

Medronate, commonly known as methylene diphosphonate or MDP, is the smallest bisphosphonates, recognized as a family of drugs used in treatment of various bone diseases [1]. Bisphosphonates are the structural analogs of native pyrophosphate with a P-C-P backbone instead of P-O-P [2]. The structural resemblance results in MDP's chelation to calcium and the consequent high affinity for osteocalcin receptors [3]. Osteocalcin is the most prominent non-collagenous protein existing in bone matrix and is produced by osteoblasts. Osteocalcin has a high affinity for calcium ions and thus is believed to be responsible for MDP's transport by osteoblasts to the bone [4]. It was hypothesized that MDP could accumulate in bone *via* adsorption to the surface of hydroxyapatite existent in the bone or absorbing into hydroxyapatite crystalline structure. Other factors were also considered for MDP's binding and interaction with the bone namely, accumulation of MDP at areas of active bone metabolism [5]. The bone-seeking potential of bisphosphonates lead to consideration of utilizing MDP radiolabelled with technetium-99m for skeletal imaging [6]. Technetium-99m emits gamma ray with the energy of 140 keV has a 6 h half-life which makes ^{99m}Tc-MDP a great diagnostic agent in bone scintigraphy, using single photon emission computed

tomography (SPECT) [7]. ^{99m}Tc-MDP specifically binds to and accumulates in bone while delineating areas of altered osteogenesis through gamma emission [8]. Moreover, ^{99m}Tc-MDP has a rapid clearance as well as high urinary excretion which results in a higher contrast between the soft tissue and the bone [9]. It is therefore valuable in early diagnosis and identification of the extent of skeletal diseases as well as locating bone metastases [10]. As the chemistry of this mechanism is quite complicated, no certain theory described the biological behavior of the ^{99m}Tc-MDP. This predicament stems from the vague and incomplete information about the manner in which MDP binds and interacts with technetium-99m. Furthermore, the exact structural mode of interaction between ^{99m}Tc-MDP and osteocalcin receptors is yet not to be understood. In addition, the physiochemical factors affecting the stability and reactivity of ^{99m}Tc-MDP kit is still wildly understudied.

This study was undertaken to shed some light on the precise molecular and structural interaction of MDP with technetium-99m as well as the exact structural manner in which ^{99m}Tc-MDP binds to and interacts with osteocalcin receptor. For this purpose, molecular docking analysis and computational chemistry methods were utilized. Finally, the physical factors affecting the stability and reactivity of ^{99m}Tc-MDP standard kit were analyzed, as well.

Experimental

Materials and computational methods

Methylene diphosphonic acid (MDP, medronic acid), Ascorbic Acid, tin (II)-chloride dihydrate were purchased from Sigma-Aldrich (St. Louis, MO, USA). The $^{99m}TcO_4Na$ was eluted from a ^{99}Mo/^{99m}Tc radionuclide generator (Pars Isotope Company, Tehran, Iran). Methanol, calcium chloride, sodium hydroxide, sodium chloride, potassium hydroxide, potassium chloride and iron chloride were obtained from Merck (Darmstadt, Germany). Solutions were prepared by following the standard procedures and using high-quality water [11]. The distribution of radioactivity on the whatman 1 chromatography papers was quantified using a lablogic mini scan TLC scanner (Sheffield, UK) and analyzed using the Lura image analysis software.

To identify the precise molecular structure of the ^{99m}Tc-MDP diagnostic radiopharmaceutical, computational studies were done using quantum mechanical (QM) methods [12-14]. Different molecular structures were considered for this nuclear medicine and optimized using density functional theory (DFT) method. All the considered molecular structures were optimized at B3LYP/Lanl2DZ basis set of theory and their total energies were compared with each other. Then,

stable molecular structures were chosen and their stability and reactivity properties were calculated based on the Frontier molecular orbitals (FMO) theory [15-17]. Also, molegro virtual docker (MVD) software was used to analyze the binding interactions of the considered molecular structures with the osteocalcin receptor (OR).

Results and discussion

Evaluation of formulation and stability of MDP kit

Many pharmaceutical molecules are designed in kit forms. A stability studies should to be developed before marketing. Also a stability analysis needs to be carried out during the marketing. This program is crucial to determine the unexpected changes of the product when the kit is produced routinely. The assays are based on the radiochemical assay of the product. Stability studies are commonly accepted during two stages in radiopharmaceutical. During development, such studies are used to estimate different formulations and to determine the conditions of storage and the shelf-life of the product. This study aims at evaluating the effect of different formulation and condition on kit stability and shelf life by assessing the radiochemical purity.

The MDP (medronic acid) kit (standard condition) contained 5 mg of medronic acid (as sodium salt), 0.8 mg of stannous chloride dihydrate, and 0.5 mg of ascorbic acid in lyophilized form. pH was adjusted to 5.5-6.5 with sodium hydroxide. Hydroxide chloride used as bulking agent.

As shown in Table 1, we changed some critical factors in the kit formulation such as the amount of $SnCl_2$, ascorbic acid and condition of storages as temperature, moisture and oxygen. Five alternative formulations B-E were prepared by applying some physical parameters after producing kits. The considered parameters are purging O_2, adding H_2O, incubation kits in high temperature, using rejected vials and rubbers. These parameters are considered to evaluate the stability of kits in comparing with the main formulation A.

The stability of the kits depends on the constant presence of the stannous content throughout the period, which in change depends on the freeze-drying conditions, remaining humidity and the storage conditions. In addition, the unique nature of radiopharmaceuticals makes it necessary to give an expiry date, that is depending both on the chemical and radiochemical stability of the product.

As described above, we changed the ratio of ascorbic acid and $SnCl_2$ in the formulation I-K. Also, we assessed other parameters by using old production date of $SnCl_2$ and NaOH, elimination of

dissolved oxygen in solution by purging N_2 and adding KOH, KCl instead of NaOH, NaCl. Each formulation kit was stored at refrigerator (5 °C) and room temperature.

For radiolabeling, to any formulations, we added sodium pertechnate ($Na^{99m}TcO_4$) containing 3-4 mL of 150-300 mCi of $^{99m}TcO_4^-$. The reaction was incubated at room temperature for 30 min. Then the radiochemical yield was determined using whatman 1 chromatography papers in different mobile phases such as normal saline and methanol 85%. In TLC, when methanol was used as mobile phase, free $^{99m}TcO_4^-$ and migrate to the solvent front (Rf = 1.0), while the MDP-bound ^{99m}Tc and colloid remain at the application point (Rf = 0.0). However, in saline system, the colloids remained near the point of spotting (Rf = 0.0–0.3) and ^{99m}Tc-MDP and free $^{99m}TcO_4^-$ moved towards the solvent front. Shelf lives (stability of labeled compounds in solution over time) of the kits were determined using TLC at various times (1 h and 4 h). In addition, the stability of lyophilized kits was monitored by TLC-scanner until 2 months.

All formulations except formulation H showed high radiochemical yield (Table 1). Any formulation remained at more than 97% at up to 4 h. Metal thranschelators was displayed to formulate H by iron cation as cation which reacts rapidly to form a metal with the ligand, then is displaced by ^{99m}Tc. In addition, after 2 months, radiochemical yield and stability was accomplished. Although stability of any formulation was accepted constant, color of appearance white lyophilized powder change to yellow in any formulation that were stored at room temperature instead of refrigerator temperature.

Despite of the importance and extensive clinical use of radiopharmaceutical agents, almost no absolute information is accessible to their chemical compositions or structures even for the complex ^{99m}Tc-MDP by quantum mechanical. The bonding and interaction between the diphosphonate ligands and technetium can be used to predict the optimum formulation and high stability or shelf life of efficacious ^{99m}Tc skeletal imaging agents. The optimum condition for high radiochemical yield and stability was accepted *via* reporting the structural characterization of a ^{99m}Tc complex with methylene diphosphonate (MDP), which is the main aim of this communication.

^{99m}Tc-MDP structural properties study

To the best of our knowledge, no exact molecular structure for this medicinal compound has been identified [1-11]. So, we used the computational chemistry methods for identify its molecular structure. In first step, different molecular structures were considered for this nuclear medicine

and then were optimized using the B3LYP/Lanl2DZ level of theory at room temperature in the isolated forms.

Table 1. Conditions of optimum formulation and stability of ^{99m}Tc-MDP complex

Formulation	MDP (mg)	$SnCl_2$ (mg)	Ascorbic acid (mg)	NaOH	NaCl	Physical and chemical changes	% Labeling yield	% Stability in solution up to 4 h
A	5	0.8	0.5	✓	✓	-	98	98
B	5	0.8	0.5	✓	✓	Temperature	98	97
C	5	0.8	0.5	✓	✓	O_2	98	98
D	5	0.8	0.5	✓	✓	Moisture	97	97
E	5	0.8	0.5	✓	✓	Rejected vial	98	98
F	5	0.8	0.5	✓	✓	Rejected rubber	98	98
G	5	0.8	0.5	✓	✓	Adding $FeCl_2$	97	98
H	5	0.8	0.5	✓	✓	Adding $CaCl_2$	60	50
I	5	0.8	1.6	✓	✓	More amount of ascorbic acid	99	99
J	5	0.5	1	✓	✓	Changing ratio of excipient	97	98
K	5	0.5	0.5	✓	✓	Less amount of $SnCl_2$	97	97
L	5	0.8	0.5	-	-	Using old NaOH	97	98
M	5	0.8	0.5	✓	✓	Using KOH and KCl	98	98
N	5	0.8	0.5	✓	✓	Purging N_2 gas	100	100
O	5	0.8	0.5	-	-	Using old $SnCl_2$	90	90

The energies of the optimized molecular structures were compared with each other. This radiopharmaceutical can have two stable molecular structures. (Figure 1) shows the most stable molecular structures of ^{99m}Tc-MDP nuclear medicine. The previous studies were showed that the Tc-O1 and Tc-O2 bonds are in front of each other and the O1-Tc-O2 bond angle is 180 degree; however, our computations showed that the Tc-O1 bond is next to the Tc-O2 bond and the O1-Tc-O2 bond angle is 104.7 degree and 102.4 degree for first and second molecular structures, respectively (Table 2). The Tc-O1 and Tc-O2 bond lengths of the first molecular structure are 1.715 A° and 1.723 A°, respectively. These bond lengths are 1.713 A° and 1.749 A° for the second molecular structure. As seen in Table 2, the Tc-O bond lengths are not similar in both optimized molecular structures. The O-Tc-O bond angles are not similar, as well. So, the cores of these structures did not show an octahedral geometry.

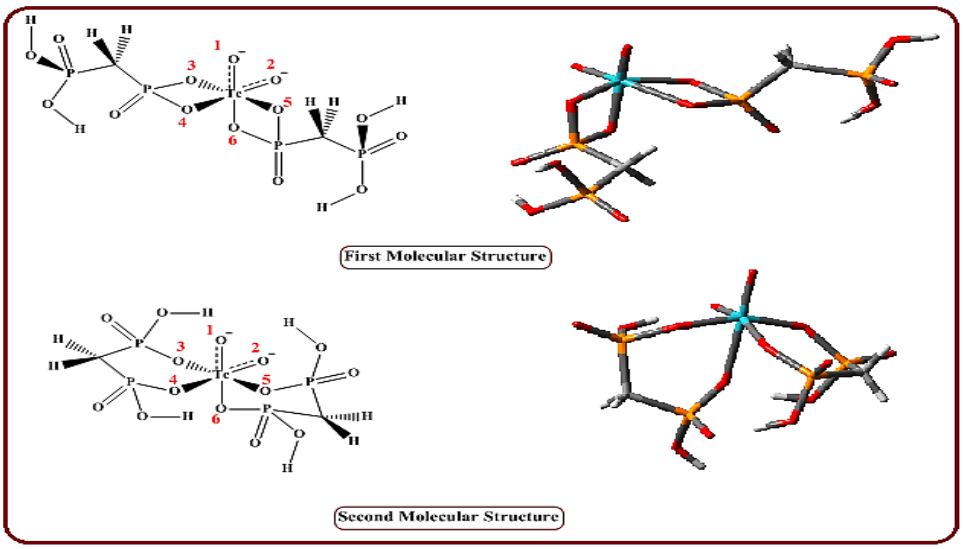


Figure 1. The theoretical geometric structures of ^{99m}Tc-MDP radiopharmaceutical

Table 2. The theoretical bond lengths and bond angles of ^{99m}Tc-MDP molecular structures

Bond lengths (A°) and bond angles (degree)	First structure	Second structure
Tc-01	1.715	1.713
Tc-02	1.723	1.749
Tc-03	2.000	1.920
Tc-04	2.105	1.935
Tc-05	1.924	1.952
Tc-06	2.355	2.413
01-Tc-02	104.69	102.36
01-Tc-03	106.57	99.57
01-Tc-04	94.86	98.63
01-Tc-05	99.63	98.85
06-Tc-02	85.94	80.22
06-Tc-03	81.36	82.57
06-Tc-04	77.29	78.79
06-Tc-05	69.55	78.72
02-Tc-03	88.36	92.32
03-Tc-04	71.39	84.62
04-Tc-05	87.17	86.16
05-Tc-02	103.99	90.13

Stability and reactivity study of optimized ^{99m}Tc-MDP molecular structures

Frontier molecular orbitals (FMOs) theory is the main concept of the molecular orbital (MO) theory [18]. The term “Frontier” refers to the orbitals that are at the outer edges of the molecules. This theory (FMO) is a powerful practical model for describing the stability and the chemical reactivity

of the organic compounds. The main aspect of this theory is related to the highest occupied molecular orbital (filled HOMO) and the lowest unoccupied molecular orbital (empty LUMO) [19].

To describe the stability and reactivity of the title molecular structures, first, their global reactivity indices should be calculated using the FMOs energies. The global reactivity descriptors including, energy gap (Eg), ionization potential (IP), electron affinity (EA), chemical hardness (η), chemical softness (S), electronegativity (χ), electronic chemical potential (μ) and electrophilicity index (ω) can be obtained from the energies of the Frontier orbitals. These reactivity indices are achieved by following equations [20-22].

$$E_g = E_{LUMO} - E_{HOMO}$$

$$IP = -E_{HOMO}$$

$$EA = -E_{LUMO}$$

$$\eta = \frac{(\varepsilon_{LUMO} - \varepsilon_{HOMO})}{2}$$

$$\chi = \frac{-(\varepsilon_{LUMO} + \varepsilon_{HOMO})}{2}$$

$$\mu = \frac{(\varepsilon_{LUMO} + \varepsilon_{HOMO})}{2}$$

$$\omega = \frac{\mu^2}{2\eta}$$

$$S = \frac{1}{\eta}$$

(Table 3) represents the global reactivity indices and Frontier molecular orbitals energies of the ^{99m}Tc-MDP molecular structures. As seen in Table 3, the HOMO and LUMO energies are -5.57 eV and -2.59 eV for the first molecular structure of the said radiopharmaceutical. These low energies of the Frontier orbitals showed the high stability of this molecular structure. In contrast, low energy gap of HOMO and LUMO (2.98 eV) indicated that, the transition of electrons can be easily done in this molecular structure (Figure 2). The density of states (DOS) graph of the first molecular structure indicated that the occupied orbitals had a great density than that of the virtual orbitals. So, this molecule probability prefers to interact with electron poor residues of the receptors. On the other hand, the energy of the HOMO and LUMO of the second molecular structure is -5.11 eV and -2.77 eV, respectively. The Frontier molecular

orbitals energy gap of this molecular structure is 2.34 eV. It can be concluded that, it is a stable compound; however, the reactivity of the second molecular structure is higher than the first molecular structure. Its DOS graph revealed that the molecular structure had a high probability to interact with the electron poor reagents as the first molecular structure. The chemical hardness, chemical softness and electrophilicity indices data indicated that the molecular orbitals of both molecular structures of ^{99m}Tc-MDP have high tendency to accept the electrons in interaction with the biomolecules. So, it can be predicted that the steric interactions play a crucial role in binding of the title radiopharmaceutical to the osteocalcin receptor.

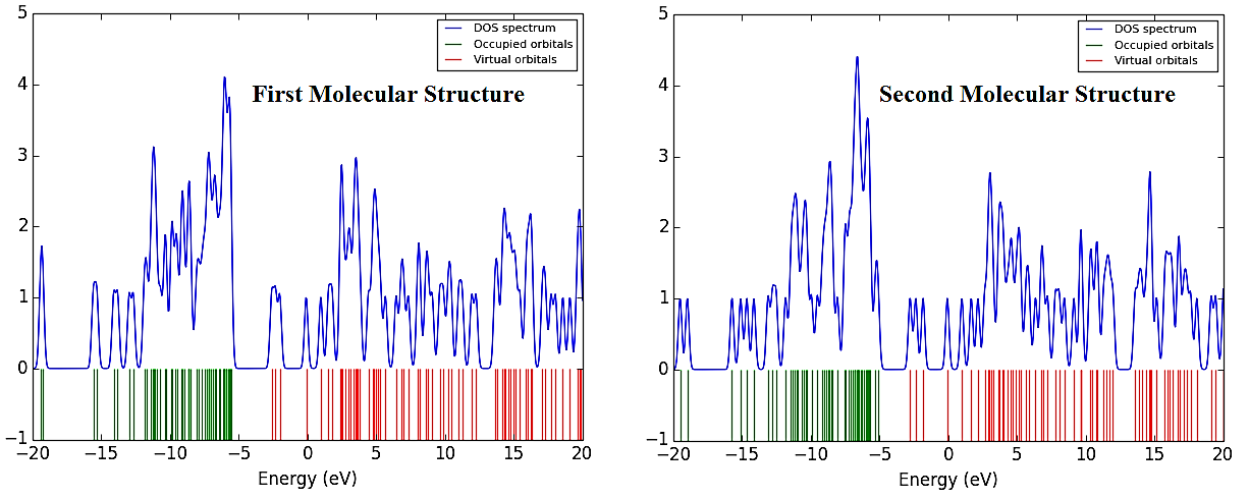


Figure 2. The density of states (DOS) graph of the ^{99m}Tc-MDP molecular structures

Table 3. Global reactivity indices of ^{99m}Tc-MDP molecular structures

Parameter	Energy value (eV)	
	First structure	Second structure
HOMO	-5.57	-5.11
LUMO	-2.59	-2.77
Ionization potential (IP)	5.57	5.11
Electron affinity (EA)	2.59	2.77
Energy gap (Eg)	2.98	2.34
Electronegativity (χ)	4.08	3.94
Chemical potential (μ)	-4.08	-3.94
Chemical hardness (η)	1.49	1.17
Chemical softness (S)	0.67	0.85
Electrophilicity index (ω)	5.59	6.63

Receptor-ligand binding analysis

Literature review clearly showed that the ^{99m}Tc-MDP radiopharmaceutical binding to the osteocalcin receptor has not been recognized until now. In this section, we docked the

optimized molecular structures into the osteocalcin receptor. Figure 3. indicates the ^{99m}Tc-MDP molecular structures embedded in the active site of the osteocalcin receptor. From the data of the Tables 4 and 5, the total energy scores of the ligand-receptor complex formation are -107 and -80 for the first and second molecular structures, respectively. So, the first molecular structure has revealed a greater tendency than the second molecular structure to make complex with the osteocalcin receptor. Steric interactions play a significant role in both molecular structures that bond to the said receptor. Figure 4. depicts that, the residues Cys 29, Leu 25 and Asn 26 make hydrogen bonds with the first molecular structure. In contrast, the second molecular structure interacts with Tyr 46 and Leu 25 residues of osteocalcin receptor by hydrogen bond interactions. On the other hand, both the molecular structures of ^{99m}Tc-MDP radiopharmaceutical can make steric interactions with Tyr 42, Phe 38, Tyr 46, Ala 33, Cys 29, Asp 30, Asn 26, and Leu 25. The residue Ala 41 makes steric interaction with second molecular structure. As can be seen in Table 6, important interactions between first molecular structure and osteocalcon receptor are related to the residues Leu 25, Asn 26, Asp 30, Cys 29, Tyr 42, and Tyr 46 and water molecules 50, 39, 16, 25, 42 and 37. The calcium cofactors do not play the main role in this complex formation. We can also see that, the complex formation between the second molecular structure and osteocalcin receptor is mainly done by the residues Leu 25, Asp 30, Tyr 46, Tyr 42, Asn 26 and Phe 38 (Table 7).

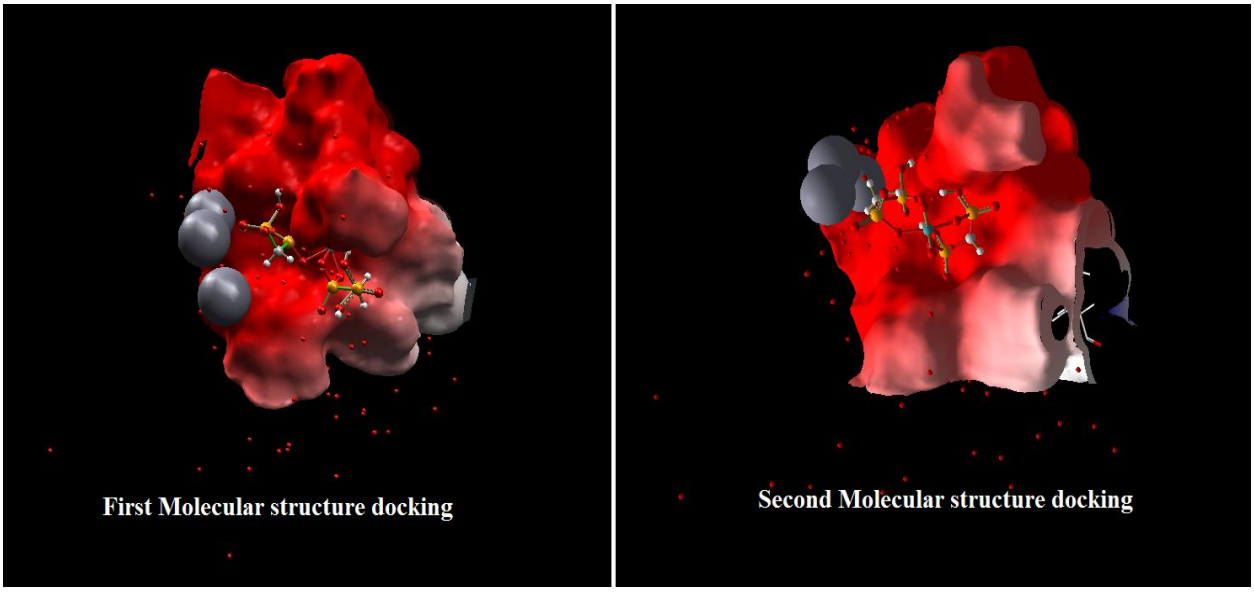


Figure 3. ^{99m}Tc-MDP molecular structures embedded in the active site of the osteocalcin receptor

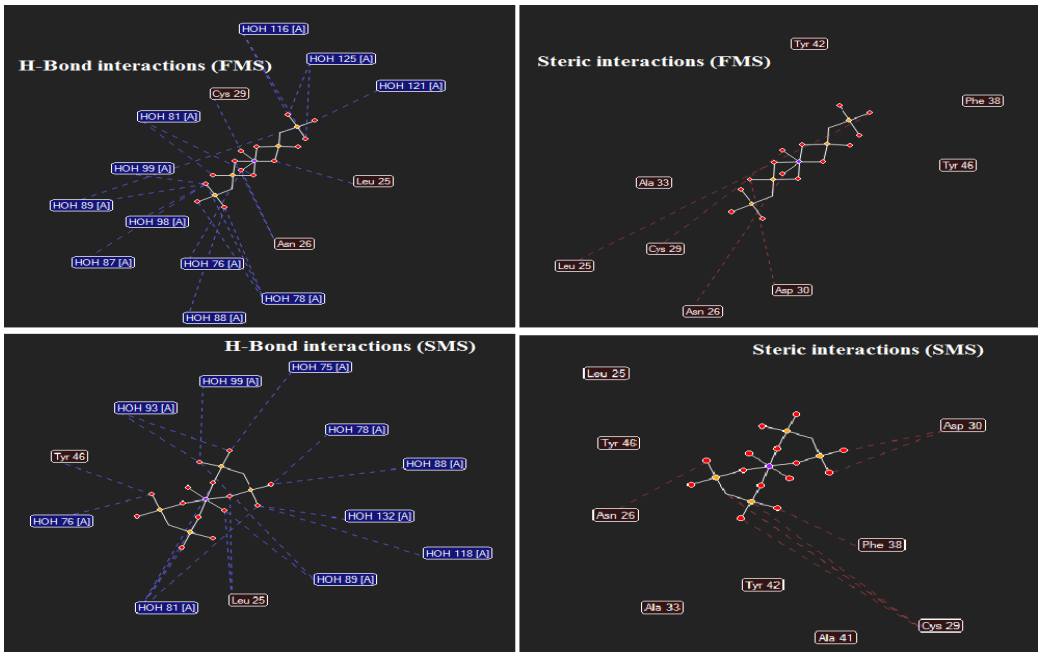


Figure 4. H-bond and steric interactions of ^{99m}Tc-MDP molecular structures embedded in the active site of osteocalcin receptor

Table 4. ^{99m}Tc-MDP first molecular structure interactions with osteocalcin receptor

Interactions		Mol dock score
Protein-ligand interactions	Steric (by PLP)	-45.668
	Steric (by LJ12-6)	-7.769
	Hydrogen bonds	-6.097
	Hydrogen bonds (no directionality)	-24.079
Cofactor-ligand interactions		-0.047
Water-ligand interactions		-46.984
Internal ligand interactions	Torsional strain	4.266
	Steric (by PLP)	-12.167
	Steric (by LJ12-6)	-2.027
External and internal ligand interactions	Total Energy	-106.696

Table 5. ^{99m}Tc-MDP second molecular structure interactions with osteocalcin receptor

Interactions		Mol dock score
Protein-ligand interactions	Steric (by PLP)	-36.422
	Steric (by LJ12-6)	72.869
	Hydrogen bonds	-3.266
	Hydrogen bonds (no directionality)	-27.484
Cofactor-ligand interactions		-2.387
Water-ligand interactions		-22.426
Internal ligand interactions	Torsional strain	0.000
	Steric (by PLP)	-15.885
	Steric (by LJ12-6)	1.960
External and internal ligand interactions	Total Energy	-80.386

Table 6. The participated osteocalcin residues in first molecular structure-receptor interactions

Residue/HOH	Total energy score
Leu 25	-14.306
Asn 26	-12.735
Asp 30	-8.704
Water HOH 50	-6.570
Water HOH 39	-5.996
Cys 29	-5.711
Water HOH 16	-5.619
Water HOH 25	-5.069
Water HOH 42	-4.000
Water HOH 37	-3.729
Tyr 42	-3.495
Tyr 46	-3.460
Water HOH 60	-3.134
Water HOH 59	-3.029
Water HOH 21	-3.016
Water HOH 48	-3.011
Water HOH 49	-2.629
Phe 38	-1.262
Cofactor (Ca) 71	-1.045
Ala 33	-0.514
Cofactor (Ca) 73	-0.506
Water HOH 32	-0.444
Cofactor (Ca) 72	1.504

Table 7. The participated osteocalcin residues in second molecular structure-receptor interactions

Residue/HOH	Total energy score
Leu 25	-10.095
Water HOH 42	-9.202
Asp 30	-7.022
Water HOH 50	-5.987
Tyr 46	-5.478
Tyr 42	-5.367
Asn 26	-4.238
Phe 38	-4.078
Water HOH 39	-3.380
Water HOH 60	-3.269
Water HOH 37	-3.045
Water HOH 32	-2.325
Water HOH 36	-2.200
Cofactor (Ca) 71	-2.165
Water HOH 18	-1.962
Ala 33	-1.805
Cofactor (Ca) 72	-1.780
Water HOH 49	-1.148
Ala 41	-1.040
Water HOH 48	-0.804
Cys 29	1.422
Cofactor (Ca) 73	1.558
Water HOH 54	11.012

Conclusions

This work is divided into two sections. The experimental section relates to the different formulations of the medronate cold kit that were made in various conditions. After 2 months, the radiochemical yield and stability was accomplished. Storing the kits at room temperature instead of refrigerator temperature (5 °C) changed the color from white lyophilized powder to yellow; however the stability of all yellow kits was constant as white kits. The theoretical section was to identify the precise molecular structures of the nuclear medicine. The quantum mechanical computations showed two most stable molecular structures for this radiopharmaceutical. The global reactivity indices indicated the high stability of the molecular structures and their tendency to interact with the biomolecules. The docking results revealed that, the main interactions between the molecular structures and osteocalcon receptor were associated with the residues Leu 25, Asn 26, Asp 30, Cys 29, Tyr 42, Tyr 46, and Phe 38.

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Conflict of Interest

We have no conflicts of interest to disclose.

References

[1] Rodan G.A., Fleisch H.A. *J. Clin. Invest.*, 1996, **97**:2692

[2] Holstein S.A. *Expert Opin. Ther. Pat.,* 2019, **27:**1

[3] Soares A.P., do Espírito Santo R.F., Line S.R., Pinto Md., Santos Pde M., Toralles M.B., do Espírito Santo A.R. *Environ. Toxicol. Pharmacol.*, 2016, **42**:212

[4] Neve A., Corrado A., Cantatore F.P. *J. Cell Physil.*, 2013, **228**:1149

[5] Radioisotopes I.A.E.A. No, R.S., Series No. 1: Technetium-99m radiopharmaceuticals: status and trends. Vienna; 2009; p 283

[6] Subramanian G., McAfee J.G., Blair R.S., Kallfelz F.A., Thomas F.D. *J. Nucl. Med.,* 1975, **16**:744

[7] Makris G., Tseligka E.D., Pirmettis I., Papadopoulos M.S., Vizirianakis I.S., Papagiannopoulou D. *Mol. Pharm.*, 2016, **13**:2301

[8] Cole L.E., Vargo-Gogola T., Roeder R.K. *Adv. Drug Deliv. Rev.,* 2016, **99**:12

[9] Blake G.M., Park-Holohan S.J., Cook G.J., Fogelman I. *Semin. Nucl. Med.,* 2001, **31**:28

[10] Kanishi D. *Oral Surg. Oral Med. Oral Pathol.*, 1993, **75**:239

[11] Sabahnoo H., Noaparast Z., Abedi S.M., Hosseinimehr S.J. *Eur. J. Med. Chem.*, 2017, **127**:1012

[12] Nabati M., Bodaghi-Namileh V. *Adv. J. Chem. A,* 2020, **3**:58

[13] Nabati M. *J. Med. Chem. Sci.,* 2020, **3**:22

[14] Nabati M., Bodaghi-Namileh V. *Int. J. New Chem.,* 2019, **6**:254

[15] Nabati M., Bodaghi-Namileh V., Sarshar S. *Prog. Chem. Biochem. Res.,* 2019, **2**:108

[16] Nabati M. *Chem. Methodol.,* 2018, **2**:223

[17] Nabati M., Kermanian M., Mohammadnejad-Mehrabani H., Kafshboran H.R., Mehmannavaz M., Sarshar S. *Chem. Methodol.,* 2018, **2**:128

[18] Nabati M., *Asian J. Green Chem.,* 2019, **3**:258

[19] Nabati M. *J. Phys. Theor. Chem. IAU Iran,* 2017, **14**:283

[20] Nabati M. *Chem. Methodol.,* 2017, **1**:121

[21] Nabati M., Sabahnoo H., Lohrasbi E., Mazidi M. *Chem. Methodol.,* 2019, **3**:377

[22] Nabati M. *Iran. Chem Commun.,* 2019, **7**:324

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