Article

Indole-3-acetamides: As Potential Antihyperglycemic and Antioxidant Agents; Synthesis, *In Vitro* α-Amylase Inhibitory Activity, Structure−Activity Relationship, and *In Silico* Studies

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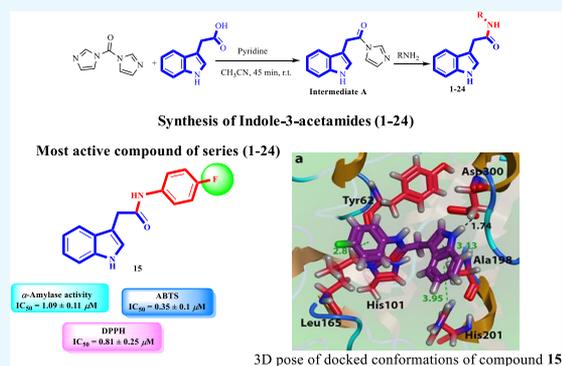
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ABSTRACT: Indole-3-acetamides (**1−24**) were synthesized via coupling of indole-3-acetic acid with various substituted anilines in the presence of coupling reagent 1,1-carbonyldiimidazole. The structures of synthetic molecules were elucidated through different spectroscopic techniques including electron ionization-mass spectroscopy (EI-MS), ^{1}H-, ^{13}C NMR, and high-resolution EI-MS (HREI-MS). These compounds were screened for their antihyperglycemic and antioxidant potentials. All compounds displayed good to moderate inhibition against α-amylase enzyme with IC$_{50}$ values ranging between 1.09 ± 0.11 and 2.84 ± 0.1 μM compared to the standard acarbose (IC$_{50}$ = 0.92 ± 0.4 μM). Compound **15** (IC$_{50}$ = 1.09 ± 0.11 μM) was the most active compound of the series and exhibited good inhibition against α-amylase; in addition, this compound also exhibited good antioxidant potential with IC$_{50}$ values of 0.35 ± 0.1 and 0.81 ± 0.25 μM in 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, respectively. The binding interactions of synthetic molecules with the enzyme's active site were confirmed via *in silico* studies. The current study had identified a number of lead molecules as potential antihyperglycemic and antioxidant agents.

Synthesis of Indole-3-acetamides (1-24)

Most active compound of series (1-24)

3D pose of docked conformations of compound **15**

INTRODUCTION:

Diabetes mellitus (DM), one of the common metabolic disorders, is usually characterized by irregular blood glucose levels. The elevated level of blood glucose is referred to as hyperglycemia, which results due to the abnormal functionality of enzyme insulin. Insulin is a peptide hormone that is secreted through the islets of Langerhans. Any damage to these cells leads to either less or no secretion of insulin, which consequently leads to uncontrolled levels of glucose in blood.[1−3] There are many pathways through which diabetes can be controlled, one of which is enzyme inhibition. Since digestive enzymes such as α-amylase have been known to increase blood glucose, one of the methods to control diabetes is the inhibition of this enzyme for controlled digestion of carbohydrates in diabetic patients.[4] α-Amylase is an enzyme (EC.3.2.1.1) that hydrolyzes α-1,4 glycoside bonds of complex carbohydrate molecules such as starch and glycogen to yield glucose and maltose. It contains calcium in its active site and is found in normal serum, saliva, and urine of humans. It is also found in bacteria and other living organisms. Primarily, α-amylase hydrolyzes complex carbohydrate molecules into small oligosaccharides and maltodextrins, and by the action of another digestive enzyme α-glucosidase, they are finally converted to glucose and absorbed in the bloodstream.[5]

Therefore, inhibition of α-amylase would be an ideal therapy to control diabetes and its related complications.[6] Prolonged diabetes mainly affects the metabolism of proteins, fats, carbohydrates, water, and electrolytes and thus leads to various structural changes in tissues of vital organ systems in the body, especially the vascular system.[7] The increased level of blood glucose prompts several metabolic signaling pathways that leads to inflammation, cytokines secretion, cell death, and subsequently diabetic complications.[8] Therefore, diabetic patients are commonly found to be associated with damage of heart, kidney, skin, eye, foot, etc. Alzheimer's disease, a common neurological disorder, is also associated with prolonged effects of this disease.[7] The high prevalence and rapid increase of diabetes have attracted the attention of many researchers, and hence many drugs have been developed for its control and cure. However, to date, no drug has been able to

completely cure this disease. Commercially available α-amylase inhibitors are associated with a number of side effects, most commonly gastrointestinal complications. Therefore, there is still a need and space for the development of cheap and less harmful drugs that can be easily available having potent antidiabetic activity.[9,10]

Among many complications associated with DM, the formation of reactive oxygen species (ROS) is also common and leads to severe damage of many vital organs. A number of free radicals and reactive oxygen species are formed during metabolism, and using antioxidants, these free radicals are neutralized without any harmful effect to our body. Free radicals may harm our immune system by a number of degenerative ailments. Free radicals are chemically unstable species with unpaired electrons. These unpaired electrons capture electrons from neighboring biomolecules like DNA, proteins, and lipids, thus initiating chain reactions in the body, which triggers the abnormal behavior of these biomolecules.[11−15] Mitochondrial respiratory chain enzymes, *e.g.*, xanthine oxidase, lipoxygenase, etc., affect the production of ROS due to hyperglycemia.[16] Thus, a high level of ROS and oxidative stress in diabetes leads to cellular death through numerous mechanisms that finally leads to tissue damage.[17−19] Therefore, diabetic complications can be controlled through the downregulation of ROS generation.[20−22]

Indole belongs to the class of heterocyclic compounds and possesses a benzene ring fused with a pyrrole ring with a 10-electron system from four double bonds and one nitrogen atom lone pair. It has been used as a primary precursor molecule for the synthesis of a variety of pharmaceuticals and fragrances.[23,24] Indomethacin and strychnine are among the most common biologically active synthetic and natural product resources that are derived from indole.[25] Indole and its related compounds possess a wide range of biological activities and are part of many medicinally important nuclei, which were widely accepted as pharmacophores.[26] Currently, indole nucleus has been found to be a part of more than 200 compounds that are marked as drugs or are undergoing clinical trials.[27] Indoles are an important class of compounds in the field of medicinal chemistry and found to have different biological activities including anticancer, anti-inflammatory, antiulcerogenic, antibacterial, antiviral, antifungal, etc.[28−31] Previously, our research group had explored and reported the potential antihyperglycemic activity of different indole derivatives.[32−35] Keeping in mind the antidiabetic potential of indoles, the synthetic molecules were considered for their α-amylase inhibition and antioxidant activity. The results showed that these compounds have dual abilities of lowering the postprandial hyperglycemia and scavenging the free radicals effectively and may serve as lead molecules in drug discovery and development (Figures 1 and 2). To the best of our knowledge, compounds **1**, **6**, **8**, and **13** are reported in the literature and the remaining compounds are new.

■ RESULTS AND DISCUSSION

Chemistry. Indole-3-acetamides (**1−24**) were synthesized via a one-pot multicomponent reaction of indole-3-acetic acid with 1,1-carbonyldiimidazole (CDI) and different substituted anilines.[36] First, indole-3-acetic acid was allowed to react with CDI in the presence of catalytic amount of pyridine in acetonitrile. Pyridine used in this reaction helps in the deprotonation of carboxylic acid and makes it more feasible to react with the coupling reagent. CO_2 gas evolved during the
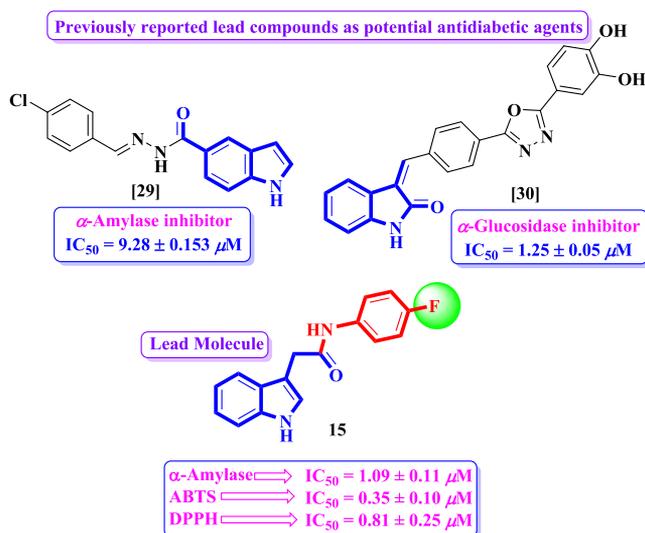

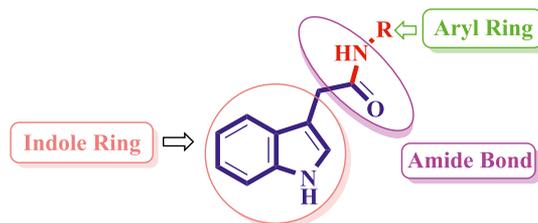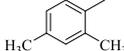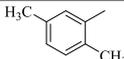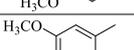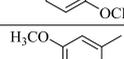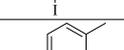**Figure 1.** Rationale of the current study.

Figure 2. General structure of synthetic compounds (**1−24**).

reaction resulted in the formation of an intermediate A, which was subsequently treated with different substituted anilines to afford indole-3-acetamides. The synthetic analogues were purified and characterized using different spectroscopic techniques including ^{1}H-, ^{13}C NMR, electron ionization-mass spectroscopy (EI-MS), and high-resolution EI-MS (HREI-MS) (Table 1).

***In Vitro* α-Amylase Inhibitory Activity and DPPH and ABTS Radical Scavenging Activities.** Twenty-four indole-3-acetamides **1−24** were synthesized and screened for antihyperglycemic and antioxidant activities. The molecules **1−24** displayed good to moderate inhibition against α-amylase enzyme with IC_{50} values ranging between 1.09 ± 0.11 and 2.84 ± 0.1 μM. However, the antioxidant potential was evaluated with DPPH and ABTS radical scavenging activities. All synthetic derivatives potentially scavenged the reactive oxygen species in the IC_{50} value ranges of 0.35 ± 0.1−2.19 ± 0.08 and 0.81 ± 0.25−2.75 ± 0.03 μM for ABTS and DPPH activities, respectively (Scheme 1).

Structure−Activity Relationship for α-Amylase Inhibition. Limited structure−activity relationship suggested that compound **1** ($IC_{50} = 2.6 \pm 0.09$ μM) bearing unsubstituted phenyl ring demonstrated moderate inhibition against α-amylase enzyme in comparison to the standard acarbose ($IC_{50} = 0.92 \pm 0.4$ μM). The addition of methyl group at the *para* position of phenyl ring resulted in slightly reduced activity in compound **2** ($IC_{50} = 2.84 \pm 0.1$ μM). Compounds **3**, **4**, and **5** with dimethyl substitutions at different positions of aryl ring displayed variable inhibitory activity. Among them, compound **3** ($IC_{50} = 2.52 \pm 0.06$ μM) with *meta*- and *para*-substituted methyl groups was moderately active. Changing the position of the methyl group from meta to ortho resulted in decreased

Table 1. *In Vitro* α-Amylase Inhibitory, DPPH, and ABTS Radical Scavenging Activities of Compounds 1−24

Comp. No.	R	α-Amylase IC$_{50}$ ± SEM[a] (μM)	ABTS IC$_{50}$ ± SEM[a] (μM)	DPPH IC$_{50}$ ± SEM[a] (μM)
1	(phenyl)	2.60 ± 0.09	1.77 ± 0.1	2.36 ± 0.2
2	(4-CH$_3$)	2.84 ± 0.1	2.19 ± 0.08	2.75 ± 0.03
3	(H$_3$C, CH$_3$)	2.52 ± 0.06	1.58 ± 0.11	2.22 ± 0.09
4	(H$_3$C, CH$_3$)	2.68 ± 0.08	1.90 ± 0.1	2.67 ± 0.12
5	(H$_3$C, CH$_3$)	2.38 ± 0.1	1.36 ± 0.15	1.79 ± 0.19
6	(C$_2$H$_5$)	2.42 ± 0.07	1.38 ± 0.26	1.92 ± 0.08
7	(C$_4$H$_9$)	2.43 ± 0.1	1.45 ± 0.15	2.02 ± 0.12
8	(H$_3$CO)	2.15 ± 0.1	0.99 ± 0.33	1.04 ± 0.1
9	(H$_3$CO, OCH$_3$)	2.42 ± 0.1	1.43 ± 0.3	2.00 ± 0.07
10	(H$_3$CO, H$_3$C)	2.24 ± 0.06	1.11 ± 0.1	1.53 ± 0.1
11	(C$_4$H$_9$O)	2.63 ± 0.1	1.78 ± 0.2	2.42 ± 0.1
12	(C$_8$H$_{17}$O)	2.64 ± 0.1	1.80 ± 0.1	2.45 ± 0.04
13	(Br)	2.10 ± 0.1	0.58 ± 0.4	1.03 ± 0.3
14	(Br)	2.27 ± 0.04	1.14 ± 0.2	1.59 ± 0.11
15	(F)	1.09 ± 0.11	0.35 ± 0.1	0.81 ± 0.25
16	(F)	2.25 ± 0.05	1.12 ± 0.2	1.53 ± 0.13
17	(F, F)	2.38 ± 0.05	1.31 ± 0.1	1.71 ± 0.12
18	(F, F)	2.2 ± 0.08	1.08 ± 0.2	1.42 ± 0.18
19	(I)	2.28 ± 0.08	1.15 ± 0.2	1.61 ± 0.11
20	(I)	2.14 ± 0.08	0.66 ± 0.2	1.04 ± 0.14
21	(Cl)	1.76 ± 0.2	0.46 ± 0.19	0.97 ± 0.19
22	(Cl, CH$_3$)	2.33 ± 0.09	1.28 ± 0.2	1.62 ± 0.15
23	(H$_3$CS)	2.48 ± 0.06	1.52 ± 0.1	2.22 ± 0.07
24	(H$_3$CS)	2.15 ± 0.09	0.94 ± 0.2	0.80 ± 0.14
Acarbose[(Std)]		0.92 ± 0.4		
Ascorbic Acid[(Std)]			0.23 ± 0.14	0.51 ± 0.18

[a]SEM is the standard error of the mean, Acarbose[(std)] is the standard inhibitor for α-amylase inhibitory activity, and Ascorbic acid[(std)] is the standard inhibitor for 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) activity.
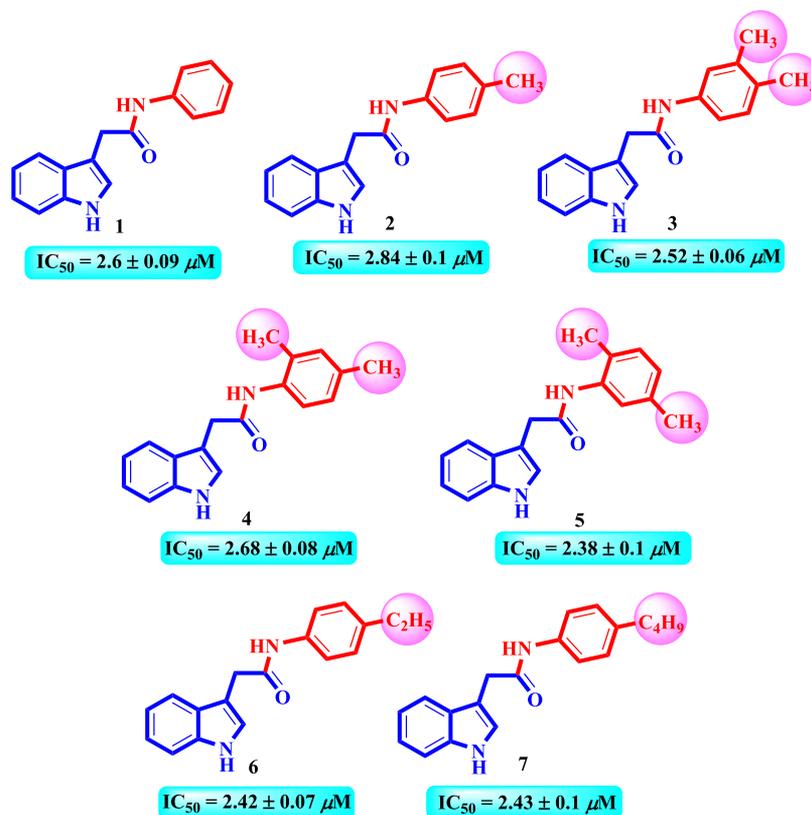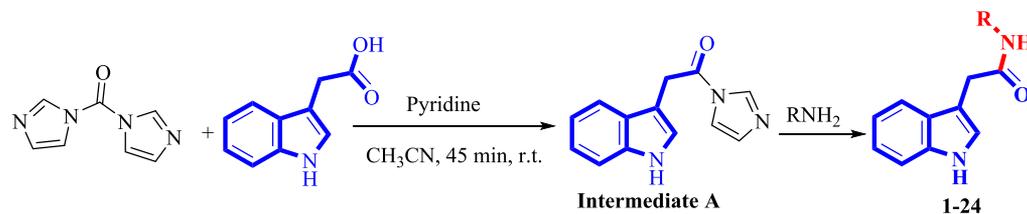
Scheme 1. Synthesis of Indole-3-acetamides (1−24)

Intermediate A

1-24

$IC_{50} = 2.6 \pm 0.09\ \mu M$ **1**

$IC_{50} = 2.84 \pm 0.1\ \mu M$ **2**

$IC_{50} = 2.52 \pm 0.06\ \mu M$ **3**

$IC_{50} = 2.68 \pm 0.08\ \mu M$ **4**

$IC_{50} = 2.38 \pm 0.1\ \mu M$ **5**

$IC_{50} = 2.42 \pm 0.07\ \mu M$ **6**

$IC_{50} = 2.43 \pm 0.1\ \mu M$ **7**

Figure 3. SAR of alkyl-substituted compounds **1−7**.

$IC_{50} = 2.15 \pm 0.1\ \mu M$ **8**

$IC_{50} = 2.42 \pm 0.1\ \mu M$ **9**

$IC_{50} = 2.24 \pm 0.06\ \mu M$ **10**

$IC_{50} = 2.63 \pm 0.1\ \mu M$ **11**
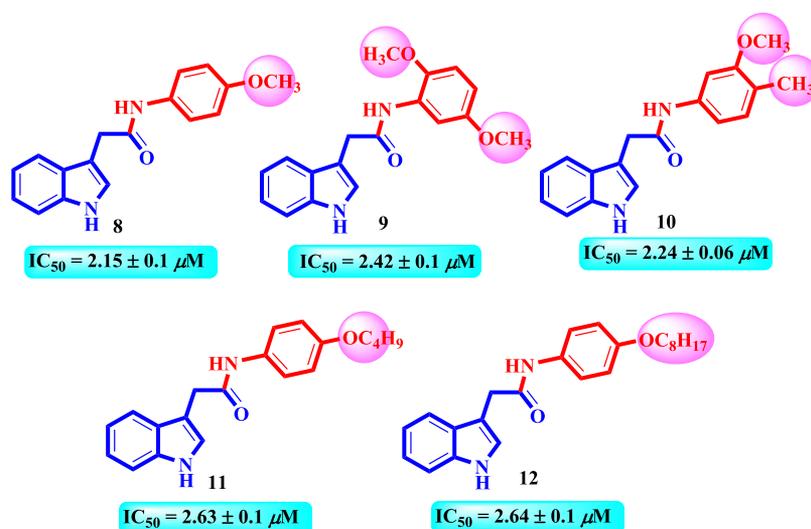
$IC_{50} = 2.64 \pm 0.1\ \mu M$ **12**

Figure 4. SAR of alkoxy-substituted compounds **8−12**.

inhibitory activity in compound **4** ($IC_{50} = 2.68 \pm 0.08\ \mu M$). Nevertheless, compound **5** ($IC_{50} = 2.38 \pm 0.1\ \mu M$) with

methyl groups para to each other showed superior activity among methyl-substituted compounds. Thus, compounds with
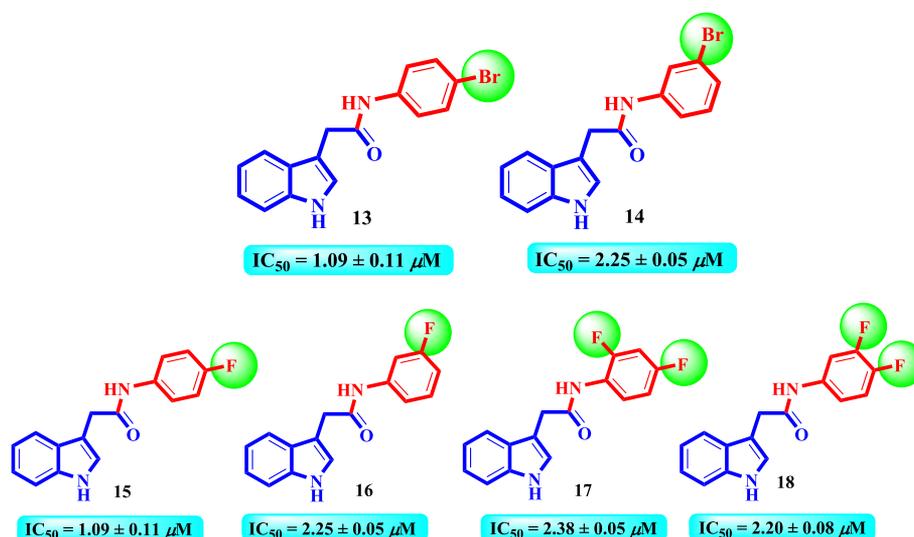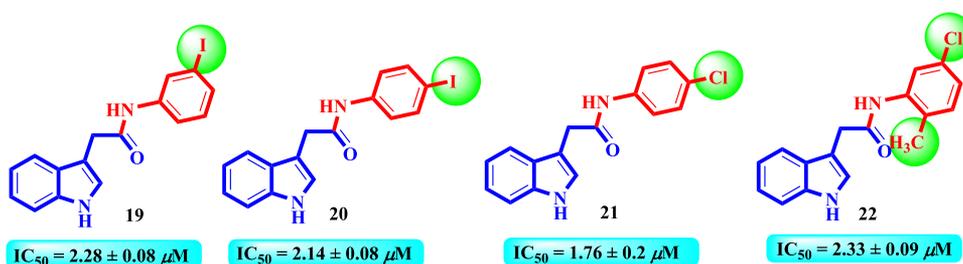
Figure 5. SAR of halogen-substituted compounds **13−18**.

Figure 6. SAR of halogen-substituted compounds **19−22**.

dimethyl substitution were more active in comparison to mono methyl- and unsubstituted compounds **1** and **2**, which might be due to the better interaction of methyl groups within the active site of enzyme. Nevertheless, compounds **6** (IC_{50} = 2.42 ± 0.07 μM) and **7** (IC_{50} = 2.43 ± 0.1 μM) with *para*-substituted ethyl and butyl groups, respectively, have shown similar activity (Figure 3).

Compound **8** (IC_{50} = 2.15 ± 0.1 μM) bearing *para* methoxy substituents displayed superior activity to methyl-substituted derivatives. Compound **9** (IC_{50} = 2.42 ± 0.07 μM) having two methoxy groups *para* to each other resulted in slightly decreased inhibition. Replacing one of the methoxy groups with methyl and changing their position in compound **10** (IC_{50} = 2.24 ± 0.06 μM) resulted in better inhibition compared to compound **9**. Interestingly, compound **11** (IC_{50} = 2.63 ± 0.1 μM) having *para*-substituted butoxy group displayed similar activity to compound **12** (IC_{50} = 2.64 ± 0.1 μM) with octyloxy substitution (Figure 4).

Among halogen-substituted derivatives, compound **13** (IC_{50} = 2.1 ± 0.1 μM) bearing bromo group at *para* substitution was slightly more active in comparison to the *meta* bromo-substituted compound **14** (IC_{50} = 2.27 ± 0.04 μM). Compound **15** (IC_{50} = 1.09 ± 0.11 μM) having *para* fluoro group was the most active compound of this series, which may be due to the inductive effect of the fluoro group. Changing the position of the fluoro group from para to meta in compound **16** (IC_{50} = 2.25 ± 0.05 μM) resulted in decreased inhibition. Nonetheless, compound **17** (IC_{50} = 2.38 ± 0.05 μM) with two fluoro atoms at ortho and para positions, respectively, demonstrated moderate inhibition in comparison to the

standard acarbose, which was also found to be less active than compound **15**, which showed that addition of another fluoro atom resulted in increased inductive effect, thus making it less available to bind with the active site of enzyme. Compound **18** (IC_{50} = 2.20 ± 0.08 μM) with changed positions of the fluoro group also displayed similar activity to compound **17** (Figure 5).

Compound **19** (IC_{50} = 2.28 ± 0.08 μM) having a *meta*-substituted iodo group was less active compared with the *para* iodo-substituted compound **20** (IC_{50} = 2.14 ± 0.08 μM). Compound **21** (IC_{50} = 1.76 ± 0.2 μM) with *para* chloro substitution was the second most active compound of the series. Compound **22** (IC_{50} = 2.33 ± 0.09 μM) bearing chloro and methyl substituents para to each other displayed weak inhibition in comparison to compound **21**. The decreased activity of this compound might be due to the addition of methyl group and change in the position of the chloro group (Figure 6).

Compounds **23** (IC_{50} = 2.48 ± 0.06 μM) and **24** (IC_{50} = 2.15 ± 0.09 μM) with meta and para thiomethyl groups, respectively, also displayed weak inhibition against α-amylase in comparison to standard acarbose (IC_{50} = 0.92 ± 0.4 μM) (Figure 7).

ABTS and DPPH Radical Scavenging Activities and Structure−Activity Relationships. Among 24 synthetic derivatives, the *para* flouro-substituted compound **15** was found to exhibit superior ABTS and DPPH radical scavenging activities in comparison to *para* chloro-, bromo-, and iodo-substituted compounds **21**, **13**, and **20**, respectively. It showed that the flouro group with a high negative inductive effect and
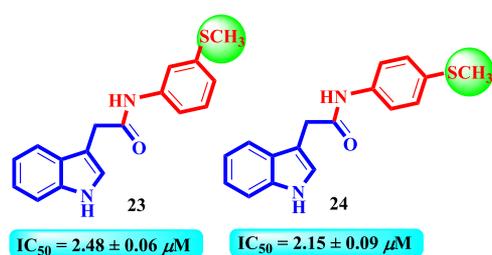
Figure 7. SAR of thiomethyl-substituted compounds **23−24**.

the smallest size among all halogen substituents was most active, and an increased size of halogen atoms and decreased inductive effect resulted in declined scavenging activity. Compounds **14**, **16**, and **19** with *meta* bromo, flouro, and iodo substituents, respectively, displayed similar activities; nonetheless, these compounds were less active in comparison to their *para*-substituted analogues. Compounds **17** and **18** bearing di-flouro substituents at ortho para and meta para positions, respectively, also displayed decreased activity in comparison to mono flouro-substituted compound **15**. This pattern showed that the number, nature, and positions of the halogen groups play a crucial role in DPPH and ABTS radical scavenging activities.

The methyl-substituted derivative **2** displayed weak antioxidant activity; nonetheless, the dimethyl-substituted compounds **3**, **4**, and **5** showed superior activity in comparison to compound **2**, which might be due to the addition of another methyl group that resulted in better activity due to the hyperconjugation effect. Compounds **6** and **7** with ethyl and butyl groups, respectively, also displayed comparable activities to methyl-substituted compounds. Compound **8** bearing a methoxy group displayed better activity than methyl-substituted compounds. However, dimethoxy-substituted

compound **9** was less active than compound **8**. The general activity pattern revealed that compounds with halogen substitutions were better antioxidizing agents compared to methyl and methoxy.

Hyperglycemia has many adverse effects along with the promotion of auto-oxidation of glucose to form free radicals. The antioxidant defense system is capable of scavenging a particular amount of free radicals; thus, the excessive production of free radicals results in macro- and microvascular dysfunction and polyneuropathy. Therefore, the drugs that can effectively scavenge the free-radical formation would be a preferable therapeutic strategy to prevent oxidative stress and the related diabetic vascular complications. Since the synthetic molecules (**1−24**) were capable of inhibiting the activity of α-amylase enzyme as well as free radicals, these molecules might act as lead candidates for drug discovery and research. Further research and modifications in the structures of these molecules might be helpful for the discovery of new drug molecules.

Molecular Docking study. *In silico* docking simulation was conducted to explore the binding modes of indole-3-acetamide derivatives **1−24** against α-amylase enzyme. The crystal structure of α-amylase (PDB ID: 1HNY) was downloaded from Protein Data Bank for docking study. Docking simulation was performed by MOE-2016 software. Using the default parameters of MOE, the α-amylase enzyme and the newly synthesized compounds were protonated and energy-minimized to get the optimized structures of the protein and compounds. These optimized structures of target protein and compounds were further used for docking studies.

Docking Results Analysis. The docking study was used to explore the binding interactions of the ligand molecules into α-amylase active-site residues. At the end of docking simulation, the binding interactions of all of the 24 compounds were discussed and their 3D images were taken. From the docking
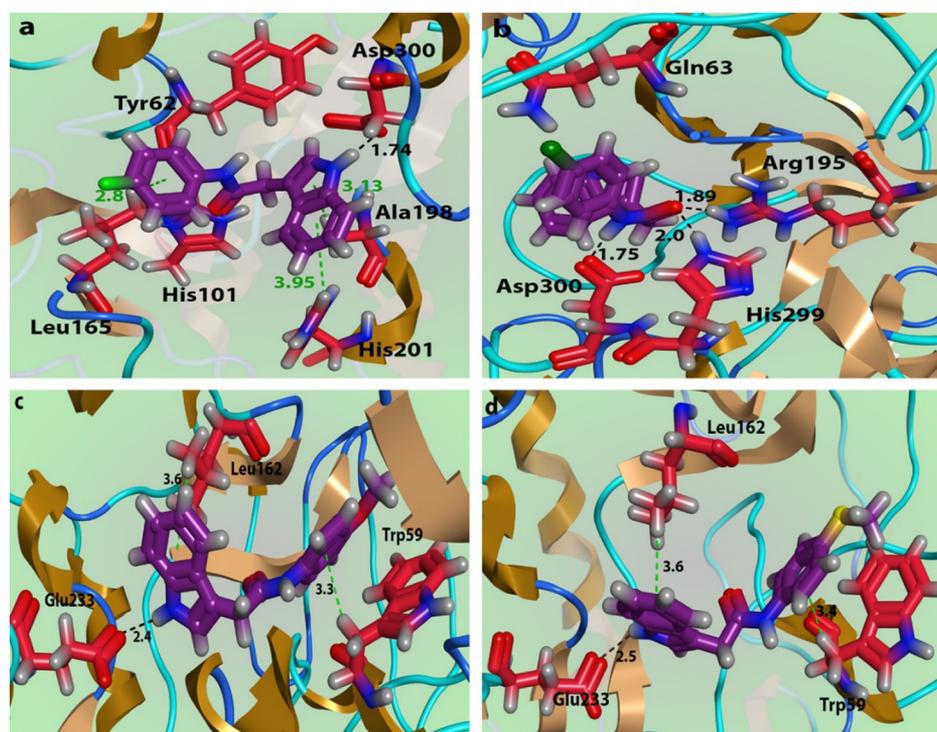

Figure 8. 3D pose of docked conformations of compounds **15** (a), **21** (b), **8** (c), and **24** (d) (purple) in the active site of α-amylase enzyme (red).

Table 2. Interaction Details, Docking Score Binding Energy, and Binding Affinity of the Compound Docked in the Active Site of α-Amylase Enzyme

C. no.	docking score	binding energy (kcal/mol)	binding affinity (kcal/mol)	interaction detail (ligands/α-amylase)	C. no.	docking score	binding energy (kcal/mol)	binding affinity (kcal/mol)	interaction detail (ligands/α-amylase)
1	−5.548	25.16	−5.29	ligand−receptor interaction distance	12				N 11 OE2 GLU 240 H-donor 3.08
				N 11 OD1 ASP 300 H-donor 3.00	13	−5.135	−29.03	−5.80	ligand−receptor interaction distance
				N 11 OD2 ASP 300 H-donor 3.24					N 11 OD1 ASP 197 H-donor 2.91
2	−5.777	−27.14	−5.66	ligand−receptor interaction distance					BR 33 OD2 ASP 356 H-donor 3.2
				N 11 OD2 ASP 300 H-donor 2.94	14	−5.212	−28.85	−5.99	ligand−receptor interaction distance
3	−5.466	−26.78	−5.30	ligand−receptor@@ interaction distance					O 22 CE1 HIS 201 H-acceptor 3.23
				N 11 OD1 ASP 300 H-donor 3.05	15	−5.625	−27.84	−5.61	ligand−receptor interaction distance N 11 OD2 ASP 300 H-donor 1.74
				6-ring 5-ring TRP 59 pi-pi 3.58					6-ring CD2 LEU 165 pi-H 2.8
				6-ring 6-ring TRP 59 pi-pi 3.99					5-ring CB ALA 198 pi-H 3.13
4	−5.767	−27.90	−5.61	ligand−receptor interaction distance					6-ring CE1 HIS 201 pi-H 3.95
				N 11 OD1 ASP 300 H-donor 2.97	16	−5.444	−27.69	−5.73	ligand−receptor interaction distance
				N 11 OD2 ASP 300 H-donor 3.26					N 11 OD1 ASP 197 H-donor 2.99
5	−5.814	−28.06	−5.62	ligand−receptor interaction distance					6-ring CD1 LEU 162 pi-H 4.79
				N 11 OD1 ASP 300 H-donor 3.05	17	−5.35	−25.44	−5.06	ligand−receptor interaction distance
				N 11 OD2 ASP 300 H-donor 3.00					N 11 OD1 ASP 300 H-donor 3.08
6	−5.165	−29.75	−5.81	ligand−receptor interaction distance	18	−5.066	−31.00	−5.78	ligand−receptor interaction distance
				N 11 OD1 ASP 197 (A) H-donor 3.02					N 20 OD1 ASP 300 H-donor 3.09
				5-ring CB ALA 198 (A) pi-H 4.67	19	−5.331	−28.92	−5.81	ligand−receptor interaction distance
7	−5.468	−30.35	−6.15	ligand−receptor interaction distance					I 33 OD2 ASP 356 H-donor 3.91
				5-ring CB TYR 62 pi-H 4.04					5-ring CB ALA 198 pi-H 4.70
8	−5.6	−34.41	−5.98	ligand−receptor interaction distance	20	−5.221	−31.46	−5.89	ligand−receptor interaction distance
				N 11 OE1 GLU 233 H-donor 2.4					N 20 OD1 ASP 300 H-donor 2.89
				6-ring CB TRP 59 pi-H 3.3	21	−5.6	−32.31	−5.99	ligand−receptor interaction distance
				6-ring CD1 LEU 162 pi-H 3.6					N 20 OD1 ASP 300 H-donor 1.75
9	−5.685	−26.76	−5.41	ligand−receptor interaction distance					O 22 NH2 ARG 195 H-acceptor 1.89
				N 11 OD1 ASP 300 (A) H-donor 3.10					O 22 NE2 HIS 299 H-acceptor 2.00
				N 11 OD2 ASP 300 (A) H-donor 2.94	22	−5.807	−32.81	−6.40	ligand−receptor interaction distance
10	−5.296	−35.44	−6.28	ligand−receptor interaction distance					N 11 OD1 ASP 300 H-donor 3.02
				N 20 OD1 ASP 300 H-donor 3.18					6-ring 5-ring TRP 59 pi-pi 3.71
				5-ring CB TRP 59 pi-H 3.87	23	−5.683	−26.56	−5.48	ligand−receptor interaction distance
11	−6.48	−33.37	−6.32	ligand−receptor interaction distance					N 11 OD2 ASP 300 H-donor 3.07
				N 11 OD1 ASP 300 H-donor 3.04					C 13 6-ring TYR 62 H-pi 4.15
				N 11 OD2 ASP 300 H-donor 3.11	24	−5.658	−32.99	−5.75	ligand−receptor interaction distance
				C 13 6-ring TYR 62 H-pi 4.22					N 11 OE1 GLU 233 H-donor 2.5
12	−6.756	−38.25	−6.92	ligand−receptor interaction distance					6-ring CB TRP 59 pi-H 3.4
									6-ring CD1 LEU 162 pi-H 3.6

study, it was revealed that the docked conformations of all of the compounds are appropriately oriented toward the catalytic residues, making sufficient interactions with the active-site residues Trp58, Trp59, Tyr62, Leu162, Arg195, Asp197, Glu233, Asp300, and His305 of the target enzyme.

Interactions of the Halides Containing Compounds. Compounds **13−22** are grouped as halide-containing compounds. All of these compounds have the same 2-(1*H*-indol-3-yl)-*N*-phenylacetamide group, whereas the phenyl ring contains different halide groups. According to the IC$_{50}$ value, compound **15** (1.09 ± 0.11 μM) is the most active compound of this group. It is clear from Figure 8a that compound **15** was bound deeply into the binding cavity of α-amylase showing four interactions with the residues Leu165, Ala198, His201, and Asp300. The hydrogen bond was observed between Asp300 and the indole moiety of compound **15**, whereas the aromatic rings of the compound show π−H interactions with the Leu165, Ala198, and His201 residues of the protein.

Compound **21** is the second most active compound (IC$_{50}$ = 1.76 ± 0.2 μM) in this group, forming three prominent hydrogen bonds with the binding-site residues Arg195, His299, and Asp300, as shown in Figure 8b.

The key structural feature observed in this group for good interaction mode and active nature of compounds is the presence of an electron-withdrawing group like halogen groups at specific positions on the phenyl ring.

Interactions of the Alkoxy- and Methanethiol-Containing Compounds. To discuss the interaction details, compounds **8−12** were placed in the second group due to the presence of the alkoxy group on the phenyl ring. Compound **8** is the most active compound (IC$_{50}$ = 2.15 ± 0.1 μM) in this group that shows hydrogen bonding with Glu233 and π−H interactions with Trp59 and Leu162, as shown in Figure 8c.

However, compounds **23** and **24** have a common methanethiol group on the phenyl ring with different *meta* and *para* positions. Compound **11** was observed in making good interactions with active-site residues Glu233, Trp59, and leu162 (Figure 8d). Compound **23** shows higher activity (IC$_{50}$ = 2.15 ± 0.09 μM) than compound **24**. It may due to the *para* position of the methanethiol group, which is the activating group.

Interactions of the Alkyl-Containing Compounds. Compounds **1−7** contain alkyl groups on the phenol moiety of the acetamide core structure at various ortho, meta, and para positions. Compound **22** of this group containing chloromethylphenyl group shows the best activity (IC$_{50}$ = 2.33 ± 0.09 μM) against α-amylase enzyme. The NH group of the indole moiety of the compound makes a hydrogen bond with Asp300, and the phenol ring shows π−π interaction with Trp59 active-site residues. The activity may be due to the presence of chloro-methylphenyl moiety Table 2.

Overall, the whole series of 2-(1*H*-indol-3-yl)-*N*-phenylacetamide derivatives shows the best activity against α-amylase enzyme. Different groups like halides, methanethiol, alkyl, and alkoxy groups at various positions of the phenol ring of the phenylacetamide compound may be responsible for their best inhibitory activity against α-amylase enzyme. Details about the interaction and docking of these compounds are given in Table 2.

CONCLUSIONS

Indole-3-acetamides (**1−24**) were synthesized and evaluated for α-amylase inhibition along with antioxidant activity. All

synthetic compounds displayed good inhibition against α-amylase. The synthetic molecules are also found to have good antioxidant potential. Among 24 derivatives, compounds **6**, **11**, **15**, and **18** were found to exhibit potential inhibition compared to the standard acarbose (IC$_{50}$ = 0.92 ± 0.40 μM). The molecular docking studies identified the types of binding interactions, which helped the synthetic compounds to interact within the active site of enzyme. Thus, this study identified many lead candidates that can act as potential antidiabetic and antioxidant agents.

EXPERIMENTAL SECTION

Materials and Methods. Thin-layer chromatography (TLC) was carried out on precoated silica gel, GF-254 (Merck, Germany). Spots were visualized under ultraviolet light at 254 and 366 nm. EI-MS and HREI-MS spectra were recorded on MAT 312 and MAT 113D mass spectrometers. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance AM spectrometer operating at 300 MHz. The chemical shift values are presented in ppm (d), relative to tetramethylsilane (TMS) as an internal standard, and the coupling constants (*J*) are in hertz. The melting points of the compounds were determined on a Stuart SMP10 melting point apparatus and are uncorrected.

General Procedure for the Synthesis of Indole-3-acetamides (1−24). Indole-3-acetic acid (0.175 g, 1 mmol), pyridine (0.8 mL), and CDI 1 equivalent (0.168 g) were taken in a reaction flask along with acetonitrile (20 mL) and stirred for 45 min at room temperature. The corresponding anilines were then added to the reaction mixture, and the mixture was continuously stirred for 2−24 h. The reaction was monitored with TLC, and the products were obtained by extraction with dichloromethane. The product was then washed with hexane to get pure product.[36]

α-Amylase Inhibition Assay. The activity was performed through modified assay from Kwon, Apostolidis, and Shetty.[37,38] Phosphate buffer (0.2 mM, pH 6.9) in 500 μL of α-amylase solution obtained from *Aspergillus oryzae* (0.5 mg/mL) was incubated with 500 μL of test sample (100, 200, 400, 800, 1000 μg/mL) at 25 °C for 10 min. Sodium phosphate buffer (0.02 M, pH 6.9) with 1% starch solution (500 μL) was added to each tube and further incubated for 10 min at 25 °C. The reaction mixture was then incubated in boiling water for 5 min after addition of 1 mL of dinitrosalicylic acid. The reaction mixture was then cooled to room temperature. The absorbance was measured at 540 nm after the addition of 10 mL of distilled water. The percentage inhibition was calculated as illustrated

$$\% \text{ inhibition} = (\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}})$$

$$/\text{absorbance}_{\text{control}} \times 100$$

Molecular Docking Studies. *In silico* docking simulation was conducted to explore the binding modes of indole-3-acetamide derivatives against α-amylase enzyme. The crystal structure of α-amylase (PDB ID: 1HNY) was downloaded from Protein Data Bank for docking study. Docking simulation was performed by MOE-2016 software. Using the default parameters of MOE, the α-amylase enzyme and the newly synthesized compounds were protonated and energy-minimized to get the optimized structures of the protein and compounds. These optimized structures of target protein and compounds were further used for docking studies.[39]

Spectral Data of Synthetic Compounds (1−24). *2-(1H-Indol-3-yl)-N-phenylacetamide (1).* Yield: 78%; mp: 186−188 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.14 (s, 1H, NH), 9.04 (s, 1H, NH), 7.62(m, 3H, H-4', H-2', H-6'), 7.38 (d, $J_{4,5}$ = 8.1 Hz, 1H, H-4), 7.32 (s, 1H, H2), 7.24 (t, J = 8.4 Hz, 2H, H-3', H-5'), 7.11 (m, 1H, H-6), 7.03 (m, 2H, H-5, H-7), 3.80 (s, 2H, H-2''); ^{13}C NMR (300 MHz, dimethyl sulfoxide (DMSO)-d_6): δ 169.6, 139.3, 136.0, 128.6, 127.1, 123.8, 122.9, 120.9, 119.0, 118.6, 118.3, 111.3, 108.5, 33.7; EI-MS m/z (% rel. abund.): 250 (M[+], 82.6), 157 (22), 130 (100), 103 (57), 93 (54), 77 (70); HREI-MS m/z: calcd for $C_{16}H_{14}N_2O$ [250.1106], found [250.1095].

2-(1H-Indol-3-yl)-N-(p-tolyl) acetamide (2). Yield: 21%; mp: 187−188 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.12 (s, 1H, NH), 8.94 (s, 1H, NH), 7.64 (d, J = 7.8 Hz, 1H, H-4), 7.47 (d, J = 8.4 Hz, 2H, H-2', H-6'), 7.38 (d, J = 7.8 Hz, 1H, H-7), 7.31 (s, 1H, H-2), 7.04 (m, 4H, H-3', H-5', H-5, H-6), 3.78 (s, 2H, H-2''), 2.22 (s, 3H, CH₃), ^{13}C NMR (300 MHz, DMSO-d_6): δ 169.4, 136.8, 136.0, 131.8, 128.9, 127.2, 123.7, 120.9, 119.0, 118.6, 118.3, 111.3, 108.6, 33.7, 20.3, EI-MS m/z (% rel. abund.): 264 (M[+], 80), 131 (53), 130 (100).

N-(3,4-Dimethylphenyl)-2-(1H-indol-3-yl)acetamide (3). Yield: 89%; mp: 158−160 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.12 (s, 1H, NH), 8.82 (s, 1H, NH), 7.63 (d, J = 8.1 Hz, 1H, H-4), 7.36 (m, 4H, H-5', H-6', H-2, H-7), 7.10 (t, J = 7.2 Hz, 1H, H-5), 7.01 (m, 2H, H-6, H-2'), 3.77 (s, 2H, H-2''), 2.14 (s, 6H, CH₃), ^{13}C NMR (300 MHz, DMSO-d_6): δ 169.3, 137.0, 136.1, 136.0, 130.6, 129.4, 127.1, 123.7, 120.9, 120.3, 118.6, 118.3, 116.5, 111.2, 108.6, 33.7, 19.5, 18.6, EI-MS m/z (% rel. abund.): 278 (M[+], 57), 131 (34), 130 (100), 121 (25), HREI-MS m/z: calcd for $C_{18}H_{18}N_2O$ [278.1419], found [278.1431].

N-(2,4-Dimethylphenyl)-2-(1H-indol-3-yl)acetamide (4). Yield: 51%; mp: 142−144 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.22 (s, 1H, NH), 8.22 (s, 1H, NH), 7.68 (d, J = 7.8 Hz, 1H, H-4), 7.44 (m, 3H, H-2, H-5', H-7), 7.12 (t, J = 7.8 Hz, 1H, H-5), 7.01 (m, 2H, H-6, H-3'), 6.90 (d, J = 7.2 Hz, 1H, H-6'), 3.84 (s, 2H, H-2''), 2.82 (s, 3H, CH₃), 1.84 (s, 3H, CH₃); ^{13}C NMR (300 MHz, DMSO-d_6): δ 169.6, 136.7, 136.2, 136.1, 130.8, 127.1, 126.6, 125.0, 123.8, 123.2, 120.9, 118.6, 118.3, 111.3, 108.7, 38.3, 38.1, 33.0; EI-MS m/z (% rel. abund.): 278 (M[+], 93), 131 (52), 130 (100), 121 (25); HREI-MS m/z: calcd for $C_{18}H_{18}N_2O$ [278.1419], found [278.1422].

N-(2,5-Dimethylphenyl)-2-(1H-indol-3-yl)acetamide (5). Yield: 53%; mp: 151−153 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.24 (s, 1H, NH), 8.08 (s, 1H, NH), 7.65 (m, 2H, H-6', H-2), 7.42 (d, J = 6.3 Hz, 2H, H-7, H-4), 7.12 (t, J = 7.2 Hz, 1H, H-5), 7.04 (t, J = 7.5 Hz, 1H, H-6), 6.94 (d, J = 7.5 Hz, 1H, H-3'), 6.78 (d, J = 7.5 Hz, 1H, H-4'), 3.84 (s, 2H, H-2''), 2.23 (s, 3H, CH₃), 1.85 (s, 3H, CH₃); ^{13}C NMR (300 MHz, DMSO-d_6): δ 169.4, 136.2, 136.1, 134.8, 129.9, 128.0, 127.1, 125.5, 125.1, 123.8, 120.9, 118.5, 118.3, 111.3, 108.7, 33.1, 20.5, 17.1; EI-MS m/z (% rel. abund.): 278 (M[+], 58), 131 (22), 130 (100), 121 (11), HREI-MS m/z: calcd for $C_{18}H_{18}N_2O$ [278.1419], found [278.1408].

N-(4-Ethylphenyl)-2-(1H-indol-3-yl)acetamide (6). Yield: 42%; mp: 160−162 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.11 (s, 1H, NH), 8.93 (s, 1H, NH),7.63 (d, J = 7.8 Hz, 1H, H-4), 7.50 (d, J = 8.4 Hz, 2H, H-2', H-6'), 7.38 (d, J = 7.8 Hz, 1H, H-7), 7.32 (s, 1H, H-2), 7.08 (m, 3H, H-3', H-5', H-5), 7.01 (t, J = 7.5 Hz, 1H, H-6), 3.78 (s, 2H, H-2''), 2.54 (m, 2H, CH₂), 1.15 (t, J = 7.5 Hz, 3H, CH₃); ^{13}C NMR (300 MHz, DMSO-d_6): δ 169.4, 138.3, 137.0, 136.0, 127.8, 127.1, 123.7,

120.9, 119.1, 118.6, 118.3, 111.3, 108.6, 33.7, 27.5, 15.6; EI-MS m/z (% rel. abund.): 278 (M[+], 33), 157 (5), 130 (100); HREI-MS m/z: calcd for $C_{18}H_{18}N_2O$ [278.1419], found [278.1400].

N-(4-Butylphenyl)-2-(1H-indol-3-yl)acetamide (7). Yield: 97%; mp: 141−143 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.13 (s, 1H, NH), 8.95 (s, 1H, NH), 7.63 (d, J = 7.5 Hz, 1H, H-4), 7.50 (d, J = 8.7 Hz, 2H, H-2', H-6'), 7.38 (d, J = 8.1 Hz, 1H, H-7), 7.32 (s, 1H, H-2), 7.06 (m, 4H, H-5, H-6, H-3',H-5'), 3.78 (s, 2H, H-2''), 2.53 (t, J = 7.5 Hz, 2H, CH₂), 1.52 (m, 2H, CH₂), 1.29 (m, 2H, CH₂), 0.88 (t, J = 7.5 Hz, 3H, CH₃); ^{13}C NMR (300 MHz, DMSO-d_6): δ 169.4, 137.0, 136.9, 136.0, 128.3, 127.1, 123.7, 120.9, 119.0, 118.6, 118.3, 111.3, 108.6, 34.1, 33.7, 33.1, 21.6, 13.7; EI-MS m/z (% rel. abund.): 306 (M[+], 86), 130 (100), 106 (33); HREI-MS m/z: calcd for $C_{20}H_{22}N_2O$ [306.1732], found [306.1699].

2-(1H-Indol-3-yl)-N-(4-methoxyphenyl)acetamide (8). Yield: 81%; M.p.: 184-185 °C, ^{1}H NMR (300 MHz, Acetone-d_6): δ 10.12 (s, 1H, NH), 8.88 (s, 1H, NH), 7.64 (d, J = 7.8 Hz, 1H, H-4), 7.50 (d, J = 9.0 Hz, 2H, H-2', H-6'), 7.38 (d, J = 7.8 Hz, 1H, H-7), 7.31 (s, 1H, H-2), 7.10 (t, J = 7.5 Hz, 1H, H-5), 7.00 (t, J = 7.2 Hz, 1H, H-6), 6.80 (d, J = 9.0 Hz, 2H, H-3', H-5'), 3.76 (s, 2H, H-2''), 3.72 (s, 3H, OCH₃); ^{13}C NMR (300 MHz, DMSO-d_6): δ 169.1, 155.0, 136.0, 132.5, 127.2, 123.7, 120.9, 120.5, 118.6, 118.3, 113.7, 111.3, 108.6, 55.1, 33.6; EI-MS m/z (% rel. abund.): 280 (M[+], 27), 130 (100), 108 (10), HREI-MS m/z: calcd for $C_{17}H_{16}N_2O_2$ [280.1212], found [280.1194].

N-(2,5-Dimethoxyphenyl)-2-(1H-indol-3-yl)acetamide (9). Yield: 35%; mp: 152−154 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.27 (s, 1H, NH), 8.37 (s, 1H, NH), 8.08 (d, J = 3 Hz, 1H, H-6'), 7.63 (d, J = 8.1 Hz, 1H, H-3'), 7.45 (s, 1H, H-2), 7.42 (m, 1H, H-4), 7.16 (m, 1H, H-5), 7.05 (m, 1H, H-6), 6.77 (d, J = 8.7 Hz, 1H, H-7), 6.48 (dd, $J_{4',6'}$ = 3 Hz, $J_{4',3'}$ = 3 Hz, 1H, H-4'), 3.87 (s, 2H, H-2''), 3.69 (s, 3H, OCH₃), 3.49 (s, 3H, OCH₃); ^{13}C NMR (300 MHz, acetone-d_6): δ 170.1, 154.7, 143.1, 137.8, 130.0, 128.2, 125.2, 122.5, 1119.9, 119.4, 112.3, 112.1, 109.4, 107.7, 106.7; EI-MS m/z (% rel. abund.): 310 (M[+], 61), 153 (24), 138 (25), 130 (100); HREI-MS m/z: calcd for $C_{18}H_{18}N_2O_3$ [310.1317], found [310.1314].

2-(1H-Indol-3-yl)-N-(3-methoxy-4-methylphenyl)-acetamide (10). Yield: 33%; mp: 102−104 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.12 (s, 1H, NH), 8.95 (s, 1H, NH), 7.64 (d, J = 7.8 Hz, 1H, H-4), 7.38 (d, J = 7.5 Hz, 2H, H-7, H-6'), 7.32 (s, 1H, H-2), 7.11 (t, J = 7.2 Hz, 1H, H-5), 7.02 (m, 3H, H-2', H-5', H-6), 3.78 (s, 2H, H-2''), 3.73 (s, 3H, OCH₃), 2.04 (m, 3H, CH₃); ^{13}C NMR (300 MHz, DMSO-d_6): δ 169.5, 157.1, 138.5, 136.0, 130.0, 127.2, 123.8, 120.9, 119.9, 118.6, 118.3, 111.3, 110.5, 108.5, 101.8, 54.9, 33.8, 15.5; EI-MS m/z (% rel. abund.): 294 (M[+], 34), 157 (5), 130 (100), 77 (8); HREI-MS m/z: calcd for $C_{18}H_{18}N_2O_2$ [294.1368], found [294.1381].

N-(4-Butoxyphenyl)-2-(1H-indol-3-yl)acetamide (11). Yield: 77%; mp: 174−177 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.11 (s, 1H, NH), 8.85 (s, 1H, NH), 7.64 (d, J = 7.8 Hz, 1H, H-4), 7.49 (d, J = 9.0 Hz, 2H, H-2', H-6'), 7.38 (d, J = 8.1 Hz, 1H, H-7), 7.31 (s, 1H, H-2), 7.10 (t, J = 7.2 Hz, 1H, H-5), 7.01 (t, J = 7.5 Hz, 1H, H-6), 6.80 (d, J = 9.0 Hz, 2H, H-3', H-5'), 3.91 (t, J = 6.3 Hz, 2H,OCH₂), 3.76 (s, 2H, CH₂), 1.71 (m, 2H, CH₂), 1.45 (m, 2H, CH₂), 0.92 (t, J = 7.5 Hz 3H, CH₃); EI-MS m/z (% rel. abund.): 322 (M[+], 96), 131 (60), 130 (100), 109 (54); HREI-MS m/z: calcd for $C_{20}H_{22}N_2O_2$ [322.1681], found [322.1703].

2-(1H-Indol-3-yl)-N-(4-(octyloxy) phenyl)acetamide (12).
Yield: 79%; M.p.: 155−157 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.12 (s, 1H, NH), 8.87 (s, 1H, NH), 7.63 (d, J = 7.8 Hz 1H, H-4), 7.49 (d, J = 8.1 Hz, 2H, H-2′, H-6′), 7.38 (d, J = 8.1 Hz, 1H, H-7), 7.31 (s, 1H, H-2), 7.10 (t, J = 7.5 Hz, 1H, H-5), 7.00 (t, J = 7.5 Hz, 1H, H-6), 6.79 (d, J = 9.0 Hz, 2H, H-3′, H-5′), 3.91 (t, J = 6.6 Hz, 2H,OCH$_2$), 3.76 (s, 2H, H-2″), 1.71 (m, 2H,CH$_2$), 1.41 (m, 2H,CH$_2$), 1.29 (m, 8H, CH$_2$, CH$_2$, CH$_2$, CH$_2$), 0.85 (m, 3H, CH$_3$); EI-MS m/z (% rel. abund.): 378 (M$^+$, 74), 131 (71), 130 (100), 109 (65), HREI-MS m/z: calcd for C$_{16}$H$_{13}$BrN$_2$O [328.0211], found [328.0233].

N-(4-Bromophenyl)-2-(1H-indol-3-yl)acetamide (13).
Yield: 62%; mp: 196−198 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.14 (s, 1H, NH), 9.19 (s, 1H, NH), 7.60 (m, 3H, H-4, H-3′, H-5′), 7.41 (m, 3H, H-2′, H-6′, H-7), 7.32 (s, 1H, H-2), 7.08 (m, 1H, H-5), 7.01 (m, 1H, H-6), 3.80 (s, 2H, H-2″); ^{13}C NMR (300 MHz, DMSO-d_6): δ 205.9, 170.4, 139.6, 132.2, 128.3, 124.6, 122.1, 121.7, 119.6, 119.3, 115.7, 112.0, 109.4, 34.9; EI-MS m/z (% rel. abund.): 328 (M$^+$, 39), 330 (M^{+2}, 7), 130 (100), 103 (11.2), 77 (9); HREI-MS m/z: calcd for C$_{16}$H$_{13}$BrN$_2$O [328.0211], found [328.0202].

N-(3-Bromophenyl)-2-(1H-indol-3-yl)acetamide (14).
Yield: 58%; mp: 133−135 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.14 (s, 1H, NH), 9.22 (s, 1H, NH), 8.03 (s, 1H, H-2′), 7.62 (d, J = 7.8 Hz, 1H, H-4′), 7.49 (m, 1H, H-6′), 7.38 (d, J = 8.1 Hz, 1H, H-7), 7.32 (s, 1H, H-2), 7.19 (m, 2H, H-4, H-5′), 7.10 (t, J = 7.8 Hz, 1H, H-5), 7.01 (t, J = 8.1 Hz, 1H, H-6), 3.81 (s, 2H, H-2″); ^{13}C NMR (300 MHz, DMSO-d_6): δ 170.0, 140.9, 136.0, 130.6, 127.1, 125.5, 123.8, 121.4, 121.3, 120.9, 118.5, 118.3, 117.7, 111.3, 108.1, 33.7; EI-MS m/z (% rel. abund.): 328 (M$^+$, 56), 330 (M^{+2}, 9), 130 (100), 103 (17); HREI-MS m/z: calcd for C$_{16}$H$_{13}$BrN$_2$O [328.0211], found [328.0233].

N-(4-Fluorophenyl)-2-(1H-indol-3-yl)acetamide (15).
Yield: 76%; mp: 108−110 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.14 (s, 1H, NH), 9.29 (s, 1H, NH), 7.69 (m, 1H, H-3′), 7.64 (m, 1H, H-5′), 7.39 (d, J = 8.5 Hz, 1H, H-4), 7.32 (s, 1H, H-2), 7.69 (m, 2H, H-2′, H-6′), 7.12 (m, 1H, H-5), 7.02 (m, 1H, H-6), 6.77 (m, 1H, H-7), 3.78 (s, 2H, H-2″); ^{13}C NMR (300 MHz, DMSO-d_6): δ 169.5, 157.1, 138.5, 136.0, 130.0, 127.2, 123.8, 120.9, 119.9, 118.6, 118.3, 111.3, 110.5, 108.5, 101.8, 54.9, 33.8, 15.5; EI-MS m/z (% rel. abund.): 268 (M$^+$, 68), 157 (2), 130 (100); HREI-MS m/z: calcd for C$_{16}$H$_{13}$FN$_2$O [268.1012], found [268.1003].

N-(3-Fluorophenyl)-2-(1H-indol-3-yl)acetamide (16).
Yield: 91%; mp: 148−150 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.13 (s, 1H, NH), 9.10 (s, 1H, NH), 7.61 (m, 3H, H-2′, H-4′, H-4), 7.38 (d, J = 8.1 Hz, 1H, H-7), 7.31 (s, 1H, H-2), 7.09 (t, J = 7.8 Hz, 1H, H-5), 7.02 (m, 3H, H-6, H-5′, H-6′), 3.79 (s, 2H, H-2″); EI-MS m/z (% rel. abund.): 268 (M$^+$, 100), 157 (7), 130 (100); HREI-MS m/z: calcd for C$_{16}$H$_{13}$FN$_2$O [268.1012], found [268.1022].

N-(2,4-Difluorophenyl)-2-(1H-indol-3-yl)acetamide (17).
Yield: 72%;mp: 133−135 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.19 (s, 1H, NH), 8.69 (s, 1H, NH), 8.14 (m, 1H, H-5′), 7.64 (d, J = 7.8 Hz, 1H, H-6′), 7.38 (m, 2H, H-2, H-4), 7.10 (t, J = 7.5 Hz, 1H, H-5), 7.04 (m, 1H, H-6), 6.95 (m, 2H, H-3′, H-7), 3.89 (s, 2H, H-2″); ^{13}C NMR (300 MHz, DMSO-d_6): δ 170.0, 136.0, 127.1, 125.6, 130.0, 127.2, 123.8, 120.9, 119.9, 118.6, 118.3, 111.3, 110.5, 108.5, 101.8, 54.9, 33.8, 15.5; EI-MS m/z (% rel. abund.): 286 (M$^+$, 17), 130 (100), 77 (18); HREI-MS m/z: calcd for C$_{16}$H$_{12}$FN$_2$O [286.0918], found [286.0912].

N-(3,4-Difluorophenyl)-2-(1H-indol-3-yl)acetamide (18).
Yield: 78%; mp: 100−102 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.15 (s, 1H, NH), 9.30 (s, 1H, NH), 7.84 (m, 1H, H-5′), 7.62 (d, J = 7.8 Hz, 1H, H-4), 7.38 (d, J = 8.1 Hz, 1H, H-7), 7.31 (s, 1H, H-2), 7.24 (m, 1H, H-2′), 7.18 (m, 1H, H-6′), 7.14 (m, 1H, H-5), 7.01 (t, J = 7.8 Hz, 1H, H-6), 3.80 (s, 2H, H-2″); EI-MS m/z (% rel. abund.): 286 (M$^+$, 92), 131 (63), 130 (100); HREI-MS m/z: calcd for C$_{16}$H$_{12}$F$_2$N$_2$O [286.0918], found [286.0943].

2-(1H-Indol-3-yl)-N-(3-iodophenyl)acetamide (19). Yield: 64%; mp: 136−138 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.13 (s, 1H, NH), 9.15 (s, 1H, NH), 8.18 (s, 1H, H-2′), 7.63 (d, J = 7.8 Hz, 1H, H-4′), 7.54 (d, J = 7.5 Hz, 1H, H-6′), 7.38 (d, J = 7.8 Hz, 1H, H-4), 7.32 (s, 1H, H-2), 7.07 (m, 4H, H-5′, H-5, H-6, H-7), 3.81 (s, 2H, H-2″), EI-MS m/z (% rel. abund.): 376 (M$^+$, 95), 131 (42), 130 (100), HREI-MS m/z: calcd for C$_{16}$H$_{13}$IN$_2$O [376.0073], found [376.0032].

2-(1H-Indol-3-yl)-N-(4-iodophenyl)acetamide (20). Yield: 16%; mp: 211−213 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.13 (s, 1H, NH), 9.16 (s, 1H, NH), 7.60 (m, 3H, H-6′, H-2′, H-4), 7.46 (d, J = 9.0 Hz, 2H, H-3′, H-5′), 7.39 (d, J = 8.1 Hz, 1H, H-7), 7.31 (s, 1H, H-2), 7.09 (t, J = 7.5 Hz, 1H, H-5), 7.01 (t, J = 7.5 Hz, 1H, H-6), 3.80 (s, 2H, H-2″); EI-MS m/z (% rel. abund.): 376 (M$^+$, 10), 218 (3), 130 (100); HREI-MS m/z: calcd for C$_{16}$H$_{13}$IN$_2$O [376.0073], found [376.0052].

N-(4-Chlorophenyl)-2-(1H-indol-3-yl)acetamide (21).
Yield: 56%; mp: 161−163 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.13 (s, 1H, NH), 9.18 (s, 1H, NH), 7.64 (m, 3H, H-5′, H-3′, H-4), 7.38 (d, J = 8.1 Hz, 1H, H-7), 7.32 (s, 1H, H-2), 7.25 (d, J = 9.0 Hz, 2H, H-2′, H-6′), 7.10 (t, J = 7.2 Hz, 1H, H-5), 7.02 (t, J = 7.5 Hz, 1H, H-6), 3.80 (s, 2H, H-2″); ^{13}C NMR (300 MHz, DMSO-d_6): δ 169.8, 138.3, 136.1, 128.5, 127.2, 126.5, 123.9, 121.0, 120.6, 118.6, 118.4, 111.4, 108.3, 33.8; EI-MS m/z (% rel. abund.): 283 (M$^+$, 16), 285 (M^{+2}, 4), 130 (100), 77(23); HREI-MS m/z: calcd for C$_{16}$H$_{13}$ClN$_2$O [284.0716], found [284.0724].

N-(5-Chloro-2-methylphenyl)-2-(1H-indol-3-yl)acetamide (22). Yield: 13%; mp: 201−203 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.26 (s, 1H, NH), 8.20 (s, 1H, NH), 8.01 (s, 1H, H-6′), 7.64 (d, J = 7.8 Hz, 1H, H-4′), 7.43 (m, 2H, H-4, H-2), 7.10 (m, 3H, H-5, H-6, H-3′), 6.98 (m, 1H, H-7), 3.88 (s, 2H, H-2″), 1.88 (s, 3H,CH$_3$); ^{13}C NMR (300 MHz, DMSO-d_6): δ 169.9, 137.7, 136.1, 131.6, 129.7, 129.4, 127.1, 124.2, 124.0, 123.4, 121.0, 118.5, 118.4, 111.3, 108.5, 33.1, 17.1; EI-MS m/z (% rel. abund.): 298 (M$^+$, 7), 300 (M^{+2}, 2), 130 (100), 76 (56); HREI-MS m/z: calcd for C$_{17}$H$_{15}$ClN$_2$O [298.0873], found [298.0873].

2-(1H-Indol-3-yl)-N-(3-(methylthio) phenyl)acetamide (23). Yield: 71%; mp: 123−125 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.13 (s, 1H, NH), 9.06 (s, 1H, NH), 7.66 (m, 1H, H-6′), 7.63(d, J = 8.1 Hz, 1H, H-4), 7.38 (d, J = 8.7 Hz, 1H, H-7), 7.35 (s, 1H, H-2), 7.31(m, 1H, H-2′), 7.18 (t, J = 8.1 Hz, 1H, H-5′), 7.07 (m, 1H, H-5), 7.01 (m, 1H, H-6), 6.91 (m, 1H, H-4′), 3.80 (s, 2H, H-2″), 2.42 (s, 3H, SCH$_3$); ^{13}C NMR (300 MHz, DMSO-d_6): δ 169.5, 157.1, 138.5, 136.0, 130.0, 127.2, 123.8, 120.9, 119.9, 118.6, 118.3, 111.3, 110.5, 108.5, 101.8, 54.9, 33.8, 15.5; EI-MS m/z (% rel. abund.): 296 (M$^+$, 9), 130 (100), 77 (28); HREI-MS m/z: calcd for C$_{17}$H$_{16}$N$_2$OS [296.0983], found [296.0970].

2-(1H-Indol-3-yl)-N-(4-(methylthio) phenyl)acetamide (24). Yield: 86%; mp: 184−186 °C, ^{1}H NMR (300 MHz, acetone-d_6): δ 10.12 (s, 1H, NH), 9.05 (s, 1H, NH), 7.63 (d, J = 7.8 Hz, 1H, H-4), 7.58 (d, J = 8.7 Hz, 2H, H-2′, H-6′),

7.38(d, J = 8.1 Hz, 1H, H-7), 7.31 (s, 1H, H-2), 7.18(m, 2H, H-3′, H-5′), 7.07 (m, 1H, H-5), 7.01 (m, 1H, H-6), 3.79 (s, 2H, H-2″), 2.42 (s, 3H, SCH$_3$); EI-MS m/z (% rel. abund.): 296 (M$^+$, 11), 130 (100), 77 (27); HREI-MS m/z: calcd for C$_{17}$H$_{16}$N$_2$OS [296.0983], found [296.0976].

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The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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