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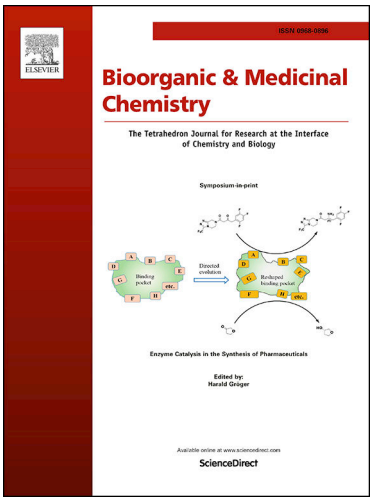

Indole acrylonitriles as potential anti-hyperglycemic agents: Synthesis, α-glucosidase inhibitory activity and molecular docking studies

Mehwish Solangi, Kanwal, Khalid Mohammed Khan, Faiza Saleem, Shehryar Hameed, Jamshed Iqbal, Zainab Shafique, Urooj Qureshi, Zaheer Ul-Haq, Muhammad Taha, Shahnaz Perveen

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Indole acrylonitriles as potential anti-hyperglycemic agents: Synthesis, α-glucosidase inhibitory activity and molecular docking studies

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Abstract: One of the most prevailing metabolic disorder diabetes mellitus has become the global health issue that has to be addressed and cured. Different marketed drugs have been made available for the treatment of diabetes but there is still a need of introducing new therapeutic agents that are economical and have lesser or no side effects. The current study deals with the synthesis of indole acrylonitriles (**3-23**) and the evaluation of these compounds for their potential for α-glucosidase inhibition. The structures of these synthetic molecules were deduced by using different spectroscopic techniques. Acarbose (IC_{50} = 2.91 ± 0.02 μM) was used as standard in this study and the synthetic molecules (**3-23**) have shown promising α-glucosidase inhibitory activity. Compounds **4**, **8**, **10**, **11**, **14**, **18**, and **21** displayed superior inhibition of α-glucosidase enzyme in the range of (IC_{50} = 0.53 ± 0.01 - 1.36 ± 0.04 μM) as compared to the standard acarbose. Compound **10** (IC_{50} = 0.53 ± 0.01 μM) was the most effective inhibitor of this library and displayed many folds enhanced activity in contrast to the standard. Molecular docking of synthetic compounds was performed to verify the binding interactions of ligand with the active site of enzyme. This study had identified a number of potential α-glucosidase inhibitors that can be used for further research to identify a potent therapeutic agent against diabetes.

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Keywords: Indole acrylonitriles; Knoevenagel condensation; Diabetes mellitus; α-Glucosidase inhibitors.

Introduction

Hormones are chemical mediators that are produced and secreted by instruct cells or tissues to regulate appropriate function of the body. The elevated levels of hormones in body are associated with both microvascular and macrovascular complications resulting in the damage of organ and tissue. Among the wide range of hormones present in the body, insulin is the main anabolic hormone synthesized in β-cells of pancreas. It is dimer peptide with two chains cross-connected by disulfide bridges. Insulin is responsible for the regulation of metabolism of carbohydrates, fats, and proteins. Glucose production by the liver and secretion into blood is strongly reliant on concentrations of insulin [1]. Disturbance in the production or secretion of insulin leads to diabetes mellitus (DM).

Diabetes mellitus (DM) is a metabolic disorder that has become a global health issue affecting children, adolescents, and adults [2]. Basically, it is classified in two key types: type-I diabetes (IDDM) and type-II diabetes (NIDDM) [3]. Type-I diabetes is insulin dependent due to immune-mediated β-cells destruction leading to insulin deficiency [4]. Type-II diabetes is non-insulin dependent, either due to the impaired insulin secretion by the β cells or defected insulin action due to insulin resistance [5]. Typical signs of diabetes are polyuria, polydipsia, polyphagia and weight loss [6].

Due to high prevalence of diabetes mellitus (DM), various therapeutic approaches have been introduced which work in combination with each other and provide different modes of action for glycemic control. Extensive interest has been observed for α-glucosidase (EC3.2.1.20) due to its essential role in carbohydrate, glycoproteins, and glycolipids processing. α-Glucosidase inhibitors (AGIs) are a unique class of antidiabetic drugs that are competitive inhibitors of this digestive enzyme. (AGIs) such as acarbose, voglibose, and miglitol; are marketed compounds used for the improvement in glycemic control by slowing down the digestion process leading to slow absorption of glucose [7]. Due to the administration of α-glucosidase inhibitors (AGIs); the augment in post-prandial glucose is diminished and delayed. The mechanism of action of the (AGIs) is that they bind competitively to the carbohydrate-binding site of α-glucosidase enzyme;

they interfere with the binding of carbohydrate to enzyme and inhibit them from splitting into their monomers [8].

Heterocyclic chemistry provides valuable source of novel compounds having diverse biological activities, mainly due to their exceptional capability to imitate the structure of peptides and to connect reversibly to proteins [9]. Nitrogenous heterocycles have broad range of bioactivities and are exceedingly versatile in nature. They are used as essential intermediates in drug design for the construction of active molecules. The indole moiety is the most broadly available nitrogen containing heterocycles in nature. The interesting molecular architecture of indole makes it suitable candidate for the drug development (Figure-1) [10]. Indole is a planar bicyclic molecule with benzene ring fused with pyrrole ring [11]. It is an essential part of the amino acid tryptophan, hormone melatonin, neurotransmitter serotonin, and also found as a plant hormone, heteroauxin [12]. Indole has wide range of biological activities such as; antioxidant, antidepressant, opioid antagonist, antihistaminic, antiinflammatory, anticancer, anti-HIV, antiviral, antimicrobial, and antituberculosis [13-15]. Indole and its derivatives also exhibit α-glucosidase inhibitory activity [16].

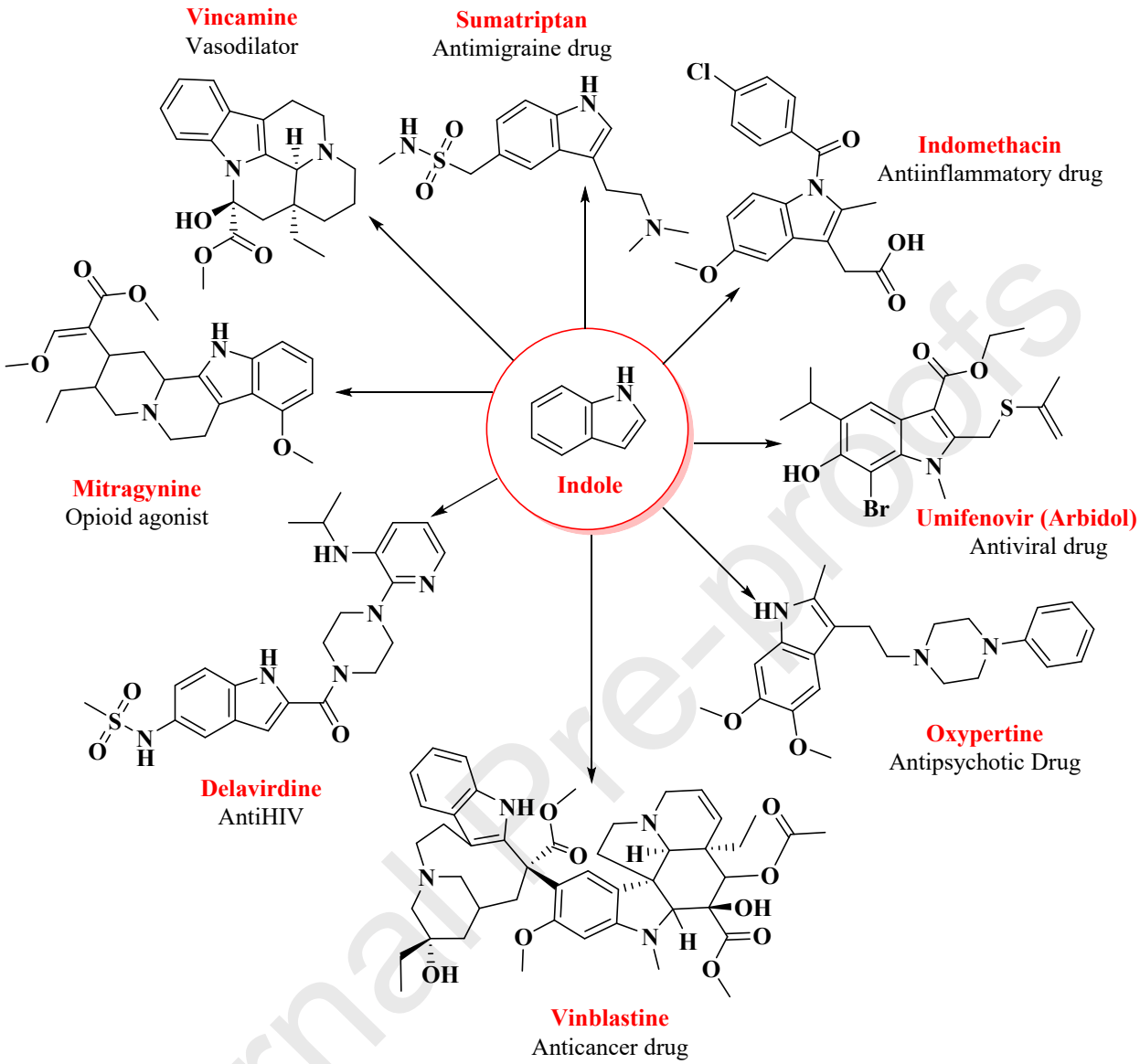


Figure-1: Indole Based Marketed Drugs.

Acrylonitrile group has significant chemical importance and is the center of attention to researchers due to the flexible properties of its conjugated system. Acrylonitrile units are privileged scaffolds due to their wide-ranging applications from medicinal agents, agrochemicals, and to functional materials [17-19]. Recently, it has been reported that the introduction of this moiety as a pharmacophore can result in very potential activities such as protein kinase inhibitors or anticancer agents [20-21].

Due to high resistance rate against available drugs; there is a dire need for new potential molecules to tackle the increasing disease problem. Our research group has been involved in search for new

classes of α-glucosidase inhibitors [22-25]. Previously reported work by our research group on indole scaffold showed exceedingly positive results in the field of medicinal chemistry. In this study, we wanted to further explore the pharmacological diversity of indole nucleus, we decided to synthesize a series of multi-substituted indole acrylonitrile derivatives for the evaluation of their α-glucosidase inhibition (AGIs) activity, and encouraging results were obtained. Ten compounds (**5**, **8-14**, **21**, **23**) in this synthetic library are new, while eleven compounds (**3**, **4**, **6**, **7**, **15-20**, **22**) are previously reported in literature [26-32] (Figure-2).

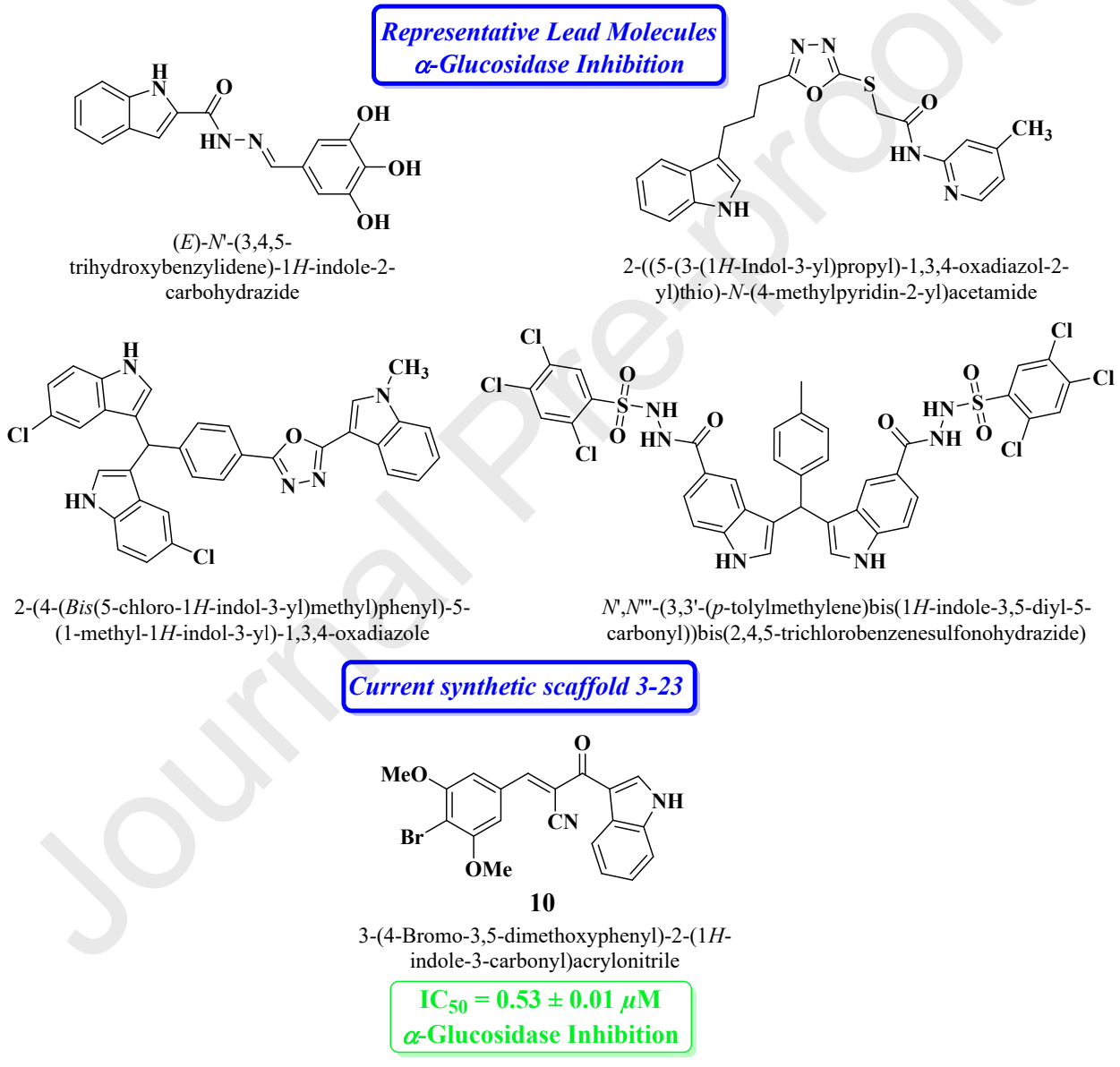
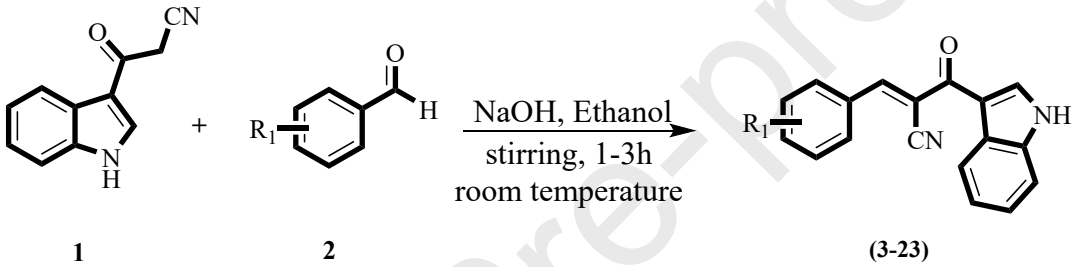


Figure-2: Rationale of Current Study.

Results and Discussion

Chemistry

A plausible mechanism for the synthesis of indole acrylonitrile is based on Knoevenagel condensation. A common synthetic method for forming carbon-carbon bond, it is a coupling reaction in which *α,β*-unsaturated ketone (a conjugated enone) is formed when an active hydrogen compound is added to a carbonyl group through nucleophilic addition, followed by dehydration. The reaction begins with base acting as nucleophile to remove the acidic proton of (3-cyanoacetyl indole). This leads to the formation of enol intermediate which attacks on the electrophilic carbonyl center of aldehyde (Scheme-1).



1 **2** **(3-23)**

Scheme-1: Synthesis of Indole Acrylonitriles (3-23).

Spectroscopic analysis of most active compound 10

Mass Spectrometry

EI-MS of most active compound **10** showed a molecular ion peak $[M]^+$ at *m/z* 410 and an isotopic peak of $[M]^{+2}$ at *m/z* 412 due to the bromine atom. $[M]^+$ undergoes the loss of cyanide to yield a cation at *m/z* 384. Loss of bromide from molecular ion gave a cation at *m/z* 331. These two fragments undergo *α*-cleavage to yeild cation at *m/z* 144 which appears as a base peak due to its stability. The inductive cleavage of base peak gives rise to neutral molecule of carbon monoxide and a radical cation of indole appearing at *m/z* 116 (Figure-S1 in Supp. Inf.).

^{1}H-NMR Spectral Study of Compound 10

^{1}H-NMR spectrum of compound **10** was recorded in DMSO-d_6 on a Bruker Avance AM 400 MHz instrument. Compound **10** possesses fifteen protons and the most downfield singlet of the indole NH resonated at δ_H 12.30, the downfield intensity of NH signal was due to the extented conjugation. The H-2 signal resonated as a singlet at δ_H 8.45, signal is downfield due to attachment

of carbonyl group at 3rd carbon of pyrrole ring. The proton of α-position appeared as a downfielded singlet at δ_H 8.23 due to the extended conjugation of sp^2 hybridized carbon with carbonyl group and the attached nitrile group. The H-4 resonated as double-doublet at δ_H 8.18 showing *ortho*- and *meta*-couplings with H-5 and H-6 with ($J_{4,5}$ = 6.8 Hz) and ($J_{4,6}$ = 2.4 Hz), respectively. The H-7 of indole ring also resonated as double-doublet at δ_H 7.56. The protons of H-2′ and H-6′ resonated at δ_H 7.47 as singlet. The signals of H-5 and H-6 appeared as an overlapping multiplet at δ_H 7.29. The six protons of methoxy groups resonated as singlet at δ_H 3.90 Hz (Figure-S2 in Supp. Inf.).

^{13}C-NMR Spectral Study of Compound 10

^{13}C-NMR spectrum of compound **10** was recorded in DMSO-d_6 on a Bruker Avance 125 MHz instrument. Compound **10** possesses twenty carbons, among them quaternary carbonyl carbon C-10 was resonated at δc 181.1 as the most downfield signal. The other two quaternary carbons 3′ and 5′ were resonated at δc 156.7; this downfield intensity was due to the methoxy groups. The α-carbon of the acrylonitrile part appeared at δc 151.5 due to the extended conjugation between sp^2 hybridized carbon and carbonyl group. C-1′ of aldehyde part resonated at δc 136.7. The methine carbon at 2nd position of indole molecule appeared at δc 136.1 due to extended conjugation with the carbonyl group. The carbon of methoxy groups was resonated at δc 56.6. All other carbons resonated in usual range (Figure-S3 in Supp. Inf.).

***In Vitro* α-Glucosidase Inhibitory Activities**

All synthetic indole acrylonitriles (**3-23**) were evaluated for their *in vitro* α-glucosidase inhibitory activity. Off twenty-one, seven compounds **4**, **8**, **10**, **11**, **14**, **18**, and **21** displayed excellent inhibitory activity against α-glucosidase enzyme ranging (IC_{50} = 0.53 ± 0.01-1.36 ± 0.04 μM) as compared to the standard acarbose (IC_{50} = 2.91 ± 0.02 μM) (Figure-3) (Table-1).

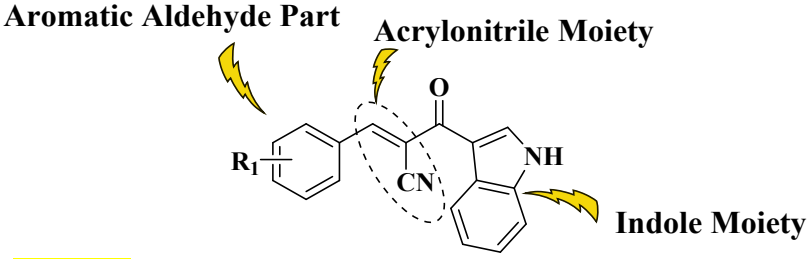
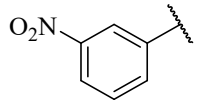
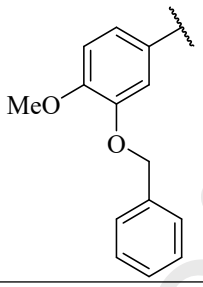
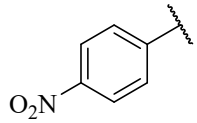
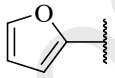
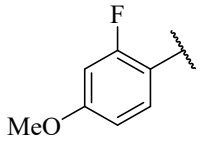
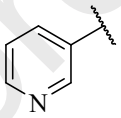
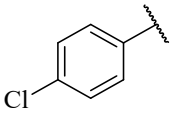
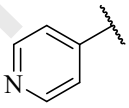
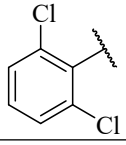
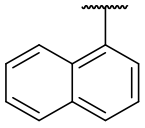
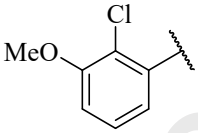
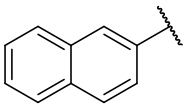
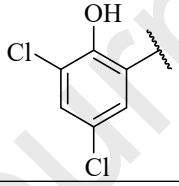
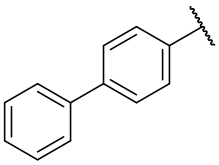
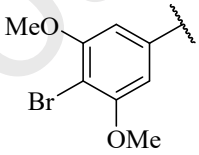
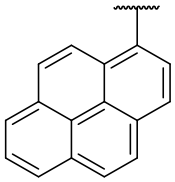
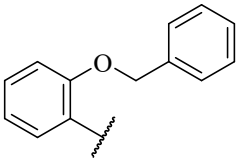
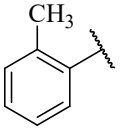
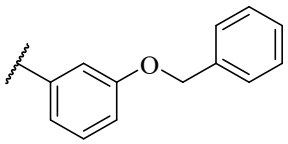
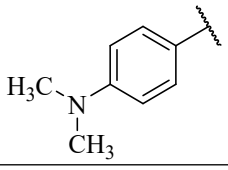
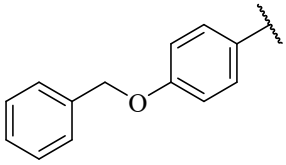


Figure-3: General Structure of Synthetic Compounds (**3-23**).

Table-1: *In vitro* α-Glucosidase Inhibitory Activity of Indole Acrylonitriles (**3-23**)

C. No.	R_1	$IC_{50} \pm SEM^a$ (μM)	C. No.	R_1	$IC_{50} \pm SEM^a$ (μM)
3		NA[b]	**14**		1.02 ± 0.06
4		0.88 ± 0.01	**15**		NA[b]
5		NA[b]	**16**		NA[b]
6		NA[b]	**17**		NA[b]
7		NA[b]	**18**		0.60 ± 0.03
8		0.95 ± 0.01	**19**		NA[b]
9		NA[b]	**20**		NA[b]
10		0.53 ± 0.01	**21**		1.36 ± 0.04
11		1.19 ± 0.06	**22**		NA[b]

12		NA[b]	**23**		NA[b]
13		NA[b]	**Standard (Acarbose)**		2.91 ± 0.02

SEM[a] (Standard error of mean); NA[b] (Not Active).

Structure-Activity Relationship

Structure-activity relationship is discussed on the basis of variable substituted "R" groups. The variation in the activity of different compounds was due to the nature and position of substituent "R" at the aldehyde ring.

Compound **4** with *para* substituted nitro group exhibited potent inhibition at IC_{50} value of 0.88 ± 0.01 μM in contrast to the standard acarbose (IC_{50} = 2.91 ± 0.02 μM), nonetheless, the *meta* substituted nitro group in compound **3** showed no activity (Figure-4).

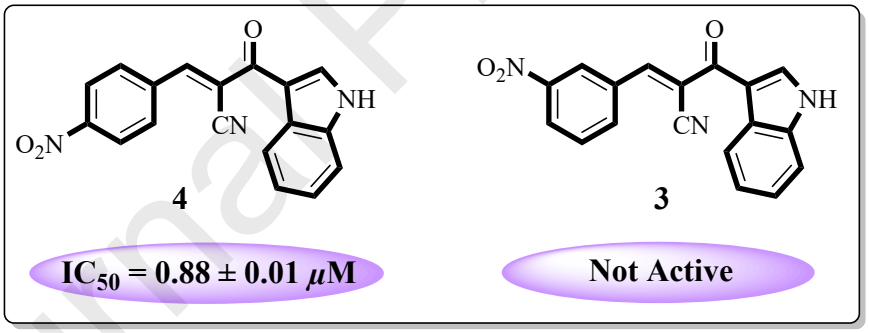


Figure-4: Structure-activity relationship (SAR) of compounds **3** and **4**.

Compounds **6**, **7**, **8**, and **9** possessed chloro substitution in combination with other functional groups as well. Amongst them, compound **8** (IC_{50} = 0.95 ± 0.01 μM) with *ortho* chloro substitutent and *meta* methoxy groups demonstrated potent inhibition (Figure-5). However, the mono and di-chloro compounds **6** and **7** were found to be inactive, in addition compound **9** in combination of chloro and hydroxy substituents was also inactive. It showed that the activity of compound **8** might be attributed to the presence of methoxy group.

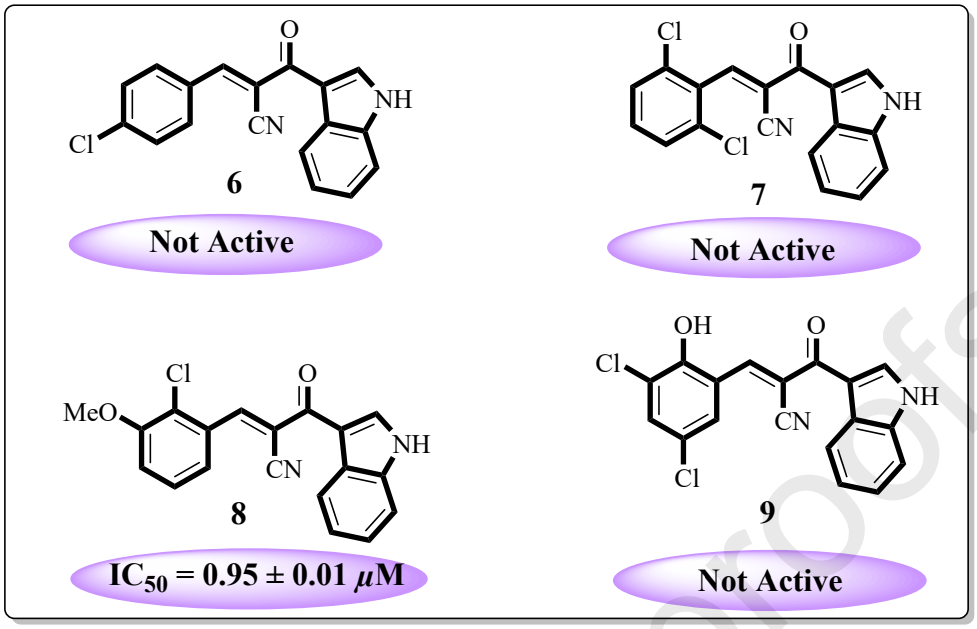


Figure-5: Structure-activity relationship (SAR) of compounds **6-9**.

Compound **10** (IC_{50} = 0.53 ± 0.01 μM) with *para* bromo and *meta* di-methoxy groups demonstrated potential inhibition against α-glucosidase and found to be the most active molecule of the series (Figure-6).

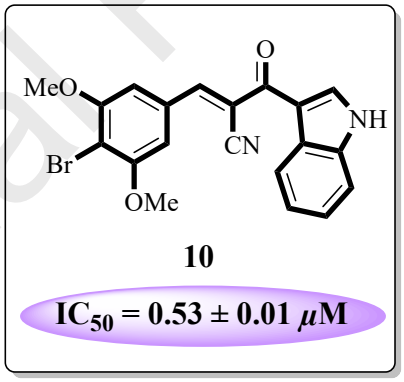


Figure-6: Structure-activity relationship (SAR) of compound **10**.

Compounds **11**, **12**, **13** and **14** bear benzyloxy groups at varying positions. Compound **11** with *ortho* benzyloxy group demonstrated superior inhibition at IC_{50} value of 1.19 ± 0.06 μM in comparison with standard, but with the change in position of benzyloxy group to *meta* and *para* in compounds **12** and **13** resulted in complete loss of activity. Compound **14** (IC_{50} = 1.02 ± 0.06 μM) with *meta* benzyloxy and *para* methoxy group showed potent activity (Figure-7).

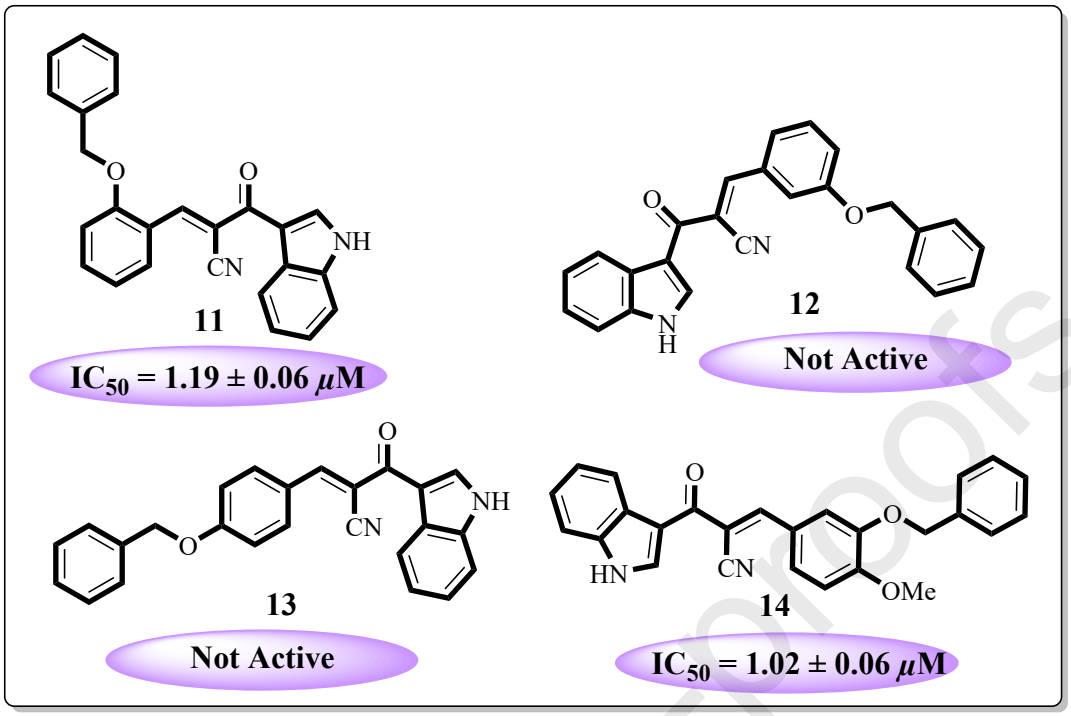


Figure-7: Structure-activity relationship (SAR) of compounds **11-14**.

Compounds **18-21** possess different aryl groups which exhibited variable inhibitory activities. Amongst them, compound **18** ($IC_{50} = 0.60 \pm 0.03$ μM) having α-naphthaldehyde group was second most potent inhibitor of α-glucosidase enzyme, while compound **19** with β-naphthaldehyde group exhibited no activity. The biphenyl substituted compound **20** also showed no activity, in contrast, compound **21** ($IC_{50} = 1.36 \pm 0.04$ μM) bearing 1-pyrenecarboxaldehyde ring displayed an excellent inhibitory activity as compared to the standard acarbose ($IC_{50} = 2.91 \pm 0.02$ μM) (Figure-8).

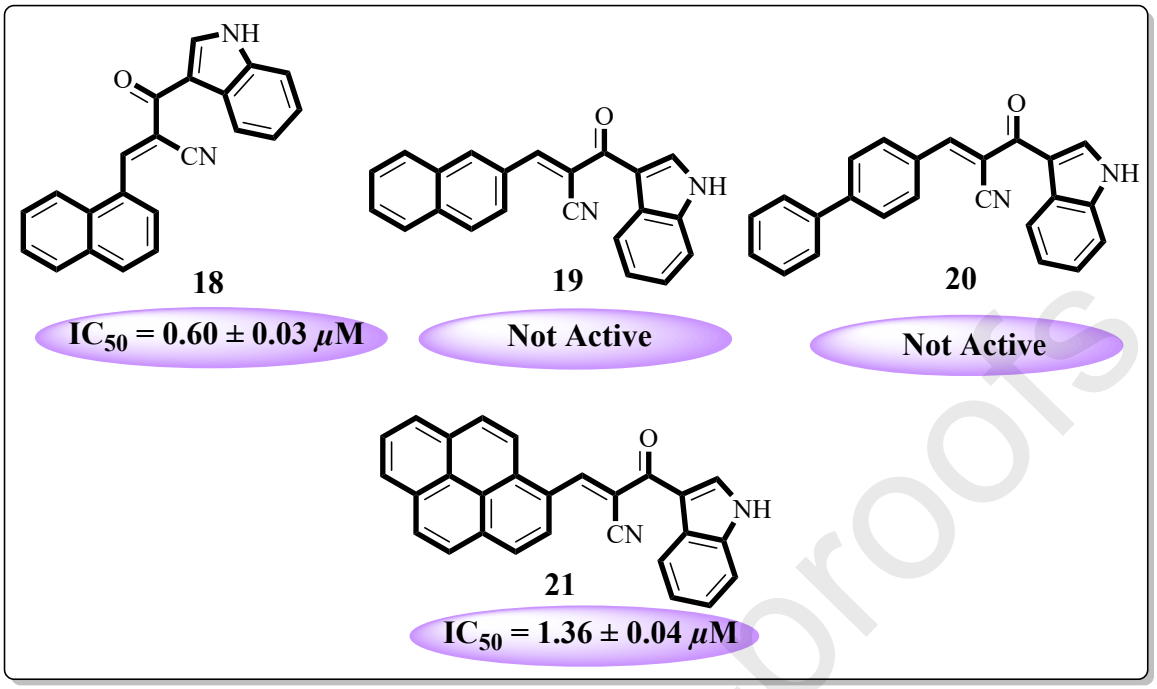


Figure-8: Structure-activity relationship (SAR) of compounds **18-21**.

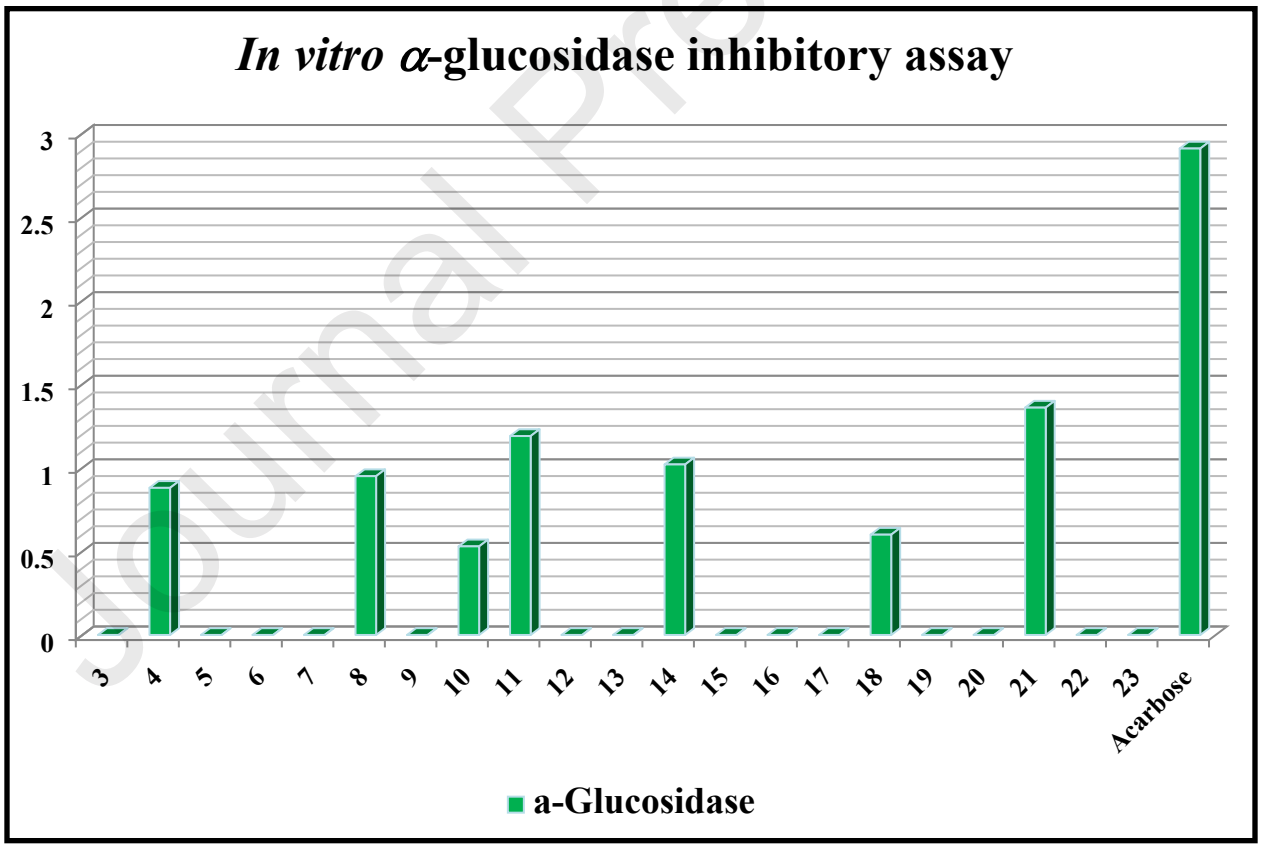


Figure-9: Comparison of α-glucosidase inhibitory activities of compounds (**3-23**) with standard.

Molecular Docking (MD) Studies:

Molecular docking simulation is one of the most frequently used methods to aid in modern drug designing. The prediction power of docking programs helps to find out suitable conformation of ligands within the binding site. In the present study, to predict the inhibitory mechanism, we employed molecular docking analysis on the newly synthesized indole acrylonitriles that displayed significant inhibitory effect for *in vitro* assay on *α*-glucosidase.

The Alpha PMI Placement method with induce fit docking protocol of MOE was employed to generate 30 conformations with affinity dG scoring of each molecule of the series that showed positive inhibitory activity along with the standard drug acarbose to identify their binding modes. The resulted poses were clustered and the conformation with highest binding affinity towards the receptor pocket was analyzed further for molecular interaction profile.

Newly synthesized indole analogues with potential *α*-glucosidase inhibitory activity explains the experimental findings of high inhibition of *α*-glucosidase. The binding affinities of respective analogues are in between the ranges from -8.59 to -6.56 (Table-2) and our standard acarbose have the binding score of -6.98. Detailed visual inspection of each active molecule and standard drug defines the interaction pattern with the crucial residues (Table-2). Ligand protein interaction profiling displayed a number of hydrogen bonds and hydrophobic interaction between indole acrylonitrile series and *α*-glucosidase enzyme. Compound **10**, **11**, and **14** gives the highest binding affinity than the standard acarbose, as they makes themselves sandwich between the most crucial residues Phe157, Phe177 and Phe300 by means of hydrophobic and π-π interactions. Additionally, hydrogen bonds by means of electronegative groups with the prime residues *i.e.* Glu181, Arg212 and His348 also facilitate the binding. Compound **10** complements the most potent inhibitory activity with dock score -8.44 by forming π-π and hydrophobic interactions with Phe157 through its indole. Whereas, Phe177 and Asp349 also contribute to maintain hydrophobic interactions. The hydrogen bonding was observed between nitrogen of acrylonitrile and the residue Arg212 validating the potency of inhibition. The π-stacking with Phe300 proves the best orientation of the molecule which was defined in our previous study (Table-2, Figure-10) [33].

Unfortunately, compound **4**, **8**, and **18** being the most potent inhibitors and compound **10** showed less binding affinity due to lack of few electronegative functionalities at the R-group for hydrogen

bonding. Whereas, compound **21** unable to fit in the cavity due to bulky group *i.e.* 1-pyrenecarboxaldehyde ring that shifts the molecule slightly to the left. However, the interaction within the pockets playing significant role in inhibition.(Table-2, Figure-11A-F). Based on the molecular docking results, the intact indole moiety and the acrylonitrile group were worthy targets for further structural modifications.

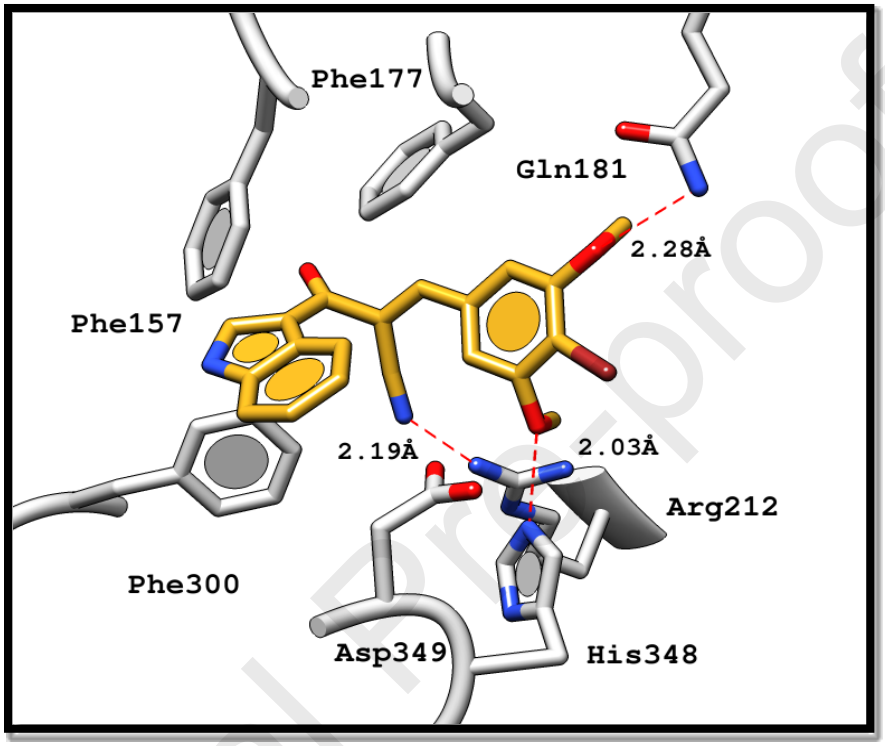


Figure-10: Molecular Interaction of one of the most potent inhibitor **10** with dock score -8.44 (IC_{50} = 0.53 ± 0.01 μM).

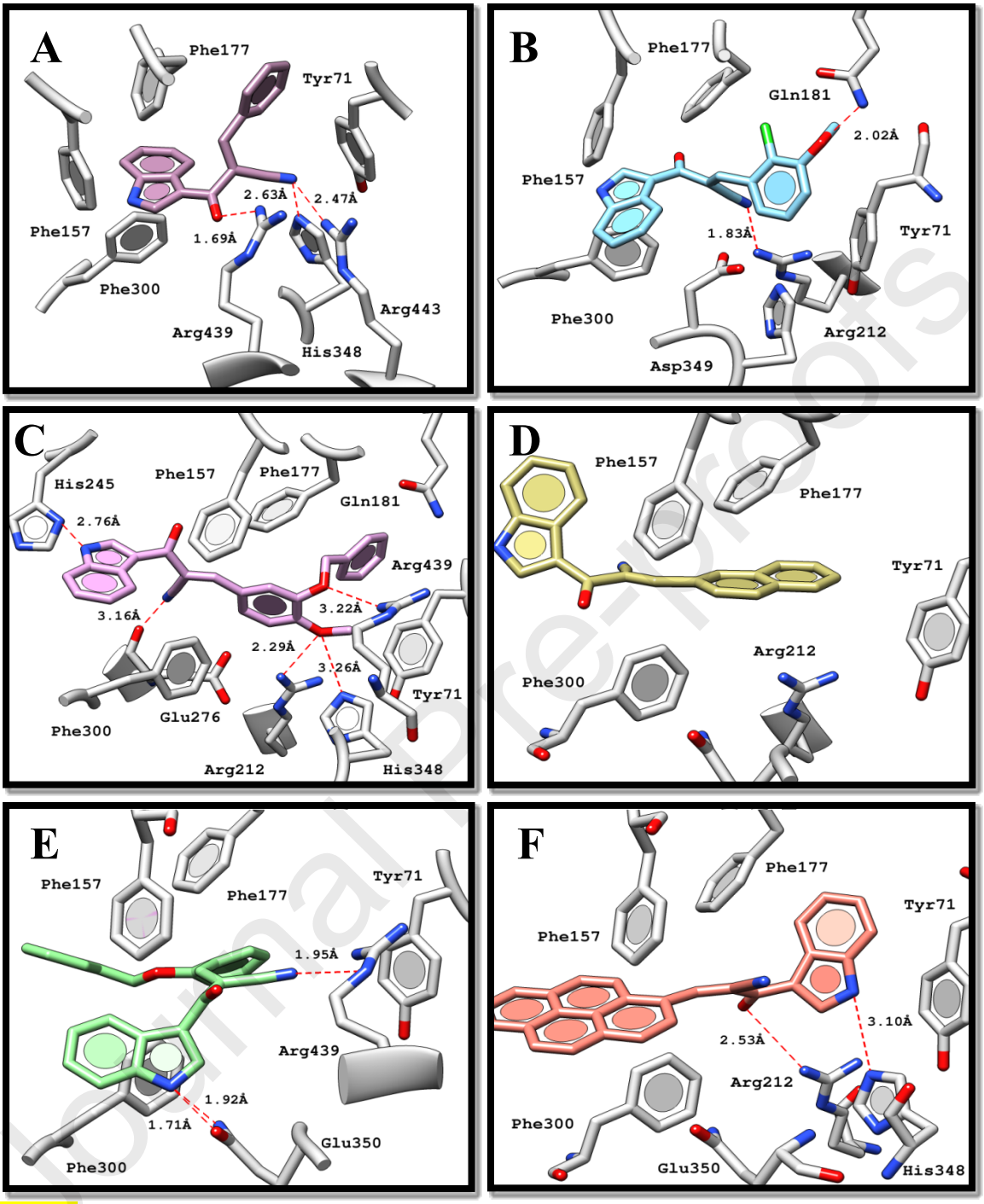


Figure-11: Binding interaction of all the active compounds with in the pocket of α-glucosidase. (**A**) Compound **4**, (**B**) Compound **8**, (**C**) Compound **14**, (**D**) Compound **18**, (**E**) Compound **11**, and (**F**) Compound **21**.

Table-2: Interaction details for synthesized indole acrylonitrile derivatives (**3-23**) against *α*-glucosidase enzyme.

C. No.	Dock Score	Hydrogen bond		Distances Å	Hydrophobic Interaction	Pi-Stacking
		Ligand	Residue			
Acarbose	-6.98	Acar:O3	Arg212:N3	3.09	Tyr71, Phe177, Asp349	-
		Acar:O3	ASP349:O2	2.38		
		Acar:O3	Glu276:O3			
		Acar:O3	Tyr71:O2			
4	-6.56	Lig4:N1	His348:NE2	2.63	Tyr71, Phe157, Phe177	Phe157, Phe177, Phe300
		Lig4:O2	Arg439:NH1	1.69		
		Lig4:N1	Arg443:NH2	2.47		
8	-6.96	Lig8:O3	Gln181:NE2	2.02	Tyr71, Phe157, Phe177	Phe300
		Lig8:N1	Arg212:NH2	1.83		
18	-7.40	-	-	-	Phe157, Phe177, Phe300	Phe157
21	-7.41	Lig21:O2	Arg212:N3	3.10	Tyr71, Phe157, Phe177, Phe300	Phe300
		Lig21:O2	Thr215:N2	3.34		
		Lig21:N1	His348:N2	2.53		
10	-8.44	Lig10:N1	Arg212:N3	2.19	Phe157, Phe177, Asp349	Phe157, Phe300
		Lig10:O3	Gln181:NE2	2.28		
		Lig10:O3	His348:NE2	2.03		
		Lig10:O3	Arg443:N3	3.26		
14	-8.58	Lig14:O3	Arg212:N3	2.29	Tyr71, Val108, Phe157, Phe177, Phe300	-
		Lig14:N1	His245:N2	2.76		
		Lig14:N1	Glu276:O3	3.16		
		Lig14:O3	His348:N2	3.26		
		Lig14:O3	Arg439:N3	3.22		
11	-8.59	Lig11:N1	Gln350:O2	1.71	Phe157, Phe177, Phe300	-
		Lig11:N1	Gln350:N3	1.92		
		Lig11:N3	Arg439:N3	1.95		

ADME Properties

ADME properties of seven active inhibitors from indole acrylonitrile series were analyzed for the quality assessment. For a compound to be a potential lead in drug discovery, it should not violate the Lipinsiki rule of five as our compounds does. For polarity assessment, topological surface area (TPSA) of all actives are in between 20 and 130 Å2 acceptable range. For lipophilicity, water octanol partition coefficient (by XLOGP3 method) with reference to the several common drugs showed favorable values and hence a measure of fitness for biliary excretion. We also need to suspect gastrointestinal absorption (GI) of these compound, to be an high orally active drug. Similarly, water solubility of a molecule greatly facilitates the ease of handling and synthesis.

Moreover, it also influences the absorption property of a drug to deliver a sufficient quantity of active ingredient in the small volume of such pharmaceutical dosage for oral administration. Herein our all actives are moderately soluble except compound **21** with poor solubility due to its large molecular size. Additionally, we also assessed the synthetic accessibility (SAR) of compounds whose molecular fragment is easily available in the market. Molecular size and complexity of a fragment contribute to summed up the SAR of a compound. The higher the SAR, implies the rare fragment and consequently, causes the difficulty in the synthesis. The SAR score ranges from 1 (very easy) to 10 (very difficult).

Moreover, PAINS (Pan Assay Interference) are the representative of those fragments in a compound that can yield false positive biological outputs and would be reactive, unstable, and toxic. As they can give response irrespective of the assay against any target. Whereas, Brenks Structural Alert which consists in a list of 105 fragments identified by Brenk *et al.* [34] to be putatively toxic, chemically reactive, metabolically unstable or to bear properties responsible for poor pharmacokinetics. Collectively, these two predictions on actives of respective series gave no PAINS alert, however, there are some Brenk alerts observed. Overall, all the mentioned parameters make our compounds as having good therapeutic properties due to acceptable physicochemical (solubility, lipophilicity, Lipinski rule of five) and pharmacokinetic (TPSA, GIT) properties. Moreover, we also suspect the medicinal property (SAR, PAINS, Structural Alerts) of active compounds that defines the no toxicity and fast synthesis of these compounds. Table-3 shows the favorable computationally predicted ADME profiles of seven compounds with good inhibitory potential.

Table-3: ADME properties of seven active inhibitors of Indole acrylonitrile series.

C. No.	Lipinski Rule of Five	TPSTRUCTURE-ACTIVITY RELATIONSHIP (20 to 130Å2)	Water Solubility	Lipophil-icity XLOGP3 (-0.7to +5.0)	GI Absorp-tion	Synthetic Accessi-bility	PAINS #alerts	Brenk #alerts
4	Yes	102.47	Moderate	3.57	High	2.73	0	3
8	Yes	65.88	Moderate	4.34	High	2.82	0	2
10	Yes	75.11	Moderate	4.38	High	2.92	0	2
11	Yes	65.88	Moderate	5.21	High	3.18	0	2

14	Yes	75.11	Moderate	5.18	High	3.25	0	2
18	Yes	56.65	Moderate	5	High	2.82	0	2
21	Yes	56.65	Poor	6.85	High	3.11	0	3

Conclusion

Twenty-one indole acrylonitriles (**3-23**) were synthesized by reacting 3-cyanoacetyl indole and aromatic aldehydes in the presence of base. The synthetic compounds were evaluated for their *in-vitro* α-glucosidase inhibitory activity. Off them, seven compounds exhibited potent activities in range (IC_{50} = 0.53 ± 0.01 - 1.36 ± 0.04 μM) against standard acarbose (IC_{50} = 2.91 ± 0.02 μM). Compound **10** (IC_{50} = 0.53 ± 0.01 μM) showed most potent inhibitory activity. *In silico* studies of synthetic compounds interpreted that the entire molecule is contributing in substantial inhibition of α-glucosidase enzyme. In conclusion, the newly identified α-glucosidase inhibitors may serve as lead candidates for advance research in order to get potent antidiabetic agents for pharmaceutical applications.

Experimental

Materials and Methods

Thin layer chromatography was carried out on pre-coated silica gel, GF-254 (Merck, Germany). Ultraviolet light of 254 and 366 nm was used to visualize spots. The ^{1}H- and ^{13}C-NMR were recorded on Bruker AM spectrometers in deuterated solvents, operating in range of 300 to 500 MHz. The chemical shift values are presented in ppm (δ), relative to internal standard tetramethylsilane (TMS) and the coupling constant (J) are in Hz. EI-MS and HREI-MS spectra were recorded on MAT 312 and MAT 113D mass spectrometers. Melting points of the compounds were determined on a Stuart® SMP10 melting point apparatus and are uncorrected.

α-Glucosidase inhibition assays

α-Glucosidase inhibition assays were performed following the literature procedures [35]. Briefly, the solutions of α-glucosidase (*Saccharomyces cerevisiae* 2.5 U mL^{-1} purchased from Sigma Aldrich, USA) and the substrate, *p*-nitrophenyl α-D-glucopyranoside (*p*-NPG) was prepared in 0.07 M phosphate buffer (pH 6.8). The assay was started with pre-incubation of 70 μL buffer, 10 μL enzyme with a test compound (10 μL) at 37 °C for 5 min. After pre-incubation, 10 μL of *p*-NPG (10 mM) was added to each well of a 96 well plate and further incubated at 37 °C for 30 min.

The reaction was stopped by adding 80 μL of 0.2 M Na_2CO_3 solution. Negative control wells contained additional 10 μL of buffer instead of test compounds and the standard drug acarbose was used as a positive control. The activity of test compounds against α-glucosidase was determined by measuring *p*-nitrophenol at a wavelength of 405 nm using microplate reader (Bio-Tek ELx 800^{TM}, Winooski, USA). The percent inhibition was calculated using the following equation:

$$\text{Percent inhibition (\%)} = [1 - (\text{Absorbance of sample}/\text{Absorbance of control}) \times 100]$$

Dose-response curves of potential inhibitors (≥ 50%) were obtained and IC_{50} was determined with the help of the GraphPad prism 5.0 Software Inc., San Diego, California, USA.

Molecular Docking (MD) Protocol

Molecular docking studies were carried out to explore α-glucosidase inhibitory mechanism of indole acrylonitrile derivatives. Due to the lack of structural information, we used the α-glucosidase model previously reported by our group [36].

Chemical structure of indole acrylonitrile series were built by Molecular Operating Environment (MOE) version 2019.01 [37] builder module, and minimized by MMFF94x forcefield. MOE-dock Program was used to explore the binding modes of each molecule. The stability of molecules was ensured by analyzing hydrogen bonding and hydrophobic interactions of molecules with the respective target through MOE-Protein Ligand Interaction Fingerprint (PLIF) Program.

ADME Profiling

Drug development involves assessment of absorption, distribution, metabolism and excretion (ADME) increasingly earlier in the discovery process, to avoid any failure in later stages. To be effective as a drug, a potent molecule must reach its target in the body in sufficient concentration, and stay there in a bioactive form long enough for the expected biologic events to occur. Here in, we used Swiss ADME web [38] tool for *in silico* ADME prediction as it is a fast yet robust predictive model to analysed physicochemical properties, pharmacokinetics, and drug-likeness of a compound.

General Procedure for the Synthesis of 2-(1H-Indole-3-ylcarbonyl)-3-heteroaryl acrylonitriles

The starting material 3-(cyanoacetyl)-indole was synthesized *via* classical Friedel-Crafts acylation reaction [39]. A mixture containing 3-(cyanoacetyl)-indole (1.0 mmol) and different substituted aryl aldehyde (1.0 mmol) in absolute ethanol (10.0 mL) was kept in ice bath to lower temperature to 0 ˚C. Catalytic amount of NaOH (0.15 mmol) was added to reaction mixture and it was stirred for 1-3 h at room temperature. The reaction progress was monitored through TLC with developing solvent system of (*n*-Hexane: Ethyl Acetate). The product was thoroughly washed with ethanol and dried. The solid mass was crystallized from hot ethanol. The synthetic derivatives were characterized by different spectroscopic techniques, such as EI-MS, HREI-MS, ^{1}H-, and ^{13}C-NMR.

Spectral data of newly synthesized compounds

3-(2-Fluoro-4-methoxyphenyl)-2-(1H-indole-3-carbonyl)acrylonitrile **(5)**

Solid; Yield: 61%; M.P.: 203-207 ˚C; IR (KBr, cm^{-1}); 3214 (Sec NH), 3022 (Alkene C-H stretch), 2242 (Nitrile), 1726 (Ketone C=O), 1615 (Alkene C=C), 1479 (Aromatic C=C), 1274 (Ester C-O) 1022 (C-F stretch);^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.26 (s, 1H, NH), 8.45 (s, 1H, H-2), 8.28 (t, $J_{6'(5'/F)}$ = 9.0 Hz, 1H, H-6'), 8.17 (s, 1H, H-α), 8.14 (dd, $J_{4,5}$ = 6.8 Hz, $J_{4,6}$ = 2.4 Hz, 1H, H-4), 7.53 (dd, $J_{7,6}$ = 7.2 Hz, $J_{7,5}$ = 2 Hz, 1H, H-7), 7.26 (m, H-2, H-5, H-6), 7.09 (d, $J_{3',5}$ = 2.4 Hz, 1H, H-3'), 7.05 (dd, $J_{5',3'}$ = 2.4 Hz, $J_{5',6'}$ = 5.6 Hz, 1H, H-5'), 3.87 (s, 3H, OCH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 181.3, 158.6, 157.9, 154.2, 138.1, 137.4, 128.2, 123.5, 122.5, 121.8, 118.4, 116.3, 113.5, 110.6, 108.1, 102.0, 55.6; EI-MS *m/z* (% rel. abd.): 320 (M^+, 96), 301 (22). 293 (34), 144 (100), 116 (26); HR-EIMS Calcd for $C_{19}H_{13}FN_2O_2$: 320.0961, Found 320.0985.

3-(2-Chloro-3-methoxyphenyl)-2-(1H-indole-3-carbonyl)acrylonitrile **(8)**

Solid; Yield: 43%; M.P.: 251-258 ˚C; IR (KBr, cm^{-1}); 3188 (Sec NH), 3056 (Aromatic CH stretch), 3011 (Alkene CH stretch), 2840 (Alkane CH stretch), 2252 (Nitrile), 1710 (Ketone C=O), 1600 (Alkene C=C stretch), 1286 (Ether C-O), 754 (C-Cl); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 12.35 (s, 1H, NH), 8.46 (s, 1H, H-2), 8.32 (s, 1H, H-α), 8.17 (dd, $J_{4,5}$ = 6.6 Hz, $J_{4,6}$ = 3.6 Hz, 1H, H-4), 7.71 (d, $J_{4',5'}$ = 7.5 Hz, 1H, H-4'), 7.55 (ovp. m, 2H, H-5', H-7), 7.36 (d, $J_{6',5'}$ = 8.4 Hz, 1H, H-6'), 7.28 (ovp. m, 2H, H-5, H-6), 3.92 (s, 3H, OCH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 180.4, 155.0, 148.3, 136.7, 136.2, 132.0, 128.2, 125.9, 123.7, 122.6, 121.9, 121.3, 121.0, 116.5, 115.0, 115.4,

113.5, 112.6, 56.5; EI-MS *m/z* (% rel. abd.): 336 (M^+, 48), 338 (M+2, 14), 301 (100), 144 (89), 116 (18); HR-EIMS Calcd for $C_{19}H_{13}N_2O_2Cl$: 336.0666, Found 336.0672.

3-(3,5-Dichloro-2-hydroxyphenyl)-2-(1H-indole-3-carbonyl)acrylonitrile **(9)**

Solid; Yield: 34%; M.P.: 267-274 ˚C; IR (KBr, cm^{-1}); 3635 (OH), 3353 (Sec NH), 3068 (Aromatic CH), 2254 (Nitrile stretch), 1707 (Ketone C=O), 1675 (Alkene C=C stretch), 744 (C-Cl) ^{1}H-NMR (300 MHz, DMSO-d_6): δ 12.25 (s, 1H, NH), 8.28 (s, 1H, H-2), 8.24 (s, 1H, H-α), 8.20 (m, 1H, H-4), 8.04 (d, $J_{4',6'}$ = 2.4 Hz, 1H, H-4'), 7.90 (d, $J_{6',4'}$ = 2.4 Hz, 1H, H-6'), 7.51 (m, 1H, H-7), 7.27 (ovp. m, 2H, H-5, H-6); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 183.8, 157.1, 148.1, 140.7, 138.1, 136.9, 131.5, 129.8, 128.2, 127.3, 125.3, 123.4, 122.5, 121.1, 120.8, 115.6, 112.5, 112.4; EI-MS *m/z* (% rel. abd.): 357 (M^+, 55), 359 (M+2, 32), 361 (M+4, 5), 144 (100), 116 (13); HR-EIMS Calcd for $C_{18}H_{10}N_2O_2Cl_2$: 357.0198, Found 357.0203.

3-(4-Bromo-3,5-dimethoxyphenyl)-2-(1H-indole-3-carbonyl)acrylonitrile **(10)**

Solid; Yield: 55%; M.P.: 252-255 ˚C; IR (KBr, cm^{-1}); 3181 (Sec NH), 3050 (Alkene C-H), 2843 (Alkane C-H), 2248 (Nitrile stretch), 1708 (Ketone C=O), 1640 (Alkene C=C stretch), 1437 (Aromatic C=C), 1185 (Ether C-O), 636 (C-Br); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.30 (s, 1H, NH), 8.45 (s, 1H, H-2), 8.23 (s, 1H, H-α), 8.18 (dd, $J_{4,5}$ = 6.8 Hz, $J_{4,6}$ = 2.4 Hz, 1H, H-4), 7.55 (dd, $J_{7,5}$ = 1.2 Hz, $J_{7,6}$ = 6.0 Hz, 1H, H-7), 7.47 (s, 2H, H-2', H-6'), 7.29 (ovp. m, 2H, H-5, H-6) 3.90 (s, 6H, O-CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 181.0, 156.7, 151.5, 136.7, 136.1, 132.7, 126.0, 123.6, 122.5, 121.3, 117.7, 113.5, 112.5, 112.2, 106.7, 104.1, 56.5; EI-MS *m/z* (% rel. abd.): 410 (M^+, 98), 412 (M+2, 97), 384 (20), 331 (16), 302 (11), 165 (29), 144 (100), 116 (46); HR-EIMS Calcd for $C_{20}H_{15}BrN_2O_3$: 410.0266, Found 410.0248.

3-(2-(Benzyloxy)phenyl)-2-(1H-indole-3-carbonyl)acrylonitrile **(11)**

Solid; Yield: 78%; M.P.: 185-187 ˚C; IR (KBr, cm^{-1}); 3222 (Sec NH), 3056 (Aromatic C-H stretch), 2946 (Alkene C-H stretch), 2258 (Nitrile stretch), 1718 (Ketone C=O), 1604 (Alkene C=C), 1510 (Aromatic C=C), 1435 (Alkane CH_2 bend), 1189 (Ether C-O); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.27 (s, 1H, NH), 8.47 (s, 1H, H-2), 8.44 (s, 1H, H-α), 8.19 (dd, $J_{4,5}$ = 8 Hz, $J_{4,6}$ =1.5 Hz, 1H, H-4), 8.15 (d, $J_{6',5'}$ =7.5 Hz, 1H, H-6'), 7.58 (t, $J_{4''(5''/3'')}$ = 8.7 Hz,1H, H-4''), 7.55 (d,

$J_{3',4'}$ = 7.5 Hz, 1H, H-3'), 7.42 (d, $J_{2",3",6",5"}$ = 7.0 Hz, 2H, H-2",H-6"), 7.35 (t, $J_{3"(2"/4"),\ 5"(6"/4")}$ = 7.5 Hz, 2H, H-3",H-5"), 7.29 (t, $J_{6(5/7),\ 5(4/6)}$ = 7.2 Hz, 2H, H-5,H-6), 7.24 (m, 2H, H-7, H-4'), 7.22 (t, $J_{5'(4'/6')}$ = 7.5 Hz, 1H, H-5'), 5.24 (s, 2H, CH_2);^{13}C-NMR (100 MHz, DMSO-d_6): δ 181.2, 157.6, 146.7, 136.6, 136.4, 135.5, 134.2, 128.5, 128.4, 128.0, 127.5, 126.0, 123.6, 122.4, 121.6, 121.3, 120.9, 117.7, 113.6, 113.5, 112.5, 111.4, 70.0; EI-MS *m/z* (% rel. abd.): 378 (M^+, 13), 349 (10), 287 (88), 271 (32), 232 (30), 144 (28), 91 (100); HR-EIMS Calcd for $C_{25}H_{18}N_2O_2$: 378.1368, Found 378.1379.

3-(3-(Benzyloxy)phenyl)-2-(1H-indole-3-carbonyl)acrylonitrile **(12)**

Solid; Yield: 65%; M.P.: 205-207 ˚C; IR (KBr, cm^{-1}); 3210 (Sec NH), 3010 (Aromatic C-H stretch), 2935 (Alkene C-H stretch), 2255 (Nitrile stretch), 1710 (Ketone C=O), 1610 (Alkene C=C), 1525 (Aromatic C=C), 1425 (Alkane CH_2 bend), 1127 (Ether C-O); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.28 (s, 1H, NH), 8.44 (s, 1H, H-2), 8.19 (s, 1H, H-α), 8.17 (dd, $J_{4,5}$ = 7 Hz, $J_{4,6}$ = 2 Hz, 1H, H-4), 7.71 (s, 1H, H-2'), 7.66 (d, $J_{4',5'}$ = 7.5 Hz, 1H, H-4'), 7.53 (dd, $J_{7,6}$ = 7.5 Hz, $J_{7,5}$ = 2 Hz, 1H, H-7), 7.50 (t, $J_{5'(4'/6')}$ = 8 Hz, 1H, H-5'), 7.46 (d, $J_{2",3",6",5"}$ = 7.5 Hz, 2H, H-2", H-6"), 7.40 (t, $J_{3"(2"/4"),\ 5"(6"/,4")}$ = 7.5 Hz, 2H, H-3", H-5"), 7.35 (d, $J_{6',5'}$ = 7.5 Hz, 1H, H-6'), 7.28 (m, 3H, H-5, H-6, H-4"), 5.16 (s, 2H, CH_2);^{13}C-NMR (100 MHz, DMSO-d_6): δ 181.2, 158.5, 151.8, 136.7, 136.6, 136.1, 133.7, 130.3, 128.5, 128.0, 127.8, 126.1, 123.6, 122.7, 122.4, 121.3, 118.8, 117.6, 116.4, 113.5, 112.5, 111.7, 69.4; EI-MS *m/z* (% rel. abd.): 378 (M^+, 40), 287 (8), 144 (16), 116 (7), 91 (100); HR-EIMS Calcd for $C_{25}H_{18}N_2O_2$: 378.1368, Found 378.1362.

3-(4-(Benzyloxy)phenyl)-2-(1H-indole-3-carbonyl)acrylonitrile **(13)**

Solid; Yield: 51%; M.P.: 214-216 ˚C; IR (KBr, cm^{-1}); 3220 (Sec NH), 3059 (Aromatic C-H stretch), 2942 (Alkene C-H stretch), 2240 (Nitrile stretch), 1720 (Ketone C=O), 1603 (Alkene C=C), 1510 (Aromatic C=C), 1439 (Alkane CH_2 bend), 1164 (Ether C-O); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.21 (s, 1H, NH), 8.42 (s, 1H, H-2), 8.18 (s, 1H, H-α), 8.16 (dd, $J_{4,5}$ = 7 Hz, $J_{4,6}$ = 1.5 Hz, 1H, H-4), 8.08 (d, $J_{2',3',\ 6',5'}$ = 8.5 Hz, 2H, H-2', H-6'), 7.52 (dd, $J_{7,6}$ = 6.5 Hz, $J_{7,5}$ = 1.5 Hz, 1H, H-7), 7.48 (d, $J_{2",3",\ 6",5"}$ = 7 Hz, 2H, H-2", H-6"), 7.40 (t, $J_{5(4/6),6(5/7)}$ = 7.2 Hz, 2H, H-5, H-6), 7.36 (m, 1H, H-4"), 7.27 (ovp. m, 2H, H-3", H-5"), 7.24 (d, $J_{3',2',\ 5',6'}$ = 9.0 Hz, 2H, H-3',H-5'), 5.23 (s, 2H, CH_2); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 181.5, 161.8, 151.9, 136.6, 136.3, 135.5, 132.8,

128.5, 128.0, 127.9, 126.1, 125.1, 123.5, 122.3, 121.4, 118.4, 115.5, 113.7, 112.4, 108.1, 69.6; EI-MS *m/z* (% rel. abd.): 378 (M^+, 85), 207 (48), 144 (34), 116 (12), 91 (100); HR-EIMS Calcd for $C_{25}H_{18}N_2O_2$: 378.1368, Found 378.1354.

3-(3-(Benzyloxy)-4-methoxyphenyl)-2-(1H-indole-3-carbonyl)acrylonitrile **(14)**

Solid; Yield: 60%; M.P.: 195-197 ˚C; IR (KBr, cm^{-1}); 3150 (Sec NH), 3051 (Aromatic C-H stretch), 2938 (Alkene C-H stretch), 2840 (Alkane C-H stretch), 2252 (Nitrile stretch), 1710 (Ketone C=O), 1618 (Alkene C=C), 1485 (Aromatic C=C), 1407 (Alkane CH_2 bend), 1188 (Ether C-O); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.23 (s, 1H, NH), 8.38 (s, 1H, H-2), 8.15 (dd, $J_{4,5}$ = 7 Hz, $J_{4,6}$ = 2 Hz, 1H, H-4), 8.13 (s, 1H, H-α), 7.85 (d, $J_{2',6'}$ = 2.0 Hz, 1H, H-2'), 7.73 (dd, $J_{6',5'}$ = 8.5 Hz, $J_{6',2'}$ = 2.0 Hz, 1H, H-6'), 7.51 (dd, $J_{7,6}$ = 7.0 Hz, $J_{7,5}$ = 1.5 Hz, 1H, H-7), 7.46 (d, $J_{2'',3''/6'',5''}$ = 7.0 Hz, 2H, H-2", H-6"), 7.40 (t, $J_{3''(2''/4'')}$, $_{5''(6''/4'')}$ = 7.25 Hz, 2H, H-3",H-5"), 7.35 (t, $J_{4''(3''/5'')}$ = 7.25 Hz, 1H, H-4"), 7.22 (m, 3H, H-5, H-6, H-5'), 5.12 (s, 2H, CH_2), 3.87 (s, 3H, OCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 181.4, 152.9, 152.0, 147.5, 136.4, 128.4, 128.0, 125.8, 124.9, 123.3, 122.1, 121.3, 118.5, 114.7, 113.6, 112.6, 112.1, 70.0, 55.9; EI-MS *m/z* (% rel. abd.): 408 (M^+, 41), 317 (11), 143 (23), 116 (6), 91 (100); HR-EIMS Calcd for $C_{26}H_{20}N_2O_3$: 408.1474, Found 408.1460.

2-(1H-Indole-3-carbonyl)-3-(pyren-1-yl)acrylonitrile **(21)**

Solid; Yield: 68%; M.P.: 278-282 ˚C; IR (KBr, cm^{-1}); IR (KBr, cm^{-1}); 3189 (Sec NH), 3048 (Aromatic C-H), 2235 (Nitrile stretch), 1720 (Ketone C=O), 1610 (Alkene C=C), 1427 (Aromatic C=C); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.30 (s, 1H, NH), 9.22 (s, 1H, H-2), 8.76 (d, $J_{8',7'}$ = 8.0 Hz, 1H, H-8'), 8.60 (s, 1H, H-α), 8.48 (d, 2H, H-4', H-5'), 8.43 (d, $J_{2',\ 3',\ 9',\ 10'}$ = 7.5 Hz, 2H, H-2', H-9'), 8.35 (d, $J_{3',\ 2',\ 10',\ 9'}$ = 7.5 Hz, 2H, H-3', H-10'), 8.30 (d, $J_{6',7'}$ = 9.0 Hz, 1H, H-6'), 8.27 (dd, $J_{4,5}$ = 6.5 Hz, $J_{4,6}$ = 4 Hz, 1H, H-4), 8.16 (t, $J_{7'(6'/5')}$ = 7.7 Hz, 1H, H-7'), 7.58 (dd, $J_{7,6}$ = 6.5 Hz, $J_{7,5}$ = 3 Hz, 1H, H-7), 7.31 (ovp. m, 2H, H-5, H-6); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 181.2, 150.2, 136.2, 133.2, 130.7, 129.5, 129.2, 127.3, 126.9, 126.7, 126.6, 126.5, 126.3, 126.2, 124.8, 123.7, 123.6, 123.4, 123.0, 122.4, 121.4, 117.9, 114.7, 113.9, 112.5; EI-MS *m/z* (% rel. abd.): 396 (M^+, 100), 367 (12), 251 (14), 144 (85), 116 (14); HR-EIMS Calcd for $C_{28}H_{16}N_2O$: 396.1263, Found 396.1250.

3-(4-(Dimethylamino)phenyl)-2-(1H-indole-3-carbonyl)acrylonitrile **(23)**

Solid; Yield: 64%; M.P.: 220-224 ˚C; IR (KBr, cm^{-1}); 3221 (Sec NH), 2935 (C-H stretch), 2250 (Nitrile), 1710 (Ketone C=O), 1475 (C=C stretch); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.12 (s, 1H, NH), 8.39 (s, 1H, H-2), 8.16 (dd, $J_{4,5}$ = 7.5 Hz, $J_{4,6}$ = 1.0 Hz, 1H, H-4), 8.10 (s, 1H, H-α), 7.99 (d, $J_{2',3',\ 6',5'}$ = 9.0 Hz, 2H, H-2', H-6'), 7.52 (dd, $J_{7,6}$ = 7.5 Hz, $J_{7,\ 5}$ = 1.0 Hz, 1H, H-7), 7.26 (ovp. m, 2H, H-5, H-6), 6.86 (d, $J_{3',2'/5',6'}$ = 9.0 Hz, 2H, H-3', H-5'), 3.07 (s, 6H, CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 181.4, 155.5, 152.8, 136.7, 136.0, 133.5, 123.4, 122.1, 118.6, 117.2, 113.7, 112.5, 41.3; EI-MS *m/z* (% rel. abd.): 315 (M^+, 78), 288 (13), 184 (20), 144 (100), 116 (27), 89 (15); HR-EIMS Calcd for $C_{20}H_{17}N_3O$: 315.1372, Found 315.1367.

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