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Discovery of novel oxindole derivatives as potent α-glucosidase inhibitors

Momin Khan [a,*], Muhammad Yousaf [a], Abdul Wadood [b], Muhammad Junaid [b], Muhammad Ashraf [c], Umber Alam [c], Muhammad Ali [d], Muhammad Arshad [d], Zahid Hussain [a], Khalid Mohammed Khan [e,*]

[a] Department of Chemistry, Abdul Wali Khan University, Mardan 23200, Pakistan
[b] Department of Biochemistry, Abdul Wali Khan University, Mardan 23200, Pakistan
[c] Department of Biochemistry and Biotechnology, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan
[d] Department of Chemistry, COMSATS Institute of Information Technology, University Road, Abbottabad 22060, Pakistan
[e] H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan

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ABSTRACT

A series of 6-chloro-3-oxindole derivatives **1**–**25** were synthesized in high yields by the reaction of 6-chlorooxindole with different aromatic aldehydes in the presence of piperidine. All the synthesized compounds were isolated with *E* configuration. The structures were confirmed using spectroscopic techniques, including ^{1}H NMR and EIMS. These compounds showed varying degree of yeast α-glucosidase inhibition and seven were found as potent inhibitors of the enzyme. Compounds **2**, **3**, **4**, **5**, **6**, **23**, and **25** exhibited IC_{50} values 2.71 ± 0.007, 11.41 ± 0.005, 37.93 ± 0.002, 15.19 ± 0.004, 24.71 ± 0.007, 17.33 ± 0.001, and 14.2 ± 0.002 μM, respectively, as compared to standard acarbose (IC_{50}, 38.25 ± 0.12 μM). Docking studies helped to find interactions between the enzyme and the active compounds. As a result of this study, oxindoles have been discovered as a new class of α-glucosidase inhibitors which have not been reported earlier.

1. Introduction

Oxindoles exhibit an extensive range of biological effects.[1] Indolin-2-one (Sunitinib) has been widely used in the treatment of gastrointestinal stromal tumors, and metastatic renal cell cancer.[2] Oxindole-Schiff base copper(II) complexes have shown potential antitumor activity towards different cells.[3] Oxindole derivatives such as indolidan and adibendan are used for the treatment of congestive heart failure as these have strong vasodilatory, positive inotropic and inodilatory actions.[4] Amino methylene oxindole derivatives are useful as antihypertensive agents.[5] 6-Chlorooxindole, 6-fluorooxindole, 5-fluorooxindole, and 5-fluoro-1-methyloxindole showed sleep inducing actions.[6] Oxindole-oxazolidinone derivatives are the most important class of antimicrobials.[7] Synthetic oxindoles moiety containing compounds exhibit useful pharmaceutical properties, including growth hormone secretagogues,[8] analgesic,[9] anti-inflammatory,[10] serotonergic.[11] Most of these compounds contain a variety of substituents at the C-3 position of oxindole, and some of them are 3-spirooxindoles[12] which possess P-glycoprotein-mediated multiple drug resistance inhibitors,[13] antibacterial, antiprotozoa.[14]

α-Glucosidase inhibitory activity of these compounds has not been reported earlier and this work describes that oxindole derivatives are potent inhibitors of this enzyme which is a target enzyme in the treatment of diabetes mellitus.

α-Glucosidases (EC3.2.1.20) is a family of enzymes located in the brush border surface of small intestinal cells and causes hydrolysis of 1,4-glycosidic bond of starch and generate α-D-glucose which on absorption enters into the blood stream. It, therefore, increases the post-prandial hyperglycemia. The inhibitors of this enzyme are thus important in the treatment of type II diabetes mellitus. A controlled digestion and absorption of monosaccharides may help to prevent diabetes, hyperlipidemia, hyper lipoproteinemia and obesity.[15,16] It also removes monosaccharides from viral glycoproteins and hence its inhibitors may bring out changes in cell-to-cell signaling and virus recognition to the cell. Therefore, these inhibitors may find applications in the treatment of viral infection and some types of cancers and as immunoregulatory agents.[15,17–19] These inhibitors proved the importance in glycosylation studies in cells.

* Corresponding authors. Tel.: +92 937929122; fax: +92 937542188 (M.K.); tel.: +92 2134824910; fax: +92 2134819018 (K.M.K.).
E-mail addresses: mominkhan@awkum.edu.pk, mominhej@yahoo.com (M. Khan), hassaan2@super.net.pk, khalid.khan@iccs.edu (K.M. Khan).

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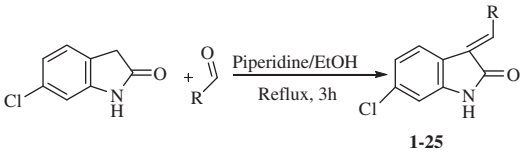
0968-0896/



Scheme 1. Synthesis of 6-chloro-3-oxindole derivatives **1**–**25**.

2. Results and discussion

2.1. Chemistry

All the synthesized compounds[20] were isolated with *E* configuration. For the determination of the structures of synthesized compounds **1**–**25**, ^{1}H NMR, and EI spectroscopy were used. All compounds gave a satisfactory CHN analysis (see Scheme 1 and Table 1).

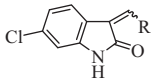
2.2. α-Glucosidase activity

All synthesized 6-chloro-3-oxindole derivatives were tested for α-glucosidase inhibition studies and all of the compounds showed >50% inhibition except one. Compounds **2**, **3**, **4**, **5**, **6**, **23**, **25** having IC_{50} values (mean ± SEM, $n = 3$), 2.71 ± 0.007, 11.41 ± 0.005, 37.93 ± 0.002, 15.19 ± 0.004, 24.71 ± 0.007, 17.33 ± 0.001, and 14.2 ± 0.002 μM, respectively, were found as potent inhibitors of the enzyme. Molecular docking studies were performed to understand the mechanism of enzyme inhibition and binding mode of these novel oxindole derivatives inside the binding pocket of α-glucosidase.

2.3. Molecular docking

From the molecular docking studies, it was observed that the top ranked conformation of all the compounds fit well in the active site and the oxindole moiety of these compounds showed interaction

Table 1
Inhibition studies of α-glucosidase of compounds **1**–**25**



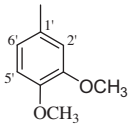
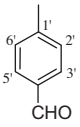
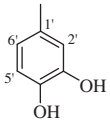
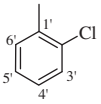
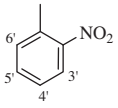
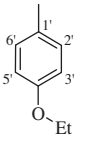
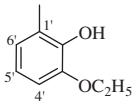
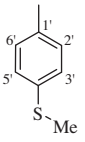
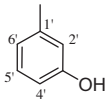
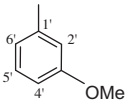
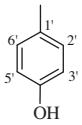
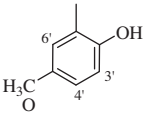
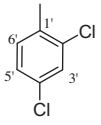
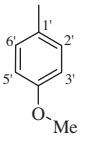
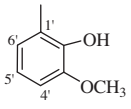
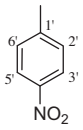
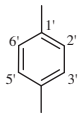
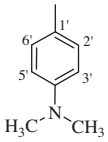
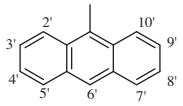
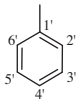
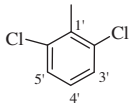
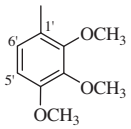
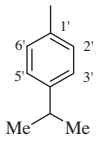
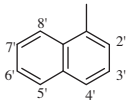
Compd #	R	IC_{50} ± SEM[a] (μM)	Compd #	R	IC_{50} ± SEM[a] (μM)
1		410.55 ± 0.91	**14**		109.98 ± 0.07
2		2.71 ± 0.007	**15**		73.11 ± 0.001
3		11.41 ± 0.005	**16**		99.72 ± 0.04
4		37.93 ± 0.002	**17**		51.81 ± 0.03
5		15.19 ± 0.004	**18**		139.23 ± 0.02
6		24.71 ± 0.007	**19**		82.15 ± 0.02
7		55.64 ± 0.09	**20**		108.31 ± 0.04
8		72.19 ± 0.008	**21**		173.32 ± 0.003

Table 1 (*continued*)

Compd #	R	IC_{50} ± SEM[a] (μM)	Compd #	R	IC_{50} ± SEM[a] (μM)
9		84.35 ± 0.001	**22**		114.73 ± 0.02
10		91.22 ± 0.009	**23**		17.33 ± 0.001
11		195.59 ± 0.05	**24**		52.87 ± 0.004
12		341.11 ± 0.12	**25**		14.27 ± 0.002
13		102.32 ± 0.003	Acarbose	Standard inhibitor	38.25 ± 0.12

[a] Data is mean of three independent experiments (mean ± SEM, $n = 3$).

with important residues of the binding pocket. In case of the most active compound (**2**) four hydrogen bonds with the catalytically active residues Asp214, Glu276, Asp349 were observed (Fig. 1). The highest activity of this compound might be due to the presence of two hydroxyl groups attached to the benzene ring. Both the hydroxyl groups are involved in hydrogen bonding. The hydroxyl groups are strong activating groups which polarize the molecule and enable it to make several interactions with other residues. Similarly, Compounds **5** and **6** having hydroxyl groups at *meta* and *para* positions respectively, exhibited the good biological activities against the enzyme. The docked conformation of compound **5** showed two hydrogen bonds with important active site residues Asp214, and Asp349 whereas two hydrogen bonds were established with other residues His348 and Arg212, which made it the fourth active compound in the series (Fig. 2). In case of compound **6**, the hydroxyl group was present in the *meta* position so, it polarized the compound which enabled it to form five hydrogen bonds and only one hydrogen bond was observed with important active

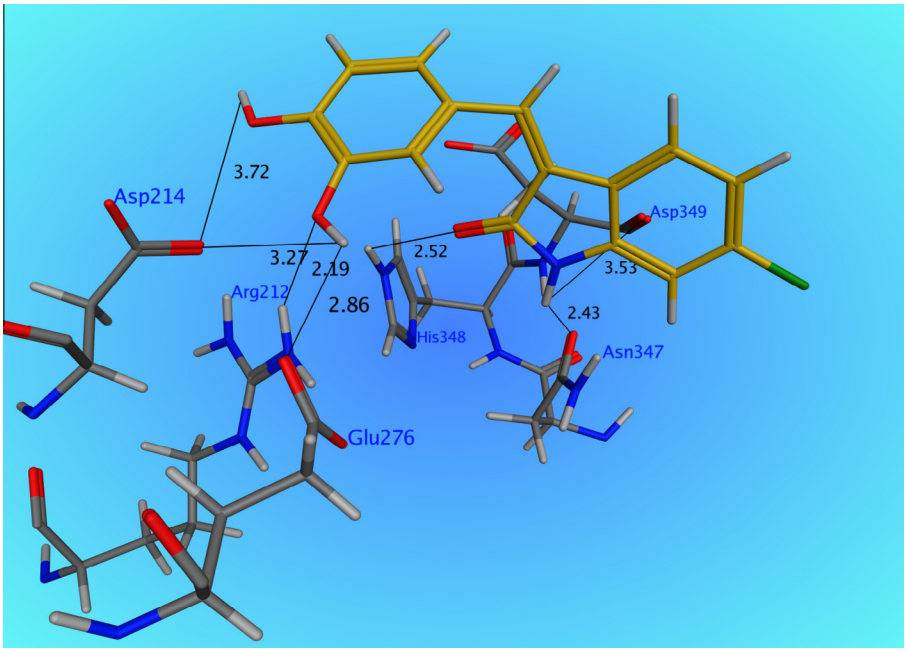


Figure 1. Binding mode of compound **2** in the active site of α-glucosidase.

residues Asp349 and four with other residues His348, Asn347, Arg212, His111 which made it the sixth active compound in the series (Fig. 3).

On the other hand, the least active compounds (**1** and **12**) contain methoxy groups which do not contain hydrogen bond donor or acceptor properties and both form one hydrogen bond with the active residues Arg439 and Asp214, respectively (Figs. 4 and 5). A Similar profile was shown by compound **11** (Fig. 6).

2.4. In Vitro assay protocol for α-glucosidase

The α-glucosidase inhibition activity was performed with slight modifications as given by Pierre et al.[21] Total volume of 100 μL reaction mixture contained, 70 μL 50 mM phosphate buffer pH 6.8, 10 μL (0.5 mM in methanol) test compound, followed by the addition of 10 μL (0.057 units, Sigma Inc.) enzyme solution in the buffer. The contents were mixed, pre-incubated for 10 min at 37 °C and pre-read at 400 nm. The reaction was initiated by the addition of 10 μL of 0.5 mM substrate (*p*-nitrophenyl glucopyranoside, Sigma Inc.). After 30 min of incubation at 37 °C, the absorbance of *p*-nitrophenol was measured at 400 nm using the Synergy HT 96-well plate reader, BioTek, USA. Acarbose was used as positive control. All experiments were carried out in triplicates (mean ± SEM, $n = 3$). Percent inhibition was calculated by the following equation:

$$\text{Inhibition}(\%) = (\text{Abs of Control} - \text{Abs of Test}/\text{Abs of Control}) \times 100$$

Active compound solutions were suitably diluted and their inhibition studies were determined. Data obtained was used for the determination of IC_{50} values (concentration at which there is 50% enzyme inhibition) using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

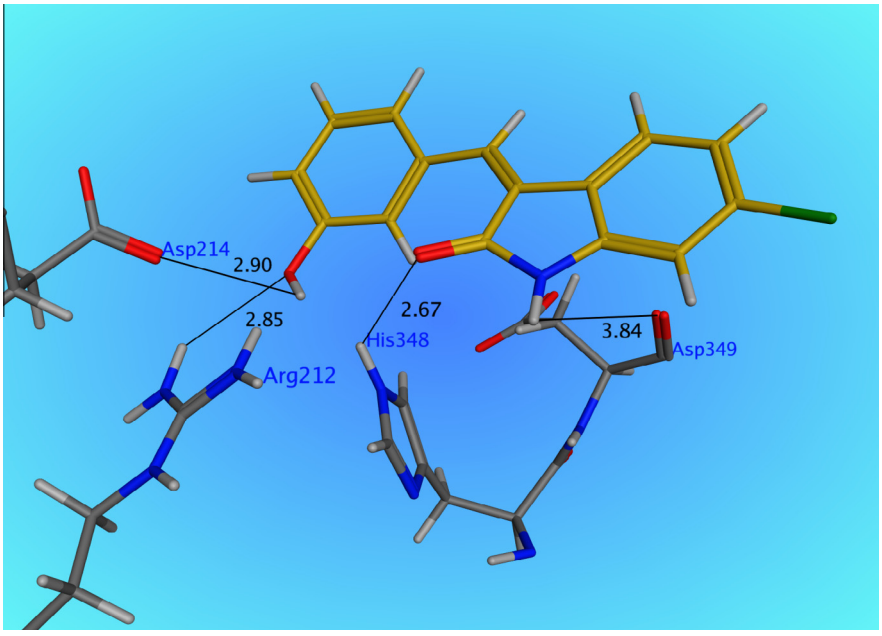


Figure 2. Binding mode of compound **5** in the active site of α-glucosidase.

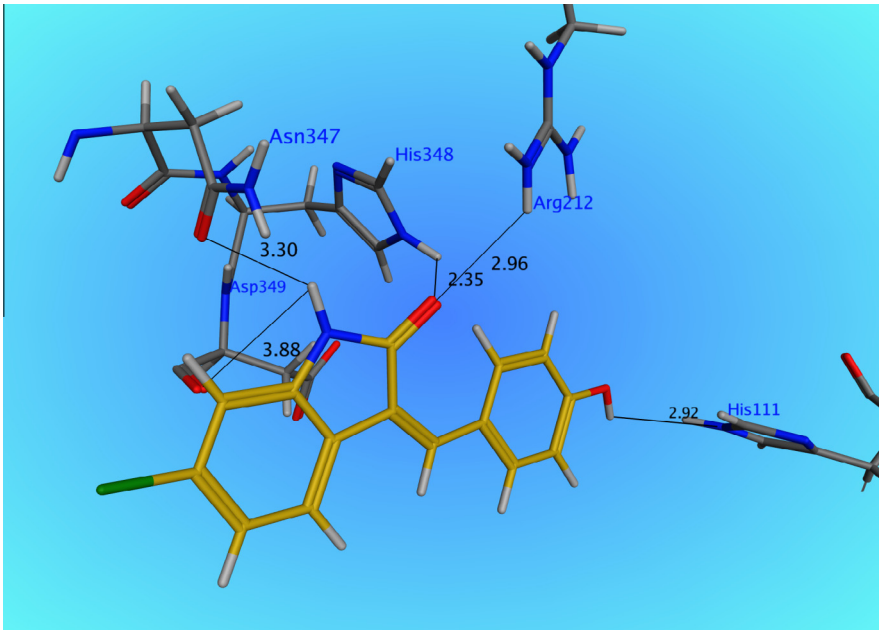


Figure 3. Binding mode of compound **6** in the active site of α-glucosidase.

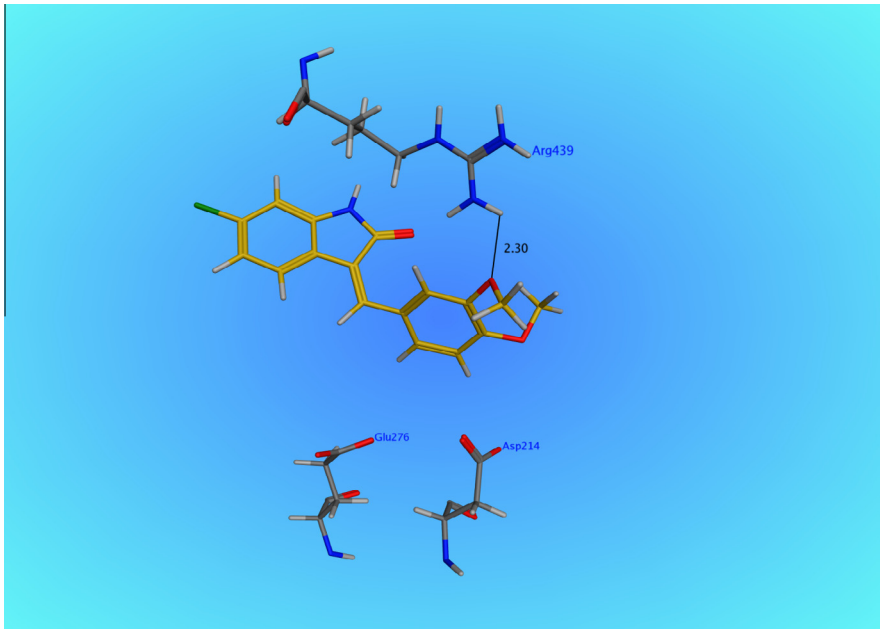


Figure 4. Binding mode of compound **1** in the active site of α-glucosidase.

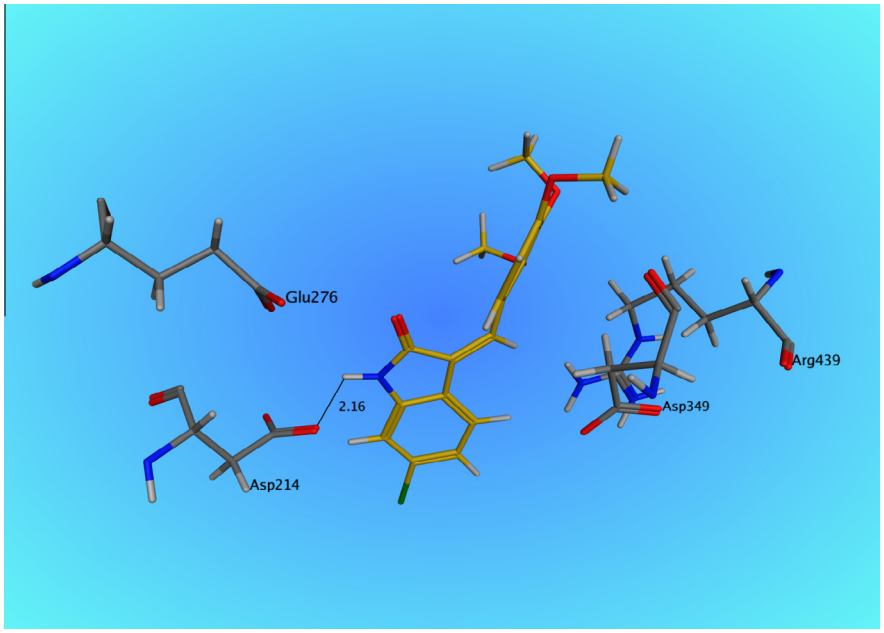


Figure 5. Binding mode of compound **12** in the active site of α-glucosidase.

2.5. Molecular docking

The 3D structure for α-glucosidase for *Saccharomyces cerevisiae* has not been solved up-to yet. However, only few homology models has been reported.[22–24] So, we developed the 3D model for α-glucosidase for *Saccharomyces cerevisiae* by a comparative homology modeling technique using the same protocol as described by Burke et al.[25] The primary sequence of α-glucosidase from *Saccharomyces cerevisiae* was retrieved from UniProt protein resource data bank (http://www.uniprot.org/) under the access code P53341. Template search was performed using MOE-Search tools against the PDB databank implemented in MOE2010.11. The crystallographic structure of *Saccharomyces cerevisiae* isomaltase (PDB code 3AJ7, 1.30 Å resolution)[26] with 72.4% of sequence identity with the target was selected as the template. The 3D structure of α-glucosidase for *Saccharomyces cerevisiae* was built using 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 MOE2010.11. Three dimensional 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 MMFF 94x force field. Energy minimization

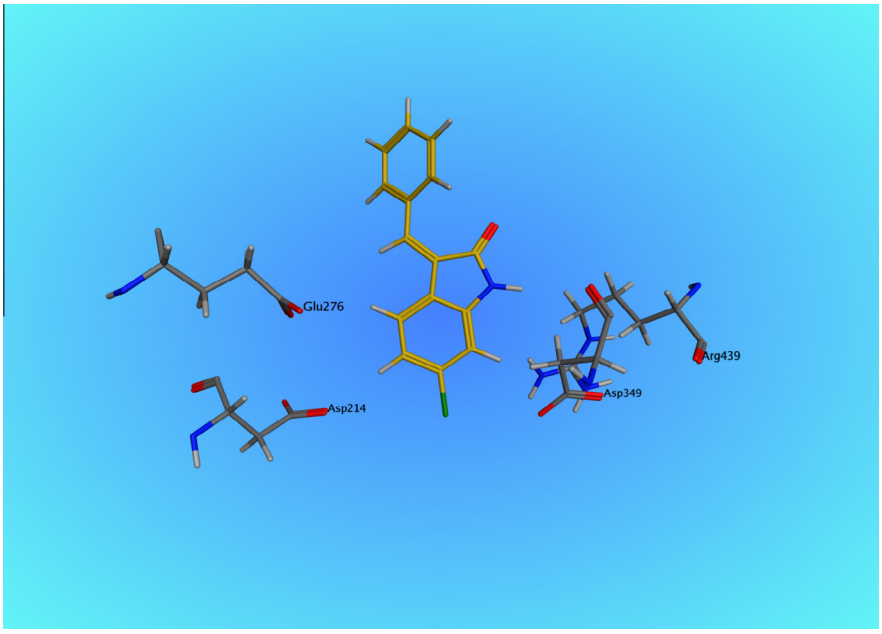


Figure 6. Binding mode of compound **11** in the active site of α-glucosidase.

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 done prior to docking using Protonate 3D tools. Protonation was followed by energy minimization up to 0.05 Gradient using Amber 99 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. Each complex was analyzed for interactions and their 3D images were taken.

3. Conclusions

6-Chloro-3-oxindole derivatives were synthesized and their inhibitory potential was evaluated against α-glucosidase enzyme. All compounds showed α-glucosidase inhibitory potential. Compounds **2**, **3**, **4**, **5**, **6**, **23**, **25** exhibited an excellent inhibition with IC_{50} values between 2.71 ± 0.007 and 37.93 ± 0.002 μM. Consequently, *in silico* studies were performed to recognize the binding mode of these compounds.

4. Materials and methods

NMR experiments were performed on Advance Bruker AM 300, 400 and 500 MHz NMR machines. Electron impact mass spectra (EIMS) were recorded on a Finnigan MAT-311A, Germany. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm.

4.1. General procedure for the synthesis of compounds 1–25

Synthesis of 6-chlorooxindole derivatives was carried out by refluxing 6-chlorooxindole with different aromatic aldehydes in ethanol in the presence of a catalytic amount of piperidine. The reaction mixture was refluxed for 3 h. After completion of reaction as determined by TLC analysis, the contents were cooled and concentrated at reduced pressure to afford solid 3-oxindole derivatives. The product was washed with equal volumes of a mixture of hexane–ethyl acetate (25 mL) and dried to afford compounds **1**–**25**.[20] ^{1}H NMR, EI spectroscopy and CHN analysis were used for the determination of their structures.

4.1.1. (*E*)-3-(3, 4-Dimethoxybenzylidene)-6-chloroindolin-2-one (1)

Yield: 0.27 g (85%); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.67 (s, 1H, NH), 8.64 (d, 1H, $J_{2',6'}$ = 1.5 Hz, H-2′), 7.83 (dd, 1H, $J_{6',2'}$ = 1.5, $J_{6',5'}$ = 8.5 Hz, H-6′), 7.78 (s, 1H, =CH), 7.69 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 7.06 (d, 1H, $J_{5',6'}$ = 8.5 Hz, H-5′),7.03 (dd, 1H, $J_{5,7}$ = 1.5, $J_{5,4}$ = 8.0 Hz, H-5), 6.83 (d, 1H, $J_{7,5}$ = 1.5 Hz, H-7), 3.83 (s, 3H, —OCH_3), 3.82 (s, 3H, —OCH_3); MS: *m*/*z* (rel. abund.%) 315 (M+, 100), 272 (25), 166 (33), 140 (27); Anal. Calcd for $C_{17}H_{14}ClNO_3$ (315.75): C, 64.67; H, 4.47; Cl, 11.23; N, 4.44; O, 15.20; Found: C, 64.65; H, 4.46; N, 4.44.

4.1.2. (*E*)-3-(3, 4-Dihydroxybenzylidene)-6-chloroindolin-2-one (2)

Yield: 0.24 g (83%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 09.65 (s, 1H, NH), 7.73 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 7.64 (d, 1H, $J_{5',6'}$ = 8.5 Hz, H-5′), 7.49 (s, 1H, =CH), 7.16 (d, 1H, $J_{2',6'}$ = 2.0 Hz, H-2′), 6.95 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 8.0 Hz, H-5), 6.86(d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 6.79(1H, $J_{6',5'}$ = 8.5 Hz, H-6′); MS: *m*/*z* (rel. abund.%) 287 (M^+, 100), 271 (4.9), 254 (10), 240 (15); Anal. Calcd for $C_{15}H_{10}ClNO_3$ (287.70): C, 62.62; H, 3.50; Cl, 12.32; N, 4.87; O, 16.68; Found: C, 62.61; H, 3.52; N, 4.88.

4.1.3. (*E*)-3-(2-Nitrobenzylidene)-6-chloroindolin-2-one (3)

Yield: 0.245 g (81%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.82 (s, 1H, NH), 8.31 (d, 1H, $J_{3',4'}$ = 8.0 Hz, H-3′), 7.88 (s, 1H, =CH), 7.86–7.75 (m, 2H, H-4/4′), 6.93–6.85 (m, 2H, H-5′/6′), 6.89 (d, 1H, $J_{5,4}$ = 8.0 Hz, H-5), 6.87 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7); MS: *m*/*z* (rel. abund.%) 300 (M^+, 3), 270 (2), 254 (23), 219 (7); Anal. Calcd for $C_{15}H_9ClN_2O_3$ (300.70): C, 59.91; H, 3.02; Cl, 11.79; N, 9.32; O, 15.96; Found: C, 59.91; H, 3.03; N, 9.33.

4.1.4. (*E*)-3-(3-Ethoxy-2-hydroxybenzylidene)-6-chloroindolin-2-one (4)

Yield: 0.23 g (73%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.69 (s, 1H, NH), 7.72 (s, 1H, =CH), 7.46 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 7.15(d,

1H, $J_{4',5'}$ = 7.5 Hz, H-4′), 7.05 (d, 1H, $J_{6',5'}$ = 7.5 Hz, H-6′), 6.92–6.82 (m, 3H, H-5′/5/7), 4.130 (q, 2H, J = 5.5 Hz, -OCH_2), 1.36 (t, 3H, J = 5.5 Hz, -CH_2CH_3); MS: m/z (rel. abund.%) 315 (M^+, 100), 298 (54), 286 (71), 270 (53); Anal. Calcd for $C_{17}H_{14}ClNO_3$ (315.75): C, 64.67; H, 4.47; Cl, 11.23; N, 4.44; O, 15.20; Found: C, 64.68; H, 4.46; N, 4.44.

4.1.5. (*E*)-3-(3-Hydroxybenzylidene)-6-chloroindolin-2-one (5)

Yield: 0.20 g (74%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.73 (s, 1H, NH), 9.90 (br.s, 1H, —OH), 7.57 (s, 1H, =CH),7.54(d, 1H, $J_{4,5}$ = 8.5 Hz, H-4), 7.31(t, 1H, $J_{5'(4',6')}$= 8.5 Hz, H-5′), 7.09–7.01 (m, 2H, H-2′/6′), 6.94–6.6.85 (m, 3H, H-4′/5/7); MS: m/z (rel. abund.%) 271 (M^+, 100), 266 (20), 243 (31), 178 (32); Anal. Calcd for $C_{15}H_{10}ClNO_2$ (271.70): C, 66.31; H, 3.71; Cl, 13.05; N, 5.16; O, 11.78; Found: C, 66.31; H, 3.70; N, 5.18.

4.1.6. (*E*)-3-(4-Hydroxybenzylidene)-6-chloroindolin-2-one (6)

Yield: 0.22 g (82%); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.67 (s, 1H, NH), 8.37 (d, 1H, $J_{4,5}$ = 8.5 Hz, H-4), 7.73–7.56 (m, 3H, H-2′/6′/=CH), 6.93–6.80 (m, 4H, H-5/7/2′/6′); MS: m/z (rel. abund.%) 271 (M^+, 100), 243 (57), 208 (27), 178 (35); Anal. Calcd for $C_{15}H_{10}ClNO_2$ (271.70): C, 66.31; H, 3.71; Cl, 13.05; N, 5.16; O, 11.78; Found: C, 66.30; H, 3.73; N, 5.17.

4.1.7. (*E*)-3-(2,4-Dichlorobenzylidene)-6-chloroindolin-2-one (7)

Yield: 0.27 g (83%); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.85 (s, 1H, NH), 7.84 (d, 1H, $J_{3',5'}$ = 2.0 Hz, H-3′), 7.77 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 7.57 (dd, 1H, $J_{5',3'}$ = 2.0, $J_{5',6'}$ = 8.5 Hz, H-5′), 7.53 (s, 1H, =CH), 7.11 (d, 1H, $J_{6',5'}$ = 8.5 Hz, H-6′), 6.89–6.88 (m, 2H, H-5/7); MS: m/z (rel. abund.%) 323 (M^+, 4), 288 (100), 253 (18), 224 (5); Anal. Calcd for $C_{15}H_8Cl_3NO$ (324.59): C, 55.50; H, 2.48; Cl, 32.77; N, 4.32; O, 4.93; Found: C, 55.48; H, 2.50; N, 4.31.

4.1.8. (*E*)-3-(2-Hydroxy-3-methoxybenzylidene)-6-chloroindolin-2-one (8)

Yield: 0.25 g (82%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.70 (s, 1H, NH), 9.41 (s, 1H, OH), 7.71 (s,1H, =CH), 7.56 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 7.16 (d, 1H, $J_{6',5'}$ = 7.5 Hz, H-6′), 7.08 (d, 1H, $J_{4',5'}$ = 7.5 Hz, H-4′), 6.92–6.85 (m, 3H, H-5/7/5′), 3.84 (s, 3H, —OCH_3); MS: m/z (rel. abund.%) 301 (M^+, 100), 284 (74), 273 (12), 230 (18); Anal. Calcd for $C_{16}H_{12}ClNO_3$ (301.72): C, 63.69; H, 4.01; Cl, 11.75; N, 4.64; O, 15.91; Found: C, 63.70; H, 4.01; N, 4.65.

4.1.9. (*E*)-3-(4-Nitrobenzylidene)-6-chloroindolin-2-one (9)

Yield: 0.24 g (81%); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.84 (s, 1H, NH), 8.35 (d, 2H, $J_{3',2'/5',6'}$ = 8.7 Hz, H-3′/5′), 7.94 (d, 2H, $J_{2',3'/6',5'}$ = 8.7 Hz, H-2′/6′), 7.71 (s, 1H, =CH), 6.90–6.85 (m, 2H, Hz, H-5/7); MS: m/z (rel. abund.%) 300 (M^+, 100), 272 (18), 254 (30), 191 (47); Anal. Calcd for $C_{15}H_9ClN_2O_3$ (300.70): C, 59.91; H, 3.02; Cl, 11.79; N, 9.32; O, 15.96; Found: C, 59.93; H, 3.00; N, 9.33.

4.1.10. (*E*)-3-(4-(Dimethylamino)benzylidene)-6-chloroindolin-2-one (10)

Yield: 0.24 g (80%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.59 (s, 1H, NH), 7.75 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4),7.63 (d, 2H, $J_{2',3'/6',5'}$ = 9.0 Hz, H-2′/6′), 7.54 (s, 1H, =CH), 6.93 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 8.0 Hz, H-5), 6.86 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 6.81(d, 2H, $J_{3',2'/5',6'}$ = 9.0 Hz, H-3′/5′),3.02 (s, 6H, N(CH3)2); MS: m/z (rel. abund.%) 298 (M^+, 100), 281 (6), 269 (7), 254 (8); Anal. Calcd for $C_{17}H_{15}ClN_2O$ (298.77): C, 68.34; H, 5.06; Cl, 11.87; N, 9.38; O, 5.36; Found: C, 68.37; H, 5.04; N, 9.40.

4.1.11. (*E*)-3-Benzylidene-6-chloroindolin-2-one (11)

Yield: 0.22 g (86%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.76 (s, 1H, NH), 7.69 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4),7.66 (s, 1H, =CH), 7.54–7.45 (m, 5H, H-2′/3′/4′/5′/6′), 6.90 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 8.0 Hz, H-5), 6.88 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7); MS: m/z (rel. abund.%) 255 (M^+, 100), 227 (34), 190 (10), 178 (37); Anal. Calcd for $C_{15}H_{10}ClNO$ (255.70): C, 70.46; H, 3.94; Cl, 13.87; N, 5.48; O, 6.26; Found: C, 70.49; H, 3.91; N, 5.48.

4.1.12. (*E*)-3-(2,3,4-Trimethoxybenzylidene)-6-chloroindolin-2-one (12)

Yield: 0.28 g (81%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.69 (s, 1H, NH), 7.61 (s, 1H, =CH), 7.49 (d, 1H, $J_{6',5'}$ = 8.5 Hz, H-6′), 7.45 (d, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 6.95 (d, 1H, $J_{5',6'}$ = 8.5 Hz, H-5′), 6.89 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 9.0 Hz, H-5), 6.87 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 3.87 (s, 3H, —OCH_3), 3.82 (s, 3H, —OCH_3), 3.78 (s, 3H, —OCH_3); MS: m/z (rel. abund.%) 345 (M^+, 100), 314 (), 299 (17), 270 (10); Anal. Calcd for $C_{18}H_{16}ClNO_4$ (345.78): C, 62.52; H, 4.66; Cl, 10.25; N, 4.05; O, 18.51; Found: C, 62.52; H, 4.65; N, 4.04.

4.1.13. (*E*)-6-Chloro-3-(naphthalene-1-ylmethylene) indolin-2-one (13)

Yield: 0.23 g (77%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.54 (s, 1H, NH), 8.26 (s,1H, H-1′), 8.04–7.93 (m, 3H, H-3′/4′/7′), 7.82 (s, 1H, =CH), 7.77 (d, 1H, $J_{5',6'}$ = 8.5 Hz, H-5′), 7.62–7.56 (m, 2H, H-4/6′), 7.52 (d, 1H, $J_{8',7'}$ = 8.5 Hz, H-8′), 6.90–6.88 (m, 2H, H-5/7); MS: m/z (rel. abund.%) 305 (M^+, 100), 277 (30), 269 (13), 241 (25); Anal. Calcd for $C_{19}H_{12}ClNO$ (305.76): C, 74.64; H, 3.96; Cl, 11.60; N, 4.58; O, 5.23; Found: C, 74.66; H, 3.97; N, 4.55.

4.1.14. (*E*)-4-((6-Chloro-2-oxoindolin-3-ylidene)methyl)benzaldehyde (14)

Yield: 0.22 g (77%); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.12 (s, 1H, NH), 10.06 (s, 1H, CHO), 8.43(d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 8.02 (d, 2H, $J_{2',3'/6',5'}$ = 7.5 Hz, H-2′/6′), 7.86 (d, 2H, $J_{3',2'/5',6'}$ = 7.5 Hz, H-3′/5′), 7.69 (s, 1H, =CH),6.90–6.84 (m, 2H, H-5/7); MS: m/z (rel. abund.%) 297 (M^+, 100), 254 (22), 191 (10), 178 (19); Anal. Calcd for $C_{16}H_{10}ClNO_2$ (283.71): C, 67.74; H, 3.55; Cl, 12.50; N, 4.94; O, 11.28; Found: C, 67.74; H, 3.54; N, 4.93.

4.1.15. (*E*)-3-(2-Chlorobenzylidene)-6-chloroindolin-2-one (15)

Yield: 0.21 g (74%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.82 (s, 1H, NH), 7.74 (d, 1H, $J_{4,5}$ = 7.0 Hz, H-4), 7.64 (dd, 1H, $J_{3',5'}$ = 1.5, $J_{3',4'}$ = 8.0 Hz, H-3′), 7.61 (s, 1H, =CH), 7.52 (td, 1H, $J_{4',6'}$ = 1.5, $J_{4'(3',5')}$ = 8.0 Hz, H-4′), 7.48 (td, 1H, $J_{5',3'}$ = 1.5, $J_{5'(4',6')}$ = 8.0 Hz, H-5′), 7.10 (d, 1H, $J_{6',5'}$ = 8.0 Hz, H-6′), 6.88–6.87 (m, 2H, H-5/7); MS: m/z (rel. abund.%) 289 (M^+, 11), 254 (100), 219 (16), 190 (12); Anal. Calcd for $C_{15}H_9Cl_2NO$ (290.14): C, 62.09; H, 3.13; Cl, 24.44; N, 4.83; O, 5.51; Found: C, 62.08; H, 3.13; N, 4.84.

4.1.16. (*E*)-3-(4-Ethoxybenzylidene)-6-chloroindolin-2-one (16)

Yield: 0.255 g (85%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.70 (s, 1H, -NH), 7.69 (d, 2H, $J_{2',6'/3',5'}$ = 8.0, H-2′/6′), 7.63 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 7.60 (s, 1H, =CH), 7.06 (d, 2H, $J_{3',5'/2',6}$ = 8.0, H-3′/5′), 7.01 (dd, 1H, $J_{5,7}$ = 1.5, $J_{5,4}$ = 8.0 Hz, H-5), 6.87 (d, 1H, $J_{7,5}$ = 1.5 Hz, H-7), 4.12 (q, 2H, $J_{5,4}$ = 7.0 Hz, —OCH_2), 1.34 (t, 3H, $J_{5,4}$ = 7.0 Hz, —CH_2CH_3); MS: m/z (rel. abund.%) 299 (M^+, 100), 271 (60), 178 (31), 152 (30); Anal. Calcd for $C_{17}H_{14}ClNO_2$ (299.75): C, 68.12; H, 4.71; Cl, 11.83; N, 4.67; O, 10.68; Found: C, 68.13; H, 4.72; N, 4.67.

4.1.17. (*E*)-3-(4-(Methylthio)benzylidene)-6-chloroindolin-2-one (17)

Yield: 0.265 g (88%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.74 (s, 1H, —NH), 8.36 (d, 1H, $J_{4,5}$ = 8.5 Hz, H-4), 7.71–7.56 (m, 3H, H-2′/6′/7), 7.39 (d, 2H, $J_{3',5'/2',6'}$ = 8.0, H-3′/5′), 6.92–6.87 (m, 2H, H-5/=CH); MS: m/z (rel. abund.%) 301 (M^+, 100), 258 (24), 178 (18), 63 (31); Anal. Calcd for $C_{16}H_{12}ClNOS$ (301.79): C, 63.68; H, 4.01;

Cl, 11.75; N, 4.64; O, 5.30; S, 10.62; Found: C, 63.66; H, 4.03; N, 4.66.

4.1.18. (*E*)-3-(3-Methoxybenzylidene)-6-chloroindolin-2-one (18)

Yield: 0.24 g (84%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.75 (s, 1H, —NH), 7.63 (s, 1H, =CH), 7.52 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 7.43 (t, 1H, $J_{5'(4',6')}$ = 8.0, H-5′), 7.24(t, 1H, $J_{4'(3',5')}$ = 8.0, H-4′), 7.23 (d, 1H, $J_{6',5'}$ = 8.0 Hz, H-6′), 7.04 (d, 1H, $J_{3',4'}$ = 8.0 Hz, H-3′), 6.93 (dd, 1H, $J_{5,7}$ = 1.5, $J_{5,4}$ = 8.0 Hz, H-5), 6.88 (d, 1H, $J_{7,5}$ = 1.5 Hz, H-7), 3.79 (s, 3H, —OMe); MS: *m*/*z* (rel. abund.%) 285 (M$^+$, 100), 178 (59), 152 (32), 63 (56); Anal. Calcd for $C_{16}H_{12}ClNO_2$ (285.72): C, 67.26; H, 4.23; Cl, 12.41; N, 4.90; O, 11.20; Found: C, 67.28; H, 4.22; N, 4.88.

4.1.19. (*E*)-3-(2-Hydroxy-5-methoxybenzylidene)-6-chloroindolin-2-one (19)

Yield: 0.25 g (83%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.68 (s, 1H, —NH), 9.53 (s, 1H, —OH), 7.68 (s, 1H, =CH), 7.52 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 7.14 (d, 1H, $J_{7,5}$ = 1.5 Hz, H-7), 6.96–6.86 (m, 4H, H-5/3′/4′/6′), 3.68 (s, 3H, —OMe); MS: *m*/*z* (rel. abund.%) 301 (M$^+$, 100), 152 (23), 75 (34), 53 (77); Anal. Calcd for $C_{16}H_{12}ClNO_3$ (301.72): C, 63.69; H, 4.01; Cl, 11.75; N, 4.64; O, 15.91; Found: C, 63.70; H, 4.00; N, 4.64.

4.1.20. (*E*)-3-(4-Methoxybenzylidene)-6-chloroindolin-2-one (20)

Yield: 0.20 g (70%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.70 (s, 1H, —NH), 7.70 (d, 2H, $J_{2',6'/3',5'}$ = 8.5, H-2′/6′), 7.61 (s, 1H, =CH), 7.08 (d, 2H, $J_{3',5'/2',6}$ = 8.5, H-3′/5′), 7.02 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 6.92 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 8.0, Hz, H-5), 6.92 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 3.83 (s, 3H, —OMe); MS: *m*/*z* (rel. abund.%) 285 (M$^+$, 100), 242 (43), 178 (44), 152 (31); Anal. Calcd for $C_{16}H_{12}ClNO_2$ (285.72): C, 67.26; H, 4.23; Cl, 12.41; N, 4.90; O, 11.20; Found: C, 67.26; H, 4.22; N, 4.89.

4.1.21. (*E*)-6-chloro-3-(furan-2-ylmethylene)indolin-2-one (21)

Yield: 0.19 g (77%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.70 (s, 1H, —NH), 8.61 (d, 1H, $J_{5',4'}$ = 3.2 Hz, H-5′), 8.38 (s, 1H, =CH), 6.71 (d, 2H, $J_{4',5'}$ = 3.2 Hz, H-4′), 6.84 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 6.47 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 8.0 Hz, H-5), 5.61 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4),2.49 (s, 3H, —CH_3); MS: *m*/*z* (rel. abund.%) 245 (M$^+$, 100), 154 (60), 127 (27), 63 (33); Anal. Calcd for $C_{13}H_8ClNO_2$ (245.66): C, 63.56; H, 3.28; Cl, 14.43; N, 5.70; O, 13.03; Found: C, 63.56; H, 3.27; N, 5.71.

4.1.22. (*E*)-3-(4-Methylbenzylidene)-6-chloroindolin-2-one (22)

Yield: 0.240 g (89%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.72 (s, 1H, —NH), 7.62 (s, 1H, =CH), 7.60 (d, 2H, $J_{2',6'/3',5'}$ = 8.0, H-2′/6′), 8.30 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 7.33 (d, 2H, $J_{3',5'/2',6'}$ = 8.0, H-3′/5′), 6.92 (dd, 1H, $J_{5,7}$ = 1.5, $J_{5,4}$ = 8.0 Hz, H-5), 6.87 (s, 1H, H-7), 2.36 (s, 3H, Ph-Me); MS: *m*/*z* (rel. abund.%) 269 (M$^+$, 100), 258 (24), 178 (18), 63 (31); Anal. Calcd for $C_{16}H_{12}ClNO$ (269.73): C, 71.25; H, 4.48; Cl, 13.14; N, 5.19; O, 5.93; Found: C, 71.24; H, 4.48; N, 5.20.

4.1.23. (*E*)-3-(Anthracen-10-ylmethylene)-6-chloroindolin-2-one (23)

Yield: 0.27 g (76%); ^{1}H NMR (500 MHz, DMSO-d_6DMSO-d_6): δ 11.48 (s, 1H, —NH), 8.77 (s, 1H, H-6′), 8.38 (s, 1H, =CH), 8.20 (d, 2H, $J_{(2',3')(10',9')}$ = 8.5, Hz, H-2′/10′), 7.95 (d, 2H, $J_{(5',6')(7',8')}$ = 8.5, Hz, H-5′/7′), 7.57 (t, 2H, $J_{3'(2',4')=9'(8',10')}$ = 8.5, H-3′/9′), 7.52 (t, 2H, $J_{4'(3',5')=8'(7',9')}$ = 8.5, H-4′/8′), 6.84 (d, 1H, $J_{7,5}$ = 2.0 Hz, H-7), 6.47 (dd, 1H, $J_{5,7}$ = 2.0, $J_{5,4}$ = 8.0 Hz, H-5), 5.61 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4); MS: *m*/*z* (rel. abund.%) 355 (M$^+$, 100), 338 (65), 291 (45), 131 (35); Anal. Calcd for $C_{23}H_{14}ClNO$ (355.82): C, 77.64; H, 3.97; Cl, 9.96; N, 3.94; O, 4.50; Found: C, 77.65; H, 3.97; N, 3.92.

4.1.24. (*E*)-3-(2,6-Dichlorobenzylidene)-6-chloroindolin-2-one (24)

Yield: 0.22 g (76%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.86 (s, 1H, —NH), 7.65 (d, 2H, $J_{4,5/5',4'}$ = 8.0 Hz, H-4/5′), 7.53 (t, 1H, $J_{4'(3',5')}$ = 8.0, H-4′), 7.46 (s, 1H, =CH), 6.89–6.87 (m, 2H, H-5′/7), 6.47 (d, 1H, $J_{3',4'}$ = 8.0 Hz, H-3′); MS:*m*/*z* (rel. abund.%) 323 (M$^+$, 05), 287 (100), 253 (22), 83 (23); Anal. Calcd for $C_{15}H_8Cl_3NO$ (324.59): C, 55.50; H, 2.48; Cl, 32.77; N, 4.32; O, 4.93; Found: C, 55.48; H, 2.48; N, 4.33.

4.1.25. (*E*)-3-(2,4-Dimethylbenzylidene)-6-chloroindolin-2-one (25)

Yield: 0.24 g (84%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.74 (s, 1H, —NH), 7.68 (s, 1H, =CH), 7.46 (d, 1H, $J_{4,5}$ = 7.5 Hz, H-4), 7.17–7.10 (m, 3H, H-5/5′/6′), 6.86–6.84 (m, 2H, H-3′/7), 2.33 (s, 3H, -Me), 2.26 (s, 3H, -Me); MS: *m*/*z* (rel. abund.%) 283 (M$^+$,58), 266 (97), 231 (100), 51 (79); Anal. Calcd for $C_{17}H_{14}ClNO$ (283.75): C, 71.96; H, 4.97; Cl, 12.49; N, 4.94; O, 5.64; Found: C, 71.96; H, 4.98; N, 4.94.

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