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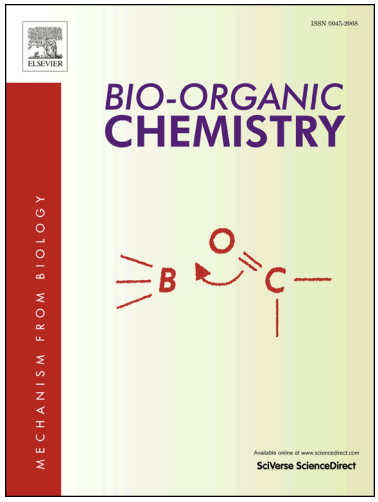

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Isatin based Schiff bases as Inhibitors of α-glucosidase: Synthesis, Characterization, *In Vitro* Evaluation and Molecular Docking Studies

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Abstract:

Isatin base Schiff bases (**1-20**) were synthesized, characterized by ^{1}HNMR and EI/MS and evaluated for α-glucosidase inhibitory potential. Out of these twenty (**20**) compounds only six analogs showed potent α-glucosidase inhibitory potential with IC_{50} value ranging in between 2.2 ± 0.25 to 83.5 ± 1.0μM when compared with the standard acarbose (IC_{50} = 840±1.73 μM). Among the series compound **2** having IC_{50} value (18.3 ± 0.56 µM), **9** (83.5 ± 1.0 µM), **11** (3.3 ± 0.25 µM), **12** (2.2 ± 0.25 µM), **14** (11.8 ± 0.15 µM), and **20** (3.0 ± 0.15µM) showed excellent inhibitory potential many fold better than the standard acarbose. The binding interactions of these active analogs were confirmed through molecular docking.

Keywords: Isatin, Schiff bases, Synthesis, α-Glucosidase Inhibition, Molecular Docking.

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1. Introduction

α-Glucosidase enzyme is located in the brush-border surface membrane of intestinal cells, responsible for catalyzing the final step in the digestive process of carbohydrates [1]. It specifically hydrolyzed the α-glucopyranoside bond, thereby releasing α-D-glucose from the non-reducing end of the sugar [2].The inhibitors of this enzyme have applications in modern medicinal chemistry. Its might be used to treat diabetics, HIV and cancers [3-5].The biological importance of α-glucosidase inhibitors ensures its uses as a vital tool to understand the mechanisms of action of *α*-glucosidase, and as therapeutic agents for some degenerative diseases [6-8]. Its inhibitors like acarbose, voglibose, and miglitol are clinically used in the effective treatment of type-2 diabetes mellitus [9].

Isatin heterocycles acts as a potent endogenous neurochemical regulator in mammalian brain [10, 11]. It's concentration in urine become a diagnostic marker for the clinical severity of Parkinson's disease in humans [12, 13]. It is a component of tribulin, a selective inhibitor of monoamine oxidase-B enzyme [14]. Isatin is well protective against certain types of infections [15]. Its derivatives exhibited activities like antibacterial [16], anti-inflammatory [17], analgesic [18], anti-viral [19], antifungal [20], anti-tubercular [21] and anti-depressant [22].Schiff bases of isatin derivatives have been reported as antiprotozoal, antibacterial, anti-HIV, anticonvulsant, antitumor, and anthelminthic activities, influence neurodegenerative diseases, participate in metabolism, acetyl cholinesterase inhibitors, and stimulate plants growth [23,24].

Herein we are going to report synthesis of isatin based Schiff bases, their *α*-glucosidase inhibitory potentials, SAR and molecular docking studies.

2. Result and Discussion

2.1. Chemistry

Isatin based Schiff bases (**1-20**) were synthesized in three steps, first an esterification carried out by reacting different carboxylic acid with methanol in sulphuric acid (2-3ml) under reflux condition for 12-16 hrs. The completion of reaction was monitored by TLC. After completion of reaction, reaction mixture was extracted with hexane to obtained pure esters. Then esters were refluxed with hydrazine hydrate in methanol with few drops of glacial acetic acid for 3 h. After completion of reaction, reaction mixture was washed with chloroform to obtained different hydrazides. These hydrazides (1mmole) each were than treated with different isatin (1mmole) in methanol having catalytic amount of glacial acetic acid for 2-4h. Reaction completion was

monitored through periodic TLC. After completion of reaction, reaction mixture were washed with n-hexane to obtain our desired products (**1-20**).

Insert Scheme-1 Here

Insert Table-1 Here

2.2. Biological activity

Compounds **1-20** showed variable degree of α-glucosidase inhibitory potential when compared with standard acarbose (IC_{50} value 840±1.73μM). Among the series only compounds **2**, **9**, **11**, **12**, **14** and **20** showed potent inhibitory potential with IC_{50} values of 18.3 ± 0.56, 83.5 ± 1.0, 3.3 ± 0.25, 2.2 ± 0.25, 11.8 ± 0.15 and 3.0 ± 0.15 μM respectively. The most active compounds are **11** (IC_{50} 3.3 ± 0.25) with 3, 4-dichloro substitution at phenyl part of hydrazide and **12** (IC_{50} 2.2 ± 0.25) with 2,4-dichloro substitution at phenyl part of hydrazide and 6-bromo on phenyl part of isatin. The compound **2** (IC_{50} 18.3 ± 0.56) with di-chloro substitution at phenyl part of hydrazide but have no bromo group at isatin and have extra alkyl group at nitrogen of isatin. The slight activity difference among the two compounds **11** and **12** is may be due to difference in position of chloro groups, while compound **2** have some decline in activity which might be due to extra alkyl group at isatin nitrogen. Compound **9** (IC_{50} 83.5 ± 1.0**)** with 5-chloro substitution and **14** (IC_{50} 11.8 ± 0.15) with 6-bromo substitution on the phenyl part of isatin also showed potent inhibitory activity as compared to standard acarbose. Compounds **20** was unique having no substitution at phenyl group but also showed potent inhibitory activity.

The results showed that the compounds having more electron withdrawing groups *i.e.* chloro or bromo such as **11** and **12** are more potent inhibitor, while those with less number of electron withdrawing groups **2**, **9** and **14** show comparatively less potent inhibitory activity. The binding interaction of these compounds was confirmed through molecular docking.

2.3. Homology modeling and docking simulation

The crystallographic structure for α-glucosidase enzyme has not been solved up-to yet. However, only few homology models have been reported [25-28] so, we developed the 3D model for α-glucosidase by comparative homology modeling technique using the same etiquette as described by Burke *et al* [29]. The primary sequence of α-glucosidase was retrieved from UniProt protein

resource data bank (http://www.uniprot.org/) under the access code P53341. Template search was performed by means of MOE-Search tools against the PDB data bank implemented in MOE 2010.11. The 1.30 Å resolving crystallographic structure of *Saccharomyces cerevisiae isomaltase* (PDB code 3AJ7,) [30] with 72.4% of sequence identity with the target were selected as the template for modeling. The 3D structure of α-glucosidase for *Saccharomyces cerevisiae* was built by means of MOE homology modeling tools. The developed 3D model was subjected to energy minimization up to 0.05 gradients. Before docking, ligands and protein were prepared using MOE 2010.11. 3D structure of all synthesized compounds was built using the Molecular Builder program implemented in MOE. Finally, a database was created in which all the ligands were converted into their respective 3D structures and this database was used as input file MOE-docking. Subsequently, the energy of compounds present in the database was minimized up to 0.05 Gradient using MMFF94x force field. Energy minimization of the database was followed by the preparation of protein for docking purposes. Most macromolecular crystal structures contain little or no hydrogen coordinate data due to limited resolution and thus protonation was accomplish prior to docking using Protonate 3D tools implemented in MOE. Protonation was followed by energy minimization up to 0.05 Gradient using Amber99 force field. The database was docked into the active site of protein using the Triangular Matching docking method and 30 conformations of each Ligand protein complex were generated with docking score (S). Each complex was analyzed for interactions and their 3D images were taken.

2.4. Molecular docking

From the docking simulation and *in vitro* validation study, it was observed that compound **12** (IC_{50} = 2.2±0.25 μM) are the most active analog among the series which are mention in this study. Compound **20** (IC_{50} 3.0±0.15 μM) also the second ranked most active compound against α-glucosidase inhibitory assay. These compounds were bound extremely into the binding cavity of α-glucosidase making interactions with the active site residues **Glu276**, **His239** and **Asp349** as described by Khan, M *et al* [31].

The most active compound **12** is fit intensely into the catalytically active residues of the enzyme making interactions with the most important active site residues **Arg212, His239, Glu276** and **Thr307** (**Figure-01**). **Arg212** was found in hydrogen interaction with the oxygen of indolenone group. **His239** make π-interaction with the Di-chloro substituted phenyl ring. **Glu276** establish

interaction with the hydrogen of the amino group of the tested compound **12**. **Thr307** was found in interaction with the oxygen of carbonyl group of the compound [32].

Insert Figure-1 Here

Similarly compound **20** also show best inhibitory activity against α-glucosidase enzyme and establish interaction with the active site residue which is readily involved in inhibition **Phe157** and **Arg212** (**Figure-02**). **Phe157** was found in making interaction with the hydrogen next attach to the nitrogen group and **Arg212** establish two interactions with the compound, one with the oxygen of the indolenone group and other with the nitrogen of the hydrazide group.

Insert Figure-2 Here

Compound **11** is ranked third most active compound among the series, which have IC_{50} value 3.3 ± 1.0. This compound established two hydrogen interactions with the active site residues of the α-glucosidase enzyme **Asn241** and **His239**. **Asn241** is most important residue and involved in inhibition, here found in making interaction with the nitrogen (**Figure-03**). **His239** make hydrogen interaction with the hydrogen next attach to nitrogen of the hydrazide moiety.

Insert Figure-3 Here

Similarly compound **14** and **02** are involved in making interaction with the active site residue **Arg212** and **His245** (**Figure-04**), and all the remaining profile of the compounds reflects no interaction (*e.g.* compound **01**, as shown in **Figure-04**), therefore these compounds were not further evaluated for further study.

Insert Figure-4 Here

2.5. α-Glucosidase assay

α-Glucosidase inhibitory activities was determined as per reported methods [33].10 μL of test samples (5 mg/mL DMSO solution)were reconstituted in 100 μL of 100 mM-phosphate buffer (pH6.8) in 96-well microplate and incubated with 50 μL of crude intestinal α-glucosidase for 5 min before 50 μL substrate (5 mM, p-nitrophenyl-α-D-glucopyranoside prepared in same buffer) was added. Release of p-nitrophenol was measured at 405 nm spectrophotometrically (SpectraMax® plus384) for5 min after incubation with substrate. Individual blanks for test

samples were prepared to correct background absorbance where substrate was replaced with 50 μL of buffer. Control sample contained 10 μL DMSO in place of test samples. Percentage of enzyme inhibition was calculated as (1-$\frac{B}{A}$) x 100 whereA represents absorbance of control without test samples, and B represents absorbance in presence of test samples.

3. Conclusion

Twenty compounds were synthesized and evaluated for α-glucosidase inhibition. Six analogs showed varied α-glucosidase inhibitory potential with IC_{50} value ranging between 2.2 ± 0.25 to 83.5 ± 1.0 μM when compared with standard acarbose (IC_{50} = 840 ± 1.73 μM). Compound **2** IC_{50} value (18.3 ± 0.56 μM), **9** (83.5 ± 1.0 μM), **11** (3.3 ± 0.25 μM), **12** (2.2 ± 0.25 μM), **14** (11.8 ± 0.15 μM), and **20** (3.0 ± 0.15 μM) showed excellent inhibitory potential much better than the standard acarbose. The binding interaction of these active compounds was confirmed through molecular docking. All other analogs were found inactive.

4. Experimental Section

4.1. General methods

Finnegan MAT-311A, Germany, spectrometer was used for electron impact mass spectra (EI/MS) analysis. An internal standard cesium iodide (CsI) was used for mass measurement. Advance Bruker AM 300 MHZ and AMX-400 MHZ spectrometers have used for NMR analysis. DMSO and acetone were used for accurate NMR analysis. All the above characterizations were performing at H.E.J Research Institute of Chemistry, Karachi University Pakistan. Column chromatography was performed on silica gel (E. Merck, type 60, 70-230 mesh). Pre-coated silica gel aluminum plates (Kieselgel 60, 20×20 and 0.5 mm thick, E. Merck, Germany) were used for TLC analysis. Light of wavelength 254 and 265 nm were used to visualize the chromatogram.

4.2. General Procedure for the synthesis of isatin based Schiff bases derivatives (1-20)

Isatin based Schiff bases (**1-20**) were synthesized in three steps, first an esterification carried out by reacting different carboxylic acid with methanol in sulphuric acid (2-3ml) under reflux

condition for 12-16 hrs. The completion of reaction was monitored by TLC. After completion of reaction, reaction mixture was extracted with hexane to obtained pure esters. Then esters were refluxed with hydrazine hydrate in methanol with few drops of glacial acetic acid for 3 h. After completion of reaction, reaction mixture was washed with chloroform to obtained different hydrazides. These hydrazides (1mmole) each were than treated with different isatin (1mmole) in methanol having catalytic amount of glacial acetic acid for 2-4h. Reaction completion was monitored through periodic TLC. After completion of reaction, reaction mixture was washed with n-hexane to obtain our desired products (**1-20**). The structure of all compounds was established through EI-MS and ^{1}HNMR.

*4.2.1. (E)-2-hydroxy-N'-(2-Oxo-1-propylindolin-3-ylidene) benzohydrazide **(1)***

Yield:79%;^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 14.3 (s, 1H, NH), 12.2 (s, 1H, OH), 8.0 (dd, $J_{7,5}$ = 1.5 Hz, $J_{7,6}$ = 8.1 Hz,1H, H-7), 7.99 (d, $J_{6',5'}$ = 7.5 Hz,1H,H-6'), 7.51 (m, 2H, H-4/6), 7.2(d, $J_{3',4'}$ = 7.8 Hz,1H, H-3'), 7.15 (m, 1H, H-5'), 7.0 (m, 1H, H-5), 6.9 (m, 1H, H-4'), 3.71 (t, 2H, NCH_2), 1.64 (m, 2H, NCH_2CH_2), 0.91 (t, 3H, CH_2CH_3).; EI-MS: *m/z* (rel. int. %): 323 (M^+, 80), 308 (69), 230 (100), 94 (35);

*4.2.2. (E)-2-(2,4-Dichlorophenyl)-N'-(2-oxo-1-propylindolin-3-ylidene)acetohydrazide **(2)***

Yield:82%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 14.1 (s, 1H, NH), 8.0 (d, $J_{3',5'}$ = 1.2 Hz,1H, H-3'), 7.7(d, $J_{4,5}$ = 7.5 Hz,1H, H-4), 7.6 (m, 3H, H-6/4'/5), 7.2 (d, $J_{6',5'}$ = 8.1 Hz, 1H, H-6'), 7.1 (m, 1H, H-3), 3.7 (t, 2H, NCH_2), 1.6 (m, 2H, NCH_2CH_2), 0.9 (t, 3H, CH_2CH_3),; EI-MS: *m/z* (rel. int. %): 389 (M^+, 60), 374 (100), 250 (78), 93 (44);

*4.2.3. (E)-2-chloro-N'-(2-Oxo-1-propylindolin-3-ylidene) benzohydrazide **(3)***

Yield:82%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ13.3 (s, 1H, NH), 7.6 (m, 4H, H-4/7/3'/6), 7.5 (m, 2H, H-5/5'), 7.2 (d, $J_{6',5'/4',5'}$= 7.8 Hz, 2H, H-6'/4'), 3.6 (t, 2H, NCH_2), 1.6 (m, 2H, NCH_2CH_2), 0.9 (t, 3H, CH_2CH_3),; EI-MS: *m/z* (rel. int. %): 341 (M^+, 35), 202 (100), 174 (55), 139 (58);

*4.2.4. (E)-3-methyl-N'-(2-Oxo-1-propylindolin-3-ylidene) benzohydrazide **(4)***

Yield:90%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 14.3 (s, 1H, NH), 8.0 (d, $J_{4,5}$ = 7.2 Hz,1H, H-4), 7.7(d, $J_{7,6}$ = 7.5 Hz,1H, H-7), 7.6 (m, 3H, H-6/6'/2'), 7.2 (d, $J_{4',5'}$ = 8.1 Hz, 1H, H-4'), 7.1 (m,

2H, H-5/5'), 3.7 (t, 2H, NCH_2), 1.6 (m, 2H, NCH_2CH_2), 0.8 (t, 3H, CH_2CH_3),; EI-MS: *m/z* (rel. int. %): 321(M^+, 45), 290 (100), 187 (60), 138 (45);

4.2.5. (E)-4-methyl-N'-(2-Oxo-1-propylindolin-3-ylidene) benzohydrazide ***(5)***

Yield:87.5%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 14.1 (s, 1H, NH), 8.0 (d, $J_{4,5}$ = 7.2 Hz,1H, H-4), 7.7(d, $J_{7,6}$ = 7.5 Hz,1H, H-7), 7.6 (m, 3H, H-6/6'/2'), 7.2(d, $J_{3',2'/5',6'}$= 8.0 Hz, 2H, H-3'/5'), 7.1 (m, 1H, H-5), 3.7 (t, 2H, NCH_2), 1.6 (m, 2H, NCH_2CH_2), 0.9 (t, 3H, CH_2CH_3),; EI-MS: *m/z* (rel. int. %): 321(M^+, 45), 290 (100), 187 (60), 138 (45);

4.2.6. (E)-2,5-dimethoxy-N'-(2-Oxo-1-propylindolin-3-ylidene)benzohydrazide ***(6)***

Yield:76%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 14.2 (s, 1H, NH), 7.6 (m, 2H, H-4/7), 7.4 (t, $J_{6/5,7}$ = 7.5 Hz,1H,H-6), 7.2(m, 4H, H-5/3'/4'/6'), 3.7 (m, 2H, NCH_2), 1.6 (m, 2H, NCH_2CH_2), 0.9 (t, 3H, CH_2CH_3),; EI-MS: *m/z* (rel. int. %): 367 (M^+, 67), 220 (100), 167 (58), 139 (45);

*4.2.7.(E)-N'-(5-Chloro-2-oxoindolin-3-ylidene)-2-(3,4-dichlorophenyl)acetohydrazide****(7)***
Yield:74.5%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 14.1 (s, 1H, NH), 13.1 (s, 1H, NH), 7.6 (s, 1H, H-4), 7.4 (s, 1H, H-2'), 7.23 (t, $J_{7,6}$= 8.1 Hz,1H, H-7), 7.2(m, 3H, H-5'/6/6'), 1.6 (s, 2H, CH_2),;EI-MS: *m/z* (rel. int. %): 381 (M^+, 60), 242 (100), 93 (60).

4.2.8. (E)-N'-(5-Chloro-2-oxoindolin-3-ylidene)-2-(2,4-dichlorophenyl)acetohydrazide ***(8)***

Yield:78%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 14.1 (s, 1H, NH), 13.1 (s, 1H, NH), 7.6 (s, 1H, H-4), 7.4 (s, 1H, H-3'), 7.2 (d, $J_{7,6}$ = 8.0 Hz,1H, H-7), 7.2(m, 3H, H-5'/6/6'), 1.6 (s, 2H, $\underline{CH_2}$);EI-MS: *m/z* (rel. int. %): 381 (M^+, 60), 242 (100), 198 (76), 93 (60);

4.2.9. (E)-N'-(5-Chloro-2-oxoindolin-3-ylidene)-4-methylbenzohydrazide ***(9)***

Yield:90%;^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 14.3 (s, 1H, NH), 13.2 (s, 1H, NH), 7.5 (s, 1H, H-4), 7.4 (d, $J_{7,6}$ = 8.0 Hz,1H, H-7), 7.2 (d, $J_{6,7}$ = 8.0 Hz,1H,H-6), 7.1 (d, $J_{6',5'/2',3'}$ = 8.0 Hz,2H, H-2'/6'), 7.1(m, 2H, H-3'/5'), 1.6 (s, 3H, CH_3),;EI-MS: *m/z* (rel. int. %): 313 (M^+, 60), 298 (56), 206 (100), 162 (45);

4.2.10. (E)-N'-(5-Bromo-2-oxoindolin-3-ylidene) dodecanehydrazide ***(10)***

Yield:82%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ14.2 (s, 1H, NH), 13.3 (s, 1H, NH), 7.4 (s, 1H, H-4), 7.3 (d, $J_{7,6}$ = 7.5 Hz,1H, H-7), 7.2 (d, $J_{6,7}$ = 7.5 Hz,1H, H-6), 1.2 (m, 23H, $(CH_2)_{10}CH_3$); EI-MS:*m/z* (rel. int. %): 377 (M^+, 67), 342 (85), 232 (100), 145 (77);

4.2.11. (E)-N'-(6-Bromo-2-oxoindolin-3-ylidene)-2-(3,4-dichlorophenyl)acetohydrazide ***(11)***

Yield:80%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 14.1 (s, 1H, NH), 13.1 (s, 1H, NH), 7.8 (s, 1H, H-2'), 7.4 (s, 1H, H-7), 7.3 (d, $J_{4,5}$ = 8.1 Hz,1H, H-4), 7.2 (d, $J_{5',6'}$ = 7.5 Hz,1H, H-5'), 7.1(m, 2H, H-4/6'), 1.6 (s, 2H, CH_2),;EI-MS: *m/z* (rel. int. %): 425 (M^+, 43), 336 (78), 277 (100), 148 (89);

4.2.12. (E)-N'-(6-Bromo-2-oxoindolin-3-ylidene)-2-(2,4-dichlorophenyl)acetohydrazide ***(12)***

Yield: 82%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 14.2 (s, 1H, NH), 12.9 (s, 1H, NH), 7.8 (s, 1H, H-3'), 7.6 (s, 1H, H-7), 7.4 (d, $J_{4,5}$ = 7.1 Hz,1H, H-4), 7.3 (d, $J_{5',6'}$ = 7.5 Hz,1H, H-5'), 7.1(d, $J_{5,4/6',5'}$ = 7.5 Hz, 2H, H-5/6'), 1.6 (s, 2H, CH_2); EI-MS: *m/z* (rel. int. %): 425 (M^+, 49), 336 (100), 277 (88), 148 (75);

4.2.13. (E)-N'-(6-Bromo-2-oxoindolin-3-ylidene)-4-methylbenzohydrazide ***(13)***

Yield:87%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 14.1 (s, 1H, NH), 13.2 (s, 1H, NH), 7.5 (s, 1H, H-4), 7.4 (d, $J_{7,6}$ = 8.1 Hz,1H, H-7), 7.3 (d, $J_{5,4}$ = 8.1 Hz,1H, H-5), 7.2 (d, $J_{6',5'/2',3'}$ = 8.0 Hz, 2H, H-2'/6'), 7.1(m, 2H, H-3'/5'), 1.6 (s, 3H, CH_3); EI-MS: *m/z* (rel. int. %): 357 (M^+, 56), 342 (75), 268 (100), 89 (66);

4.2.14. (E)-N'-(6-Bromo-2-oxoindolin-3-ylidene)-2-hydroxybenzohydrazide ***(14)***

Yield:83%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 14.1 (s, 1H, NH), 13.2 (s, 1H, NH), 10.2 (s, 1H, OH), 7.5 (s, 1H, H-7), 7.4 (d, $J_{4,5}$ = 8.0 Hz,1H, H-4), 7.3 (d, $J_{5,4}$ = 8.0 Hz,1H, H-5), 7.2 (d, $J_{3',4'/6',5'}$ = 8.0 Hz, 2H, H-3'/6'), 7.1(m, 2H, H-4'/5'); EI-MS: *m/z* (rel. int. %): 359 (M^+, 100), 342 (65), 270 (78), 89 (34);

4.2.15. (E)-N'-(6-Bromo-2-oxoindolin-3-ylidene) dodecanehydrazide ***(15)***

Yield:76%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 14.1 (s, 1H, NH), 13.2 (s, 1H, NH), 7.5 (s, 1H, H-7), 7.3 (d, $J_{4,5}$ = 7.1 Hz,1H, H-7), 7.2 (d, $J_{5,6}$ = 7.1 Hz,1H, H-5), 1.8 (m, 2H, CH_2), 1.2 (m, 23H, $(CH_2)_{10}CH_3$),;EI-MS: *m/z* (rel. int. %): 421 (M^+, 69), 332 (100), 276 (85), 93 (70);

4.2.16. (E)-2-(3,4-Dichlorophenyl)-N'-(2-oxoindolin-3-ylidene)acetohydrazide ***(16)***

Yield: 85%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 14.2 (s, 1H, NH), 13.2 (s, 1H, NH), 7.7 (s, 1H, H-2'), 7.5 (d, $J_{5',6'}$ = 8.0 Hz,1H, H-5'), 7.4 (d, $J_{6',5'}$ = 8.0 Hz,1H, H-6'), 7.3 (d, $J_{4,5}$ = 7.5 Hz,1H, H-4), 7.2(m, 3H, H-5/6/7), 1.6 (s, 2H, CH_2),;EI-MS: *m/z* (rel. int. %): 347 (M^+, 67), 312 (100), 202 (80), 145 (70);

4.2.17. (E)-2-(2,4-Dichlorophenyl)-N'-(2-oxoindolin-3-ylidene)acetohydrazide ***(17)***

Yield: 83%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 14.1 (s, 1H, NH), 12.7 (s, 1H, NH), 7.7 (s, 1H, H-3'), 7.6 (d, $J_{5',6'}$ = 7.5 Hz,1H, H-5'), 7.5 (d, $J_{6',5'}$ = 7.5 Hz,1H, H-6'), 7.4 (d, $J_{4,5}$ = 7.1 Hz,1H, H-4), 7.3 (d, $J_{7,6}$= 7.1 Hz,1H, H-7), 7.1(m, 2H,H-5/6), 1.6 (s, 2H, CH_2),;EI-MS: *m/z* (rel. int. %): 347 (M^+, 65), 312 (87), 202 (100), 145 (71);

4.2.18. (E)-4-methyl-N'-(2-Oxoindolin-3-ylidene) benzohydrazide ***(18)***

Yield: 84%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 14.1 (s, 1H, NH), 13.2 (s, 1H, NH), 7.5 (d, $J_{4,5}$ = 8.0 Hz,1H, H-4), 7.4 (d, $J_{7,6}$ = 7.5 Hz,1H, H-7), 7.3 (d, $J_{2',3'/6',5'}$= 8.1 Hz,2H, H-2'/6'), 7.2 (d, $J_{3',2'/5',6'}$ = 8.0 Hz,2H, H-3'/5'), 7.1(m, 2H, H-5/6), 1.6 (s, 3H, CH_3),;EI-MS: *m/z* (rel. int. %): 279 (M^+, 88), 264 (100), 188 (50), 91(78);

4.2.19. (E)-2-hydroxy-N'-(2-Oxoindolin-3-ylidene) benzohydrazide ***(19)***

Yield: 80%;^{1}H-NMR: (DMSO-d_6, 300 MHz):δ 14.3 (s, 1H, NH), 13.1 (s, 1H, NH), 10.4 (s, 1H, OH), 7.5 (s, $J_{4,5}$ = 8.0 Hz,1H, H-4), 7.4 (d, $J_{7,6}$ = 8.1 Hz,1H, H-7), 7.3 (d, $J_{6',5'}$ = 7.5 Hz,1H, H-6'), 7.2 (m, 3H, H-3'/5/6), 7.1(m, 2H, H-4'/5'); EI-MS: *m/z* (rel. int. %): 281 (M^+, 80), 264 (100), 198 (60), 83 (79);

4.2.20. (E)-N'-(2-Oxoindolin-3-ylidene) dodecanehydrazide ***(20)***

Yield: 88%;[1]H-NMR: (DMSO-d_6, 300 MHz): δ 14.3 (s, 1H, NH), 13.1 (s, 1H, NH), 7.5 (d, $J_{4,5}$ = 7.1 Hz,1H, H-4), 7.3 (d, $J_{7,6}$ = 7.1 Hz,1H, H-7), 7.2 (m,1H,H-5/6), 1.8 (m, 2H, CH_2), 1.2 (m, 23H, $(CH_2)_{10}CH_3$); EI-MS: *m/z* (rel. int. %): 343 (M^+, 60), 228 (76), 198 (100), 93 (70);

Acknowledgments

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Caption

Scheme-1: synthesis of Schiff based isatin derivatives (**1-20)**

Table-1: α-glucosidase inhibitor activity of isatin Schiff bases (**1-20)**

Figure-1: The binding modes and molecular interactions of Isatin Schiff basescompound**12** in the active sites of α-glucosidase.

Figure-2: The binding modes and molecular interactions of isatin Schiff basescompound**20** in the active sites of α-glucosidase.

Figure-3: The binding modes and molecular interactions of Isatin Schiff basescompound**11** in the active sites of α-glucosidase.

Figure-4: 2-Dimensional pose of the compound **01**, **02** and **14** in the active site of α-glucosidase enzyme

Scheme-1:

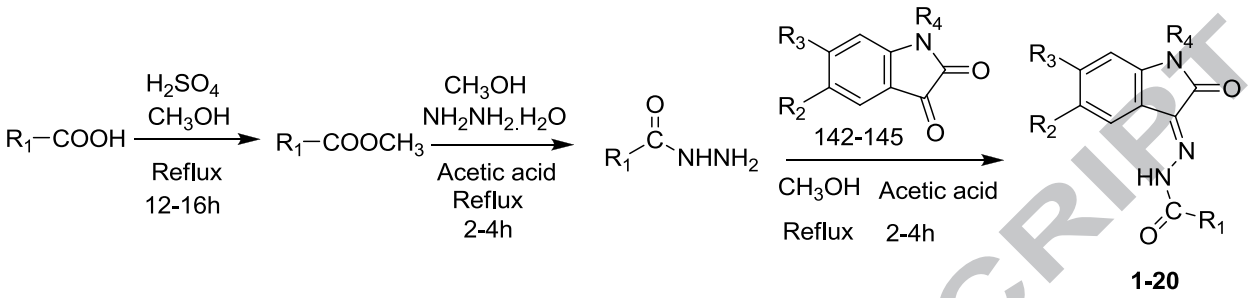


Figure-1:

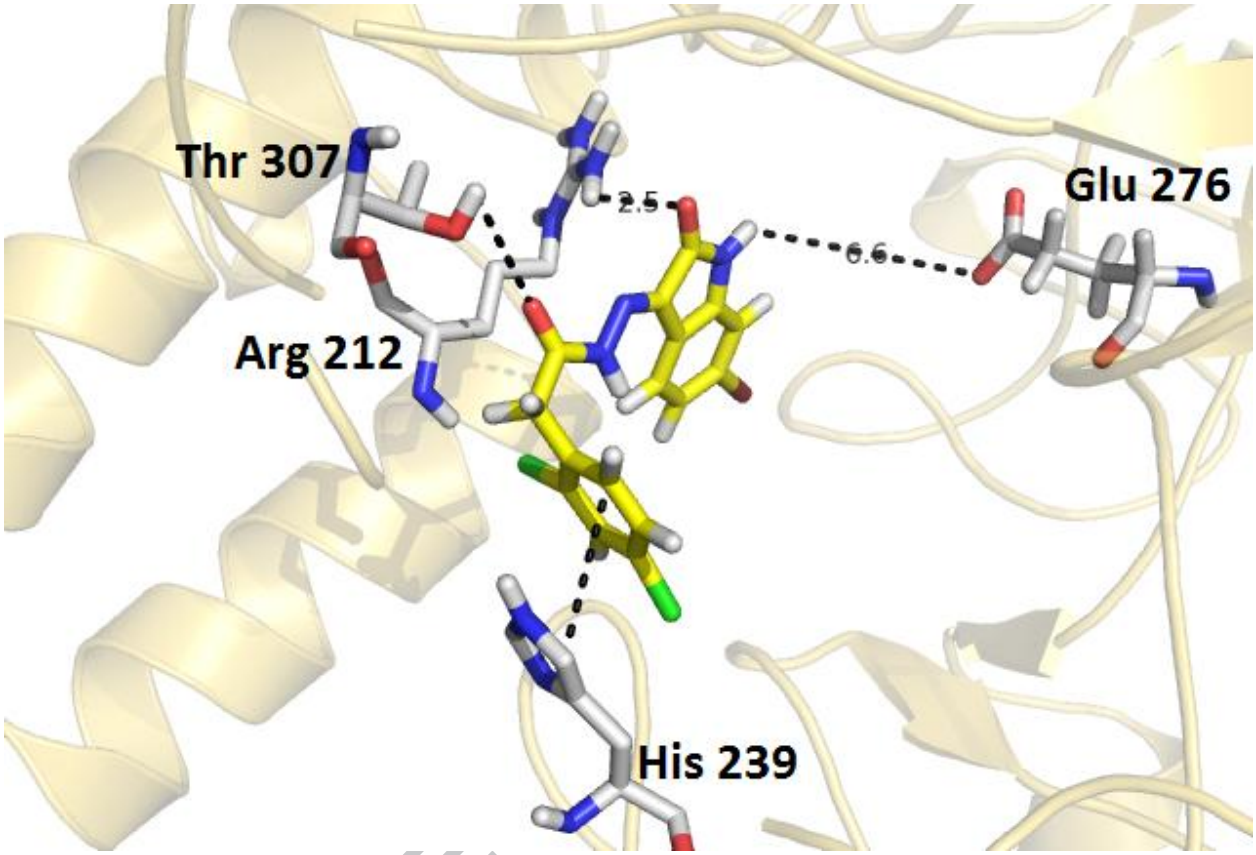


Figure-2:

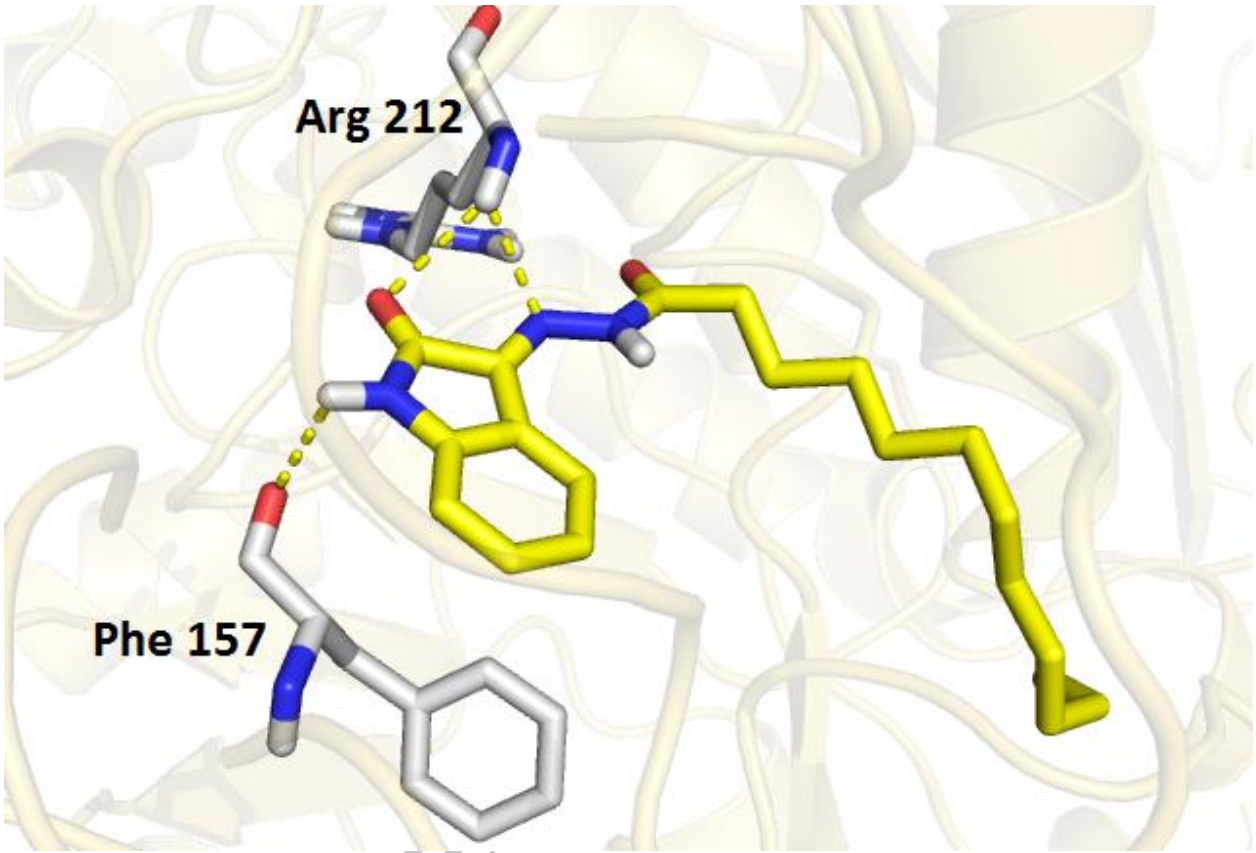


Figure-3:

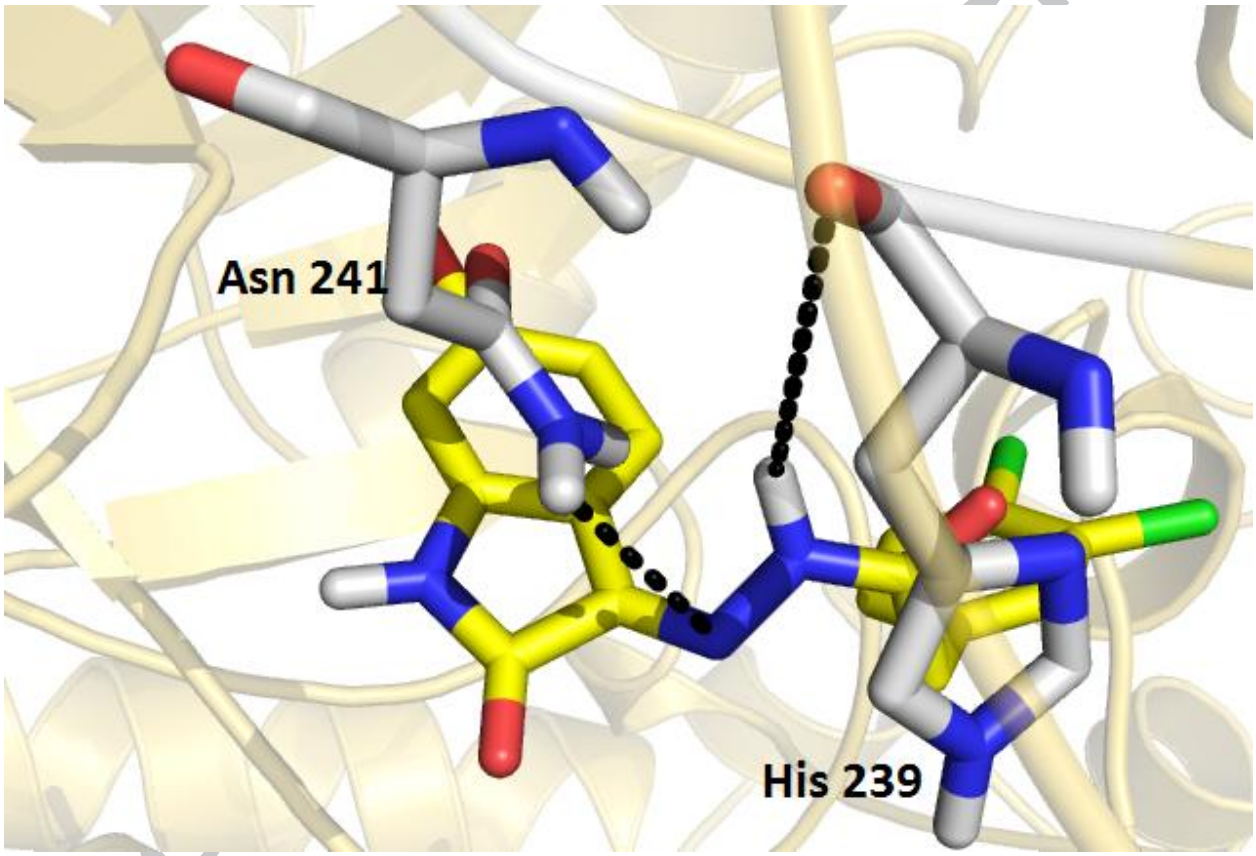


Figure-4:

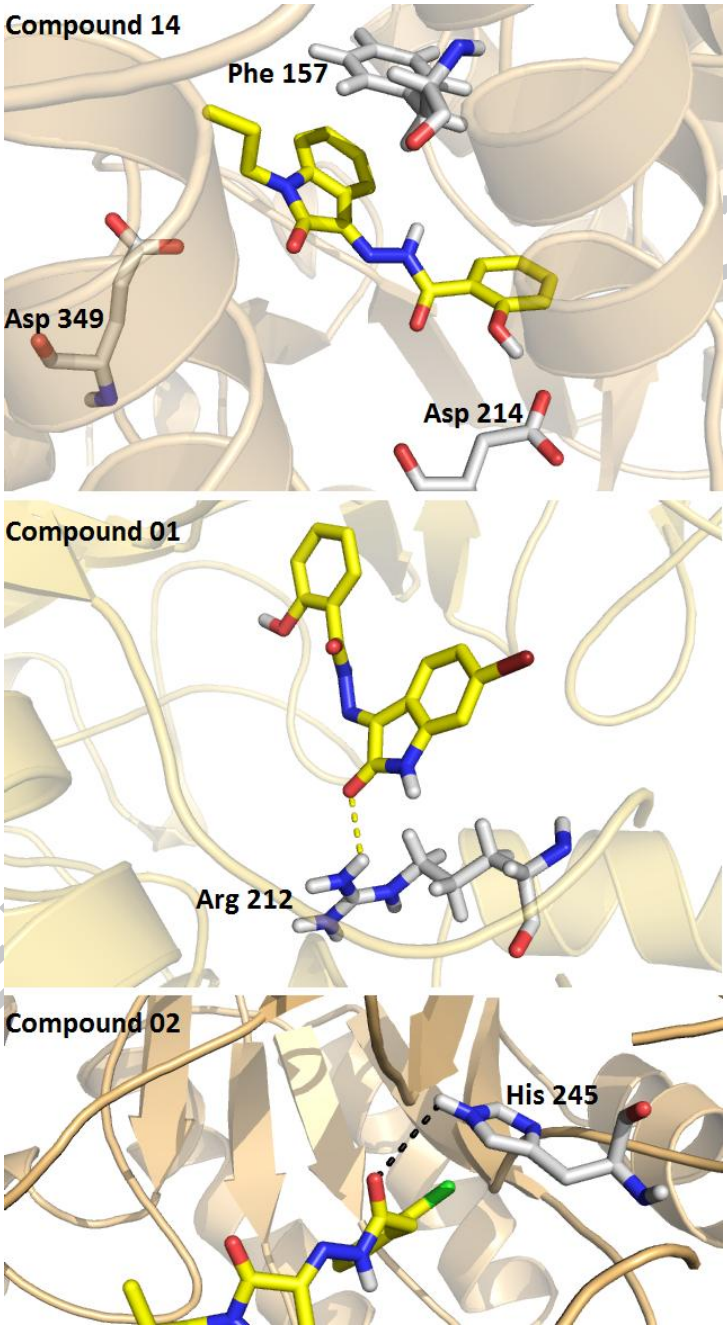
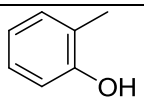
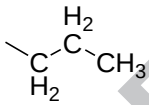
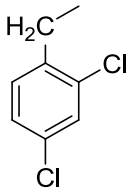
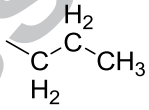
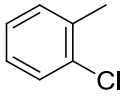
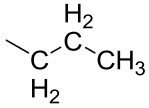
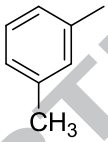
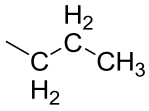
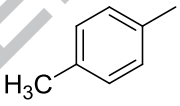
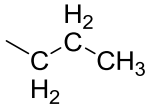
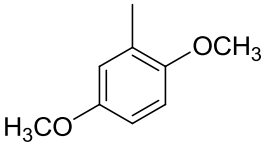
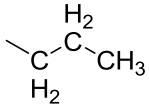
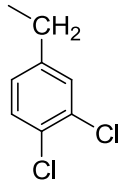
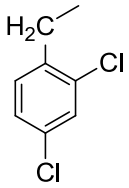
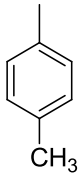
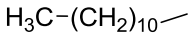
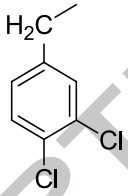
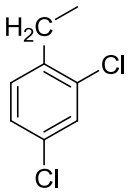
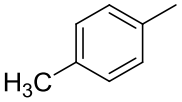
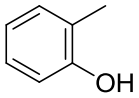
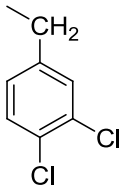
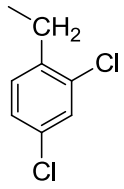
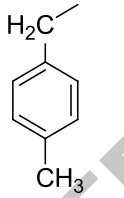
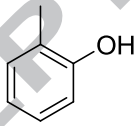


Table-1

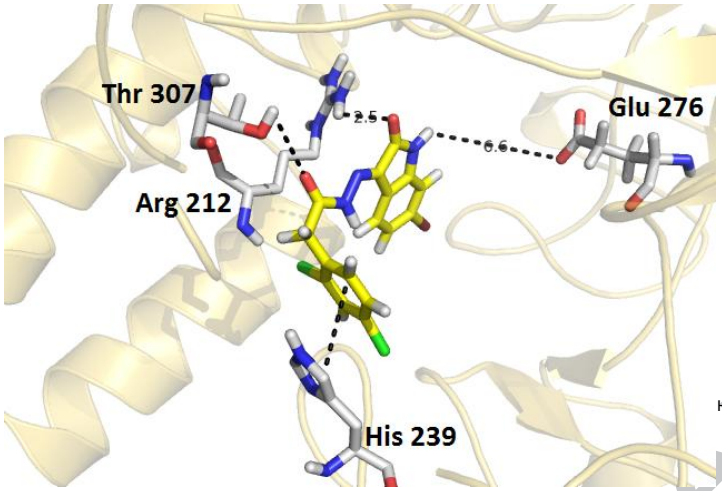
Compounds	**R_1**	**R_2**	**R_3**	**R_4**	**IC_{50} ± SEM [μM]**
1		H	H		NA
2		H	H		18.3 ± 0.56
3		H	H		NA
4		H	H		NA
5		H	H		NA
6		H	H		NA

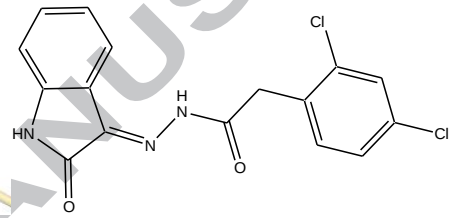
7		Cl	H	H	NA
8		Cl	H	H	NA
9		Cl	H	H	83.5 ± 1.0
10		Cl	H	H	NA
11		H	Br	H	3.3 ± 0.25
12		H	Br	H	2.2 ± 0.25
13		H	Br	H	NA

14		H	Br	H	11.8 ± 0.15
15		H	Br	H	NA
16		H	H	H	NA
17		H	H	H	NA
18		H	H	H	NA
19		H	H	H	NA
20		H	H	H	3.0 ± 0.15
Acarbose					840±1.73

SEM is stand error of the mean, NA not active; acarbose is standard for α-glucosidase inhibition activity

Graphical abstract





Compound 12

(IC_{50}, 2.2 ± 0.25 μM)

Potent Inhibitors of α-glucosidase

Highlights:

- Synthesis of **Isatin based Schiff bases**
- *In vitro α-glucosidase* inhibitory activity
- Identification of a novel class of α-glucosidase inhibitors
- Structure-activity Relationship established
- Molecular docking