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ARTICLE

Green Synthesis of Barbituric Acid Derivative *via* Goldsmith Effluent Initiated Gold Nanoparticles and its Molecular Docking Study against Alzheimer Drug Target

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Asian Journal of Organic & Medicinal Chemistry

Volume: 6 Year: 2021
Issue: 3 Month: July–September
pp: 175-180
DOI: https://doi.org/10.14233/ajomc.2021.AJOMC-P332

Received: 24 July 2021
Accepted: 30 July 2021
Published: 30 September 2021

ABSTRACT

A convenient and efficient synthesis of 1,3-dimethyl-5-benzylidene-barbituric acid derivatives *via* gold nanoparticles is carried out. The gold nanoparticles were initiated from novel and low-cost goldsmith effluent source using green reducing agent D-glucose. By mediating autoclave at 121 ºC and 15 lb/cm^2 pressure, these particles were further uniformly synthesized by using microwave radiation. The catalyst was analyzed using UV, IR and scanning electron microscopic techniques. Synthesized 1,3-dimethyl-5-benzylidene-barbituric acid was assayed to study its inhibitory action against TAU protein.

KEYWORDS

Green synthesis, Gold nanoparticles, 1,3-Dimethyl-5-benzylidene, Barbituric acid.

INTRODUCTION

Derivatives of benzylidine barbituric acid are the important class of heterocyclic compound. Though they are originated from barbituric acid, which is actually pharmaceutically inactive one but its derivatives were practically verified drug moiety [1-3]. On the account of this pharmacological potential, they are widely used in variety of disease treatment. Apart from this application, they found as intermediates in organic synthesis [4] and the synthesis of other bioactive molecules like pigment, vitamin B_{12}, *etc.* [5,6]. With all these applications barbituric acid derivatives attract much attention of researchers. However, the reported methods of synthesis have encountered some limitations, such as long time to complete reaction, reduced yields, extreme reaction conditions, the requirement of high price catalysts and needs special kind of apparatus. So, to overcome the stated restrictions, the finding of an altered and proficient process for the production of 1,3-dimethyl-5-benzylidene-barbituric acid derivatives is of foremost concern.

In the past decade, green synthesis of nanoparticles has evolved as an important branch of nanotechnology because of its potential application in the biomedical, magnetics, energy science and aerospace industries [7]. Due to Lewis acid nature of gold, it has gained tremendous attention in organic synthesis [8]. The nanosized gold particles shows excellent catalytic activity than its bulk structure [9,10]. Gold nanoparticles has found significant catalyst in nearly all organic reactions, parti-

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cularly benzannulation [11], oxidation reaction [12], reduction of aromatic amine [13], C-C Ullman coupling reaction [14], Biginelli reaction [15], *etc*.

Numerous studies have reported the synthesis protocols for AuNPs [16-20], out of those physical method microwave-based synthesis was one of the recent approaches for green synthesis [21,22]. In synthesis and assembly strategies of nanoparticles, precursors from liquids, solids or gas phase are used applying physical and chemical deposition approach [23]. The green synthesis of gold nanoparticles involves three main steps *viz*. selection of solvent medium, selection of environmental benign reducing agent and selection of the non-toxic substances [24,25].

Alzheimer's disease, progressive supranuclear palsy (PSP) and Parkinson's disease are common form of dementia. These dementias considered under tauopathy; it is a neurodegenerative diseases class caused by misfolding of the TAU protein. It is estimated that more than 45 million people worldwide are living with dementia and this number is expected to increase to more than 130 million people by 2050 [26]. Consequently, understanding the physiological and pathological roles of tau in health and disease is important to identify new therapeutic targets

In present study, 1,3-dimethyl benzilydene barbituric acid derivatives were synthesized by using green and cost-effective gold nanoparticles fabricated from gold smith effluent as a low-cost and abundant source using autoclave microwave composite treatment to get uniform distribution of nanoparticles. D-glucose is used as the reducing reagent and starch as the capping reagent for gold nanoparticles synthesis. Furthermore, we checked the molecular docking study of barbituric acid derivative against TAU protein as an anti-alzheimer drug target.

EXPERIMENTAL

Gold smith effluent, starch, D-glucose, aromatic aldehyde and 1,3-dimethyl barbituric acid were procured commerically and used as such.

General procedure for synthesis of gold nanoparticles

Optimization of autoclave treatment time for gold nanoparticles synthesis: A 2% starch (Himedia) solution (50 mL) was prepared in double distilled water. D-glucose (Himedia, 1 mM) and 250 mL goldsmith effluent collected from local goldsmith shop outlet was added. This solution was autoclaved at 15 lb/inch^2 pressure for 10, 15 and 20 min and analyzed by UV spectroscopy. After cooling, solution pH was made to alkaline by using 1 mM NaOH.

Optimization of microwave treatment cycles for gold nanoparticles synthesis: Microwave radiation treatment for autoclaved solution was given at 2450 MHz, frequency in domestic microwave oven (LG make) in cycles. Each cycle consists of 15 s exposure to microwave irradiation followed by cooling time interval of 15 s. Maximum 15 cycles had given. Resulting solution was centrifuged at 14000 rpm for 20 min. Pellet was washed with distilled water and then by 70% ethanol three times.

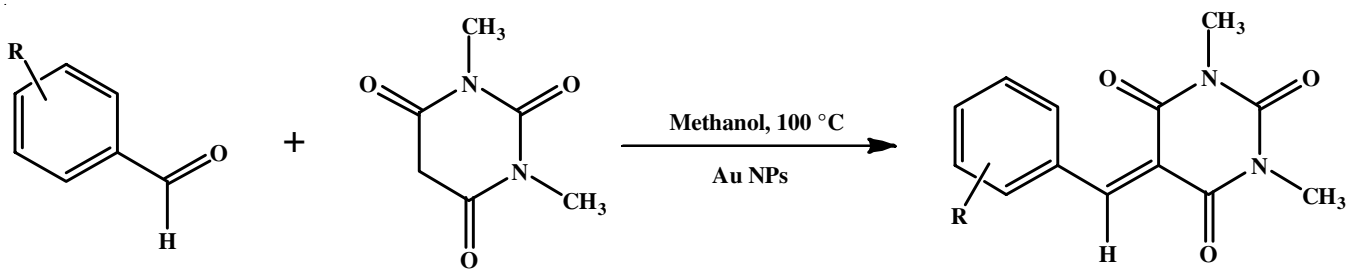
Characterization of gold nanoparticles: Harvested particles were dried at room temperature and made into fine powder. This powder was dissolved in distilled water and analyzed on the UV spectrophotometer (ELICO) from 400 to 750 nm range. Same powder was analyzed by using scanning electron microscopy (SEM).

General procedure for preparation of 1,3-dimethyl-5-(benzylidene)barbituric acid: A mixture of aromatic aldehyde (1 mmol), 1,3-dimethyl barbituric acid (1 mmol) and catalyst gold nanoparticle (AuNP) (0.4 mol%) was stirred for appropriate time. The progress and completion of reaction was checked and monitored by TLC. After completion of the reaction, the mixture was cooled to room temperature. The mixture was poured in cold water. The solid separated was filtered by suction to afford crude product. Pure product was obtained by further crystallization from ethanol to get the desired compound in pure form (**Scheme-I**). The structure of all the products was confirmed by physical and spectroscopic data.

1,3-Dimethyl-5-(4-chlorobenzylidene)barbituric acid (3b): ^{1}H NMR (400 MHz, DMSO) δ ppm: 3.08 (s, 3H) 3.12 (s, 3H) 7.49 (d, 2H) 8.10 (d, 2H) 8.24 (s 1H). ^{13}C NMR (100 MHz, DMSO); δ_C (ppm): 27.09, 28.18, 118.29, 127.83, 130.10, 133.58, 149.25, 151.70, 160.84, 166.59. MS (*m/z*) 278.69 (M+1).

1,3-Dimethyl-5-(2,5-dimethoxybenzylidene)barbituric acid (3g): ^{1}H NMR (400 MHz, DMSO) δ (ppm): 3.29 (s, 3H) 3.37 (s, 3H) 3.82 (s, 3H) 3.89 (s, 3H) 7.11 (d, 1H) 7.92 (dd, 1H) 8.29 (d 1H) 8.33 (s 1H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 28.50, 33.18, 49.07, 104.65, 121.08, 126.48, 127.46, 130.28, 137.83, 145.35, 157.60, 162.54, 165.58. MS (*m/z*) 305.11 (M+1).

General procedure for the molecular docking study: Receptor preparation -TAU as a receptor was prepared using AUTODOCK 4.2 tools. The Autodock tools package version 1.4.6 was used to generate the docking input files. Co-crystallized ligand molecule was removed from the enzyme active site. Binding site was detected from PDB pocket finder. For the docking, a grid spacing of 0.375 Å and 75 × 69 × 76 number of points was used. Before docking all water molecules



Scheme-I: Synthetic scheme for1,3-dimethyl-5-benzylidene-barbituric acid

were removed from the protein structure followed by addition of hydrogen atoms to receptor and merging non-polar hydrogen. Receptor protein was assigned by Kollman united atom charges and solvation parameters while ligands were assigned by Gasteiger charge. Rigid roots were also assigned to the ligand and five bonds were made rotatable.

Ligand preparation: All the synthesized compounds 5-arylidine barbiturate were taken for prediction of 3D structures by using Cambridge software. The energy minimization was done by using open babel in Pyrx virtual screening tool with UFF force field and conjugate gradient optimization algorithm. Receptor grid generation was done by Autodock 4.2. In the receptor grid generation, the receptor structure was defined by excluding co-crystallized ligand, which confirmed the position and size of the active site represented by receptor grids.

Docking simulation: Docking simulation was done using Autodock-Vina suite as molecular-docking tool. The default optimization parameters were used with the Lamarckian Genetic Algorithm with a population size of 150 dockings. A default protocol were applied with mutation rate of 0.02 and a cross-over rate of 0.8. The grid box used for specifying the search space was set at 75 × 60 × 75 centered on of TAU with a default grid point spacing of 0.375 Å. Auto Grid was used to obtain pre-calculated grid maps. After completion of docking, most suitable conformation was chosen based on lowest docked energy.

RESULTS AND DISCUSSION

Goldsmith effluent as a source of green synthesis of gold nanoparticles was used. In alkaline condition, glucose oxidizes itself by reducing gold solution in water. This reaction was performed in autoclave at 15 lb/inch pressure for 15 min at 121 ºC. Comparative gold nanoparticle synthesis was analyzed by observing the absorbance at 523 nm. Fig. 1 shows gold nanoparticles synthesis at various time duration. Increase in autoclave treatment time showed increase in absorbance at 523 nm. Maximum absorbance is observed at 15 min autoclave treatment time. Further increase in autoclave treatment time did not show significant increase in absorbance. SEM images confirmed the synthesis of nanoparticles (Fig. 2).

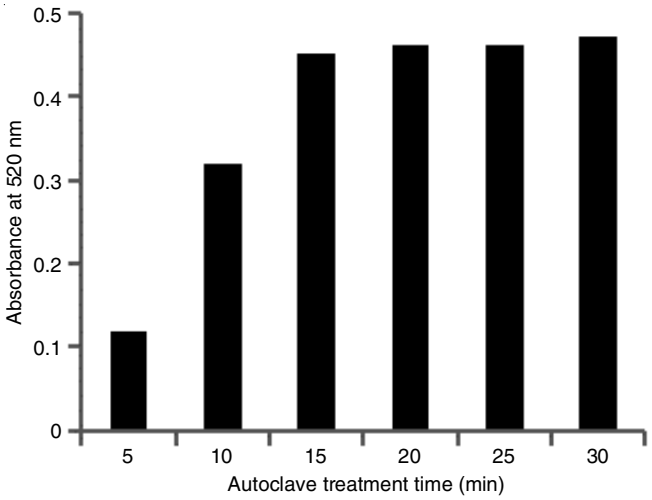


Fig. 1. Optimization of autoclave treatment time for gold nanoparticles synthesis

After the successful synthesis of gold nanoparticles, we have proposed its application as a catalyst in Knoevenagel condensation reaction of 1,3-dimethyl barbituric acid, which having active methylene group with aromatic aldehyde that produce 1,3-dimethyl-5-benzylidene-barbituric acid. Owing to improve yield of resulted product of model reaction (Table-1). We optimized different reaction parameters like temperature, solvent and amount of catalyst. After evaluating efficiency of catalyst, we have also monitored the amount of catalyst in order to get maximum yield. Use of 0.4 mol % of catalyst gave high yield with less time. Selection of solvent and temperature is also important parameters of reaction. Where we have been found methanol was best solvent with respect to yield and reaction time and 100 ºC offer best results for the model reaction. With this optimized reaction conditions further, we have synthesized wide range of derivatives of 1,3-dimethyl-5-benzylidene-barbituric acid using structurally diverse aromatic aldehydes (Table-2).

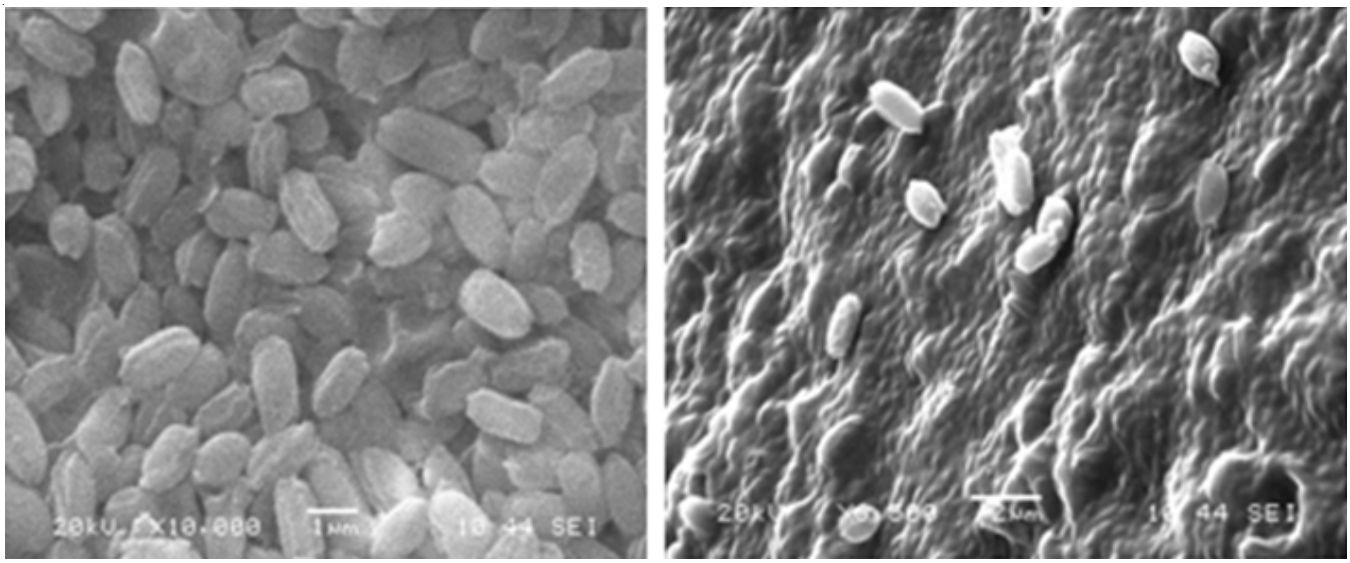


Fig. 2. SEM images of synthesized gold nanoparticles

TABLE-1
OPTIMIZATION DATA OF THE REACTION CONDITIONS

Entry	Catalyst	Mol (%) of catalyst	Solvent	Temperature (°C)	Time (min)	Yield (%)
1	Without catalyst	–	Ethanol	60	240	Reaction incomplete
2	Au NPs	0.5	Ethanol	60	120	42
3	Au NPs	0.5	THF	70	75	45
4	Au NPs	0.5	DMF	80	75	60
5	Au NPs	0.5	Methanol	100	55	78
6	Au NPs	2.0	Methanol	100	50	80
7	Au NPs	3.0	Methanol	100	50	84
8	Au NPs	4.0	Methanol	100	35	96

TABLE-2
SYNTHESIS OF DIFFERENT 1,3-DIMETHYL-5-BENZYLIDENE-BARBITURIC ACID DERIVATIVES CATALYZED BY Au NPs

Entry	R group	Time (min)	Yield (%)	m.p. (°C) (observed)
3a	H	40	89	159-160
3b	4-Cl	35	96	154-156
3c	4-Br	25	88	150-151
3d	4-F	25	90	145-146
3e	2,5-OMe	30	96	230-231
3f	4-OH	25	95	297-298
3g	4-NO_2	35	92	190-193

The recovery and reusability are major advantages of gold nanopaticles as heterogeneous catalyst after taking part in reaction. The catalyst was employed in reaction, washed with acetone and kept in the oven at 80 ºC. This regenerated activating catalyst results similar yield up to 4th cycle, which proved there is no further loss in its catalytic activity (Fig. 3).

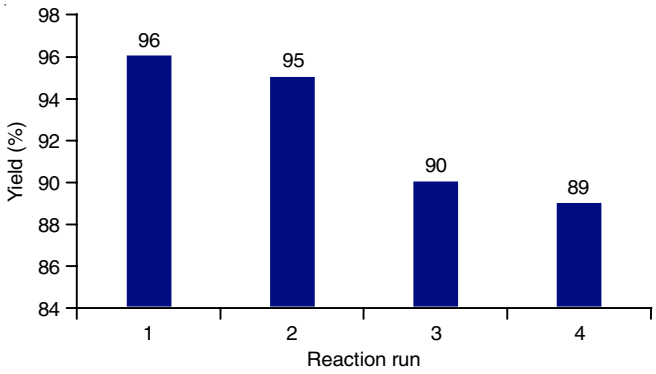


Fig. 3. Reusability of catalyst in synthesis of 3-dimethyl-5-benzylidene-barbituric acid derivatives

Molecular docking studies: Docking protocol was verified by docking with reference molecule donepezil in the vicinity of the same active site of TAU protein. Selected conformations were analyzed using Pymol and Discovery Studio softwares. All derivatives showed better binding affinity than existing reference octopamine (Table-3). Compound **3f** molecule showed highest binding affinity with lowest energy -8.7 kcal/mol and compound **3b** showed lowest binding affinity with energy -6.9 kcal/mol. ADME analysis was performed using Swiss ADME tool. ADME study predict that compounds **3a**, **3b**, **3c**, **3d**, **3e**, **3f** and **3g** molecules will pass through central nervous system as all are not substrate of P-glycoprotein. It further predicts **3b**, **3c** and **3d** will pass through blood brain barrier, but **3d** molecule shows highest binding affinity with lowest energy -8.3 kcal/mol (Fig. 4). All the molecules showed maximum 55%, oral bioavailability, chemical stability at acidic pH less than 2, have good passive absorption (> 70%) against intestinal barrier with 100% passive absorption with *trans*-cellular route. Molecular descriptor studies revealed that all selected ligands were passed and acted as hydrophilic or moderately hydrophobic basic drug molecule by their adherence to the properties.

Conclusion

Gold nanoparticles catalyzed efficient synthesis of 1,3-dimethyl-5-benzylidene-barbituric acid and its derivatives through one pot two component Knovengel condensation reaction was reported. Synthesized 1,3-dimethyl-5-benzylidene-barbituric acid derivatives were assayed to study its inhibitory action against TAU protein. Experimental evidence as PFGR inhibitor led to further molecular docking study. Molecular docking studies was done for the analysis with training set composed of the newly synthesized compound whose inhibitory activity is unknown against TAU protein as an anti-Alzheimer drug target. On the basis of molecular docking, oral bioavailability, chemical stability at acidic pH, absorption against intestinal barrier and ability to cross blood brain barrier; it is concluded that 1,3-dimethyl-5-(4-flurobenzylidene)-

TABLE-3
ADME AND PHARMACOKINETIC STUDY OF 3-DIMETHYL-5-BENZYLIDENE-BARBITURIC ACID DERIVATIVES

Molecule	m.w. (g/mol)	HD	HA	TPSA (Å)	BBB permeability	PGP substrate	Bioavailability score	Binding affinity (Kcal/mol)	W log P
Donepezil	153.18	3	3	66.48	No	No	0.55	-5.0	0.06
3a	244.25	0	3	57.69	No	No	0.55	-8.1	0.25
3b	244.25	0	3	57.69	Yes	No	0.55	-6.9	0.25
3c	323.14	0	3	57.69	Yes	No	0.55	-8.1	1.01
3d	262.24	0	4	57.69	Yes	No	0.55	-8.3	0.81
3e	260.25	1	4	77.92	No	No	0.55	-8.2	0.04
3f	291.26	2	5	101.39	No	No	0.55	-8.7	0.17
3g	276.24	2	5	93.73	No	No	0.55	-7.8	0.42

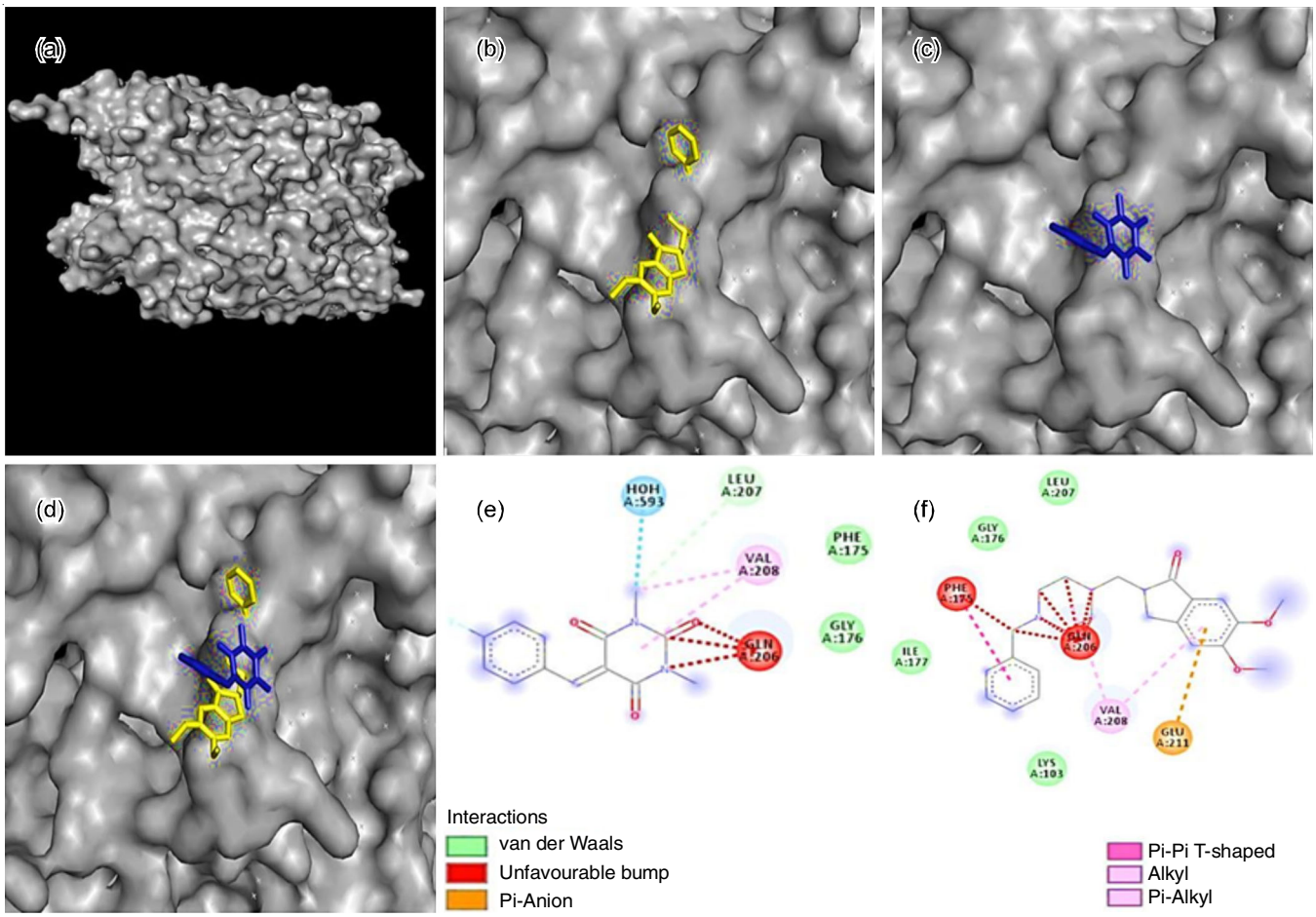


Fig. 4. Molecular docking study of **3d** barbiturate derivative against TAU protein; (a) TAU protein crystal structure with binding site, (b) Docked **3d** molecule, (c) Docked donepezil as standard, (d) **3d** molecule and donepezil docked at same binding site amino acids, (e) **3d** interaction with binding site amino acids, (f) donepezil interaction with binding site amino acids

barbituric acid (**3d**) can act as best inhibitor drug molecule among all synthesized derivatives resulting in anti-Alzheimer activity.

ACKNOWLEDGEMENTS

The authors are thankful to Management and Principal of our college for providing all necessary research facilities. The authors are also thankful to Savitribai Phule Pune University, Pune and SAIF Chandigarh for providing analysis facility.

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