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Article

Mannich bases derivatives of 2-Phenyl-5-Benzimidazole sulfonic acid; Synthesis, Characterization, Computational studies and Biological evaluation

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A new series of N-Mannich bases of 2-Phenyl-5-benzimidazole sulfonic acid have been synthesized through amino methylation reaction with secondary amines. The two moieties were held together through a methylene bridge, which comes from formaldehyde (Formalin Solution 37%) used in the reaction. Chemical structures of the newly synthesized compounds have been confirmed using FT-IR, ^{1}HNMR and ^{13}CNMR. Different *in vitro* assays including Anti-oxidant, Enzyme inhibition, Anti-microbial and Cytotoxicity assay were performed to evaluate the biological potential with reference to the standard drug. Among the synthesized library, compound 3a shows maximum alpha-glucosidase inhibition with an IC_{50} value of 66.66 µg/ml, compound 3d was found most toxic with LC_{50} value of 10.17 µg/ml. ADME evaluation studies were performed with the help of Molinspiration online software. Docking calculations were also performed. Given the importance of the nucleus involved, the synthesized compound might find extensive medicinal applications as reported in the literature.

Keywords: Benzimidazole. Mannich bases. Antioxidant activity. Cytotoxicity activity. α-glucosidase activity. α-Amylase activity. Molecular Docking.

INTRODUCTION

Benzimidazole, a miracle pharmacophore which is the core structure of several drugs approved for clinical use including PPI,s (Proton pump inhibitors), Anticancer (Bendamustine), ARBS (Angiotensin receptor blockers), Antiemetics/Antipsychotics (Droperidol) and Anthelmintic drugs (Albendazole, Mebendazole). The importance of benzimidazole arises from the fact that it is a structural component of vitamin B12 and structural analogy to aminopurines make it a suitable candidate to act as an antimetabolite, therefore, serve as a ligand for a diverse array of receptors (Hodgkin *et al*., 1955). Considering the importance of benzimidazole scaffold we perform aminomethylation of benzimidazole derivative with different secondary amines having biological importance to synthesize novel Mannich bases which were further evaluated *in vitro* to determine the pharmacological potential. In addition, Mannich bases have several interesting biological activities due to the following properties. It enhances hydrophilicity and hence reduces the toxicity of the drug (Saab et al., 1990). It could act as a prodrug, deamination (Huttunen, Rautio, 2011) leads to the release of active drug. Mannich bases of ciprofloxacin are shown to be more effective than their parent compounds (Notz *et al*., 2003). Synthesis of Telavancin also uses aminomethylation to reduce the toxicity of the final compound (Bérdy, 2012). Mannich bases of sulfonamide and Tetracycline were found to be more effective and less toxic than their parent compounds (Joshi, Manikpuri, Tiwari, 2007). The aim of the current study was an appendage-oriented synthesis of small molecules capable of perturbing any disease-related biological pathway leading eventually to the identification of therapeutic protein targets. Owing to the Benzimidazole affinity for several receptors, joining the therapeutic potential of Mannich bases, synthesized

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ligands were expected to be pharmacologically active therefore after synthesis, compounds were screened by using different *in vitro* assays including Antimicrobial, Antioxidant, cytotoxicity and enzyme inhibition assays. Furthermore, docking calculations were also performed against the α-glucosidase enzyme.

MATERIAL AND METHODS:

Material

All Reagents used in this research were obtained commercially from Merck, Daejung and Sigma Aldrich. The melting point was determined using a Gallenkamp apparatus. The IR spectra of the synthesized compounds were taken on Perkin-Elmer (Model spectrum 65) spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded on an Agilent VNMR 400 (^{1}H NMR: 400MHz, ^{13}C (NMR: 101 MHz) or an Agilent VNMR 600 (^{1}H NMR: 600MHz, ^{13}C NMR: 151 MHz) spectrometer at 36°C with TMS (tetramethylsilane) as standard. The chemical shifts are given in parts per million (ppm) relative to the residual solvent peak of the nondeutrated solvent ($CHCl_3$: ^{1}H NMR: δ= 7.26ppm; ^{13}C NMR: δ= 77.00 ppm). A microplate reader was used to measure the absorbance for calculations in Antioxidant and enzyme inhibition assays. The completion of the reaction was checked by TLC obtained from Merck.

Experimental:

General procedure for aminomethylation reaction

The compounds were synthesized as per given scheme 1. Secondary amine and formaldehyde were added to 20ml of absolute ethanol with gentle stirring and heating. After 1 hour 2-Phenyl-5-Benzimidazole sulfonic acid was added to the reaction mixture and refluxed until the completion of the reaction, which was determined with the help of TLC using n-hexane: ethyl acetate: methanol in a ratio of 3:2:1. The final reaction mixture was filtered, and the filtrate was dried at room temperature to get the final product. FTIR, NMR was used to characterize synthesized compounds.

Antioxidant assay

Free Radical scavenging assay (FRSA)

Few modifications were made to the protocol reported by (Bibi et al., 2011). DPPH reagent was used to check the antioxidant potential of the compound with possible free radical scavenging ability. The samples were analyzed at 200, 66.66, 22.22, 7.40 µg/ml. Ascorbic acid and DMSO were used as positive control and negative control, respectively. Absorbance was noted at 517 nm by using a microplate reader. The procedure was repeated three times. To calculate % radical scavenging activity following formula was used.

$$\% \text{ radical scavenging activity} = (1\text{-}Ab_s/Ab_c) \times 100$$

Where
Ab_s = Absorbance of sample
Ab_c = Absorbance of negative control

Total antioxidant capacity determination (TAC)

To check the total antioxidant capacity of the synthesized library the procedure reported by (Fatima et al., 2015) was employed. With the help of a micropipette 100 µl of the sample was added to the Eppendorf tube. After that, 900 ml of TAC reagent consisting of (4 mM ammonium molybdate, sulphuric acid (0.6 M) and sodium phosphate (28 mM) was transferred to the Eppendorf tubes. The water bath was used for the incubation at 95^{0}C for 90 min. cool the reaction mixture at room temperature. Ascorbic acid and DMSO were used as positive and negative control respectively. A microplate reader was used to measure the absorbance at 630 nm. The assay was performed in triplicate.

Total reducing power determination (TRP)

The total reducing power of the compound was checked using the procedure reported by (Fatima et al., 2015). The test sample (100 µl) along with 400 µl of 0.2 Molar phosphate buffer (pH 6.6) and potassium ferric cyanide (1% w/v) was added to the Eppendorf tubes and incubated in a water bath at 50^{0}C for 20 min followed by the addition of 400 µl of trichloroacetic acid (10% w/v).

The mixture was centrifuged at 3000 rpm for 10 min. The supernatant (150 µl) of every mixture was transferred to their respective wells of a 96 well plate containing 50 µl of ferric cyanide solution (0.1% w/v). To measure the absorbance microplate reader was used at 630nm. For both positive and negative same procedure was followed as discussed earlier. The total reducing power (TRP) of the compounds was shown as µg AAE (ascorbic acid equivalent) per mg compound.

Antimicrobial assays

Antibacterial assay

In vitro antibacterial potential of each synthesized compound was evaluated by disc diffusion protocol narrated by (Zahra *et al*., 2017. The refreshed culture of bacterial strains with pre adjusted seeding density of 1 × 106 CFU/ml was used to make bacterial lawn on nutrient agar plates. An aliquot of each test extract (5 µl from 20 mg/ml DMSO) was impregnated on sterile filter paper discs while Cefixime and roxithromycin (5 µl from 4 mg/ml DMSO) were applied as positive control and DMSO (5 µl) as negative control. The discs were then placed on properly labeled seeded agar plates followed by 24 h incubation at 37°C and the zone of inhibition around each disc was measured by Vernier caliper. The assay was run in triplicate.

Antifungal assay

Antifungal assay was performed as previously illustrated by (Zahra *et al*., 2017). Petri plates having sterile sabouraud dextrose agar (20-25 ml) were swabbed with 100µl refreshed inoculum. The sterile filter paper discs loaded with test extracts (5 µl, 20 mg/ml DMSO), clotrimazole (5 µl, 4 mg/ml DMSO) and DMSO (5 µl) were placed on seeded SDA plates. The petri plates were then incubated for period 24-48 hours at 28-30°C and the zone of inhibition around each disc was measured by vernier caliper. The assay was run in triplicate.

Cytotoxicity assay

Brine shrimp lethality assay:

Brine shrimp lethality assay was performed according to the protocol narrated by (Ahmed *et al*., 2017. *Artemia salina* eggs were hatched in a specially designed bicompartmental perforated tank that was filled with simulated seawater. The compartment containing eggs was completely covered with aluminum foil while the other was lightened with a light source. The tank was incubated at 30-32ºC for 24-48 hrs. After a specified incubation period, the eggs were hatched and nauplii started moving towards the lightened compartment of the tank through small perforations. The hatched nauplii were then collected with Pasteur pipette and placed in a beaker containing seawater. Two-fold serial dilution of test extracts was made up to the final concentrations of 200, 100, 50 and 25 µg/ml. To each well, 10 mature nauplii were transferred and 150 µl of seawater was added. The corresponding volume of each test sample containing (not more than 1% DMSO in sea water) was added to the wells containing seawater and shrimp larvae. The seawater was used to make the final volume of each well up to 300 µl. Doxorubicin (10, 5, 2.5, 1.25 µg/ml) was used as positive control while 1% DMSO as negative control. After incubating the 96 well plate at 37°C for 24 hrs, the dead nauplii were counted by using an inverted microscope. The whole experiment was performed thrice and % mortality was calculated by following formula while LC50 was calculated by graph pad prism 5 software. The percent lethality of each extract was determined using the given formula:

% mortality = no. of dead shrimps/total no. of shrimps × 100

Enzyme inhibition potential

Protein kinase inhibition assay

Previously established and practiced protocol was used. *Streptomyces* 85E was used as a test strain (Waters et al., 2002). Refreshed culture of *Streptomyces* 85 E strain was prepared. 1ooµl of culture was added to the plates containing the ISP4 medium. Samples (5 µl) were poured on sterile filter paper disc (6 mm) and placed in plates properly labeled. Surfactin and DMSO loaded filter paper discs were used as positive and negative, respectively. After the incubation period of 48hours at $28C^0$ plates were examined for hyphae formation.

Indication for the inhibition of phosphorylation, spores and mycelia formation was the appearance of the clear and bald zone around the disc. Vernier caliper was used to measure ZOI to the nearest mm. Bald zones with diameter ≥12mm were considered significant. Clear zones show the cytotoxic potential of compounds and killing of the test strain while bald zones indicate the test sample inhibitory capacity for hyphae formation.

Alpha Amylase inhibition assay

The procedure reported by (Kim, kwon, Son, 2000) was used in this study with slight changes. The following reagents were added to the corresponding well of 96 well plates; Phosphate buffer (15 µl), alpha amylase the enzyme (25 µl), sample (10 µl) and starch (40 µl). Incubation was done for 30 min at $50C^{0.}$ At the end of the incubation period 1M HCl (20 µl) and 90 µl of iodine solution was added. Acarbose and DMSO act as positive and negative control, respectively. Blank contained buffer solution and starch instead of samples. Absorbance was measured using a microplate reader. The following formula was used to calculate percent inhibition.

Percent enzyme inhibition= $[(OD_x - ODy) \div (ODz \neg ODy)] \times 100$

Where
ODx = Absorbance value of the sample
ODy = Absorbance value of negative control
ODZ = Absorbance value of the blank
Alpha glucosidase inhibition assay

This assay was performed using the protocol reported by (Nair, Kavrekar, Mishra, 2013). Substrate solution (25 µl, 20 mM), phosphate buffer (69 µl, 50 Mm, pH 6.8) and test sample (5 µl, 4 mg/ml DMSO) were added to the respective wells of 96 well plate followed by the addition of enzyme solution (1µl) to the respective wells. A microplate reader (405nm) was used to take the initial readings. It was then incubated for at 37˚C for 30min. At the end of the incubation period, sodium bicarbonate solution (100 µl, 0.5 mM) was added to the wells of 96 well plate to stop the reaction and the final reading was taken at 405nm immediately.

% enzyme inhibition was determined by employing the formula as given below:
% inhibition = Ac-As/Ac × 100
Ac = Absorbance of control, As = Absorbance of sample

In negative control, the same amount of DMSO replaces the sample with the remaining procedure remains the same. Acarbose was added instead of test compounds in case of positive control and the whole assay was carried out in triplicate.

Pharmacokinetic studies

Molinspiration, an online free software was used to assess different pharmacokinetic parameters. It predicts oral bioavailability based on different parameters. The principle of this type of study involves the assessment of compounds for obeying Lipinski's rule of five. Consistent with the rule for newly design molecules to be orally bioavailable (Husain et al., 2016).

i) The compounds must have a molecular weight of less than 500.
ii) Log P (Octanol-water partition coefficient) <5.
iii) Hydrogen bond donor's ≤5.
iv) Hydrogen bond acceptors ≤10.
v) The number of rotatable bonds ≤10.
vi) The topological polar surface area ≤160 (Lipinski *et al*., 1997; Faizi *et al.,* 2017).

Compounds that are not complying with more than one of the above-mentioned rules will lead to problems in oral bioavailability (Faizi et al., 2017). The percentage of absorption (% Abs) can be calculated using topological polar surface area (TPSA) by the formula, % Abs=109−[0.345×TPSA] (Husain et al., 2016)

Docking Studies

Docking calculations were performed using pyRx 0.8 version. Ligand and protein preparation was done with the help of Autodock tools and Discovery studio visualizer.

RESULTS

Chemistry

Mannich reaction is a single pot nucleophilic substitution in which a compound containing an active hydrogen atom, in our study (2-Phenyl-5-Benzimidazole sulfonic acid) undergoes reaction with formalin (37%) and secondary amines (Piperazine, Piperidine, Morpholine, Diphenylamine and Dipropyl amine as shown in Figure 1. Absolute ethanol was used as a solvent while a few drops of conc. HCl was used to speed up the reaction. The final product is an amino methylated compound. Synthesized compounds were soluble in polar organic solvents like methanol, ethanol, chloroform, ethyl acetate, DMF and DMSO. The purity of the compounds was checked with TLC by using n-hexane, ethyl acetate, and methanol in the ratio of 3:2:1 respectively and detected with the help of UV light. FTIR results as given below for compounds 3a,3b,3c,3d,3e respectively was used for the structure elucidation.

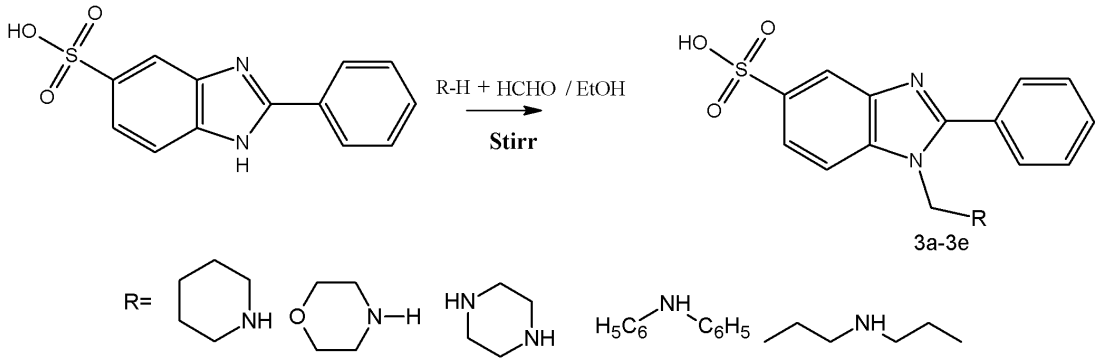

FIGURE 1 - General Reaction for the synthesis of Mannich bases 3a-3e.

compound 3a: 2-phenyl-1-(piperazin-1-ylmethyl)-1H-benzo[d]imidazole-5-sulfonic acid

Yield 78% MP 220−225C° IR (cm^{-1}); 3475 (OH), 3006 (Ar, C−H), 2850 (C−H), 1619 (S=O), 1409 (C=N) ^{1}H NMR (600 MHz, $CDCl_3$) **δ** 9.73 (s, 1H), 7.26 (s, 5H), 7.29 – 7.21 (m, 1H), 6.96 (s, 2H), 6.52 (d, *J* = 8.5 Hz, 1H), 4.29 (s, 2H), 3.71 – 3.64 (m, 1H), 2.95 (s, 1H), 2.72 (t, *J* = 7.5 Hz, 3H) ^{13}C NMR (151 MHz, Chloroform-*d*) **δ** 171.03, 67.02, 60.30, 55.00, 53.38, 20.95, 14.12.

compound 3b: 2-phenyl-1-(piperidin-1-ylmethyl)-1H-benzo[d]imidazole-5-sulfonic acid

Yield 67% MP 210−215 C° IR (cm^{-1}); 3474 (OH), 3006(Ar, C−H), 2850 (C−H), 1619 (S=O), 1409 (C=N) ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.05 (dq, *J* = 9.9, 8.2, 7.8 Hz, 1H), 7.02 – 6.91 (m, 2H), 6.93 – 6.80 (m, 1H), 6.63 (dtd, *J* = 10.4, 7.3, 1.1 Hz, 1H), 4.89 – 4.78 (m, 1H), 3.71 (dt, *J* = 7.8, 4.6 Hz, 5H), 2.81 – 2.74 (m, 3H), 2.50 (q, *J* = 6.2, 4.5 Hz, 4H), 2.00 – 1.90 (m, 3H) ^{13}C NMR (151 MHz, $CDCl_3$) δ 145.46, 144.92, 130.67, 129.59, 129.20, 129.17, 126.87, 123.17, 112.64, 77.25, 77.03, 76.82, 66.84, 66.76, 50.79, 27.89, 22.40.

compound 3c: 1-(morpholinomethyl)-2-phenyl-1H-benzo[d]imidazole-5-sulfonic acid

Yield 56% MP 205−210 C° IR (cm^{-1}); 3475 (OH), 3006 (Ar, C−H), 2850 (C−H), 1619 (S=O), 1409 (C=N) ^{1}H NMR (600 MHz,) δ 7.98 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.63 – 7.56 (m, 1H), 7.52 (dd, *J* = 8.3, 7.0 Hz, 2H), 3.65 (t, *J* = 4.7 Hz, 6H), 3.07 (s, 3H) ^{13}C NMR (151 MHz, Chloroform-*d*) δ 143.46, 133.02, 129.23, 127.63, 106.55, 81.61, 68.02, 66.96, 51.96, 46.40, 46.13.

compound 3d: 1-((dipropylamino)methyl)-2-phenyl-1H-benzo[d]imidazole-5-sulfonic acid

Yield 56% MP 205−210 C° IR (cm^{-1}); 3475 (OH), 3006 (Ar, C−H), 2850 (C−H), 1619 (S=O), 1409 (C=N) ^{1}H

NMR (600 MHz, $CDCl_3$) **δ** 8.01 (s, 1H), 7.93 (td, *J* = 8.6, 1.3 Hz, 1H), 7.63 – 7.47 (m, 6H), 6.76 (s, 6H), 3.14 – 3.07 (m, 2H), 3.11 (s, 5H), 1.99 (s, 0H) ^{13}C NMR (151 MHz, Chloroform-*d*) **δ** 143.51, 133.04, 132.70, 129.25, 129.16, 128.54, 127.66, 46.16, 45.69, 45.25

compound 3e: 1-((diphenylamino)methyl)-2-phenyl-1H-benzo[d]imidazole-5-sulfonic acid

Yield 82% MP 70−75 C° IR (cm^{-1}); 3391 (OH), 3151 (Ar, C−H), 2807 (C−H), 1630 (S=O), 1463 (C=N) ^1H NMR (600 MHz, $CDCl_3$) **δ** 7.01 (ddd, *J* = 28.5, 15.7, 8.2 Hz, 1H), 6.90 – 6.79 (m, 1H), 4.96 – 4.85 (m, 1H), 4.87 – 4.76 (m, 1H), 4.29 – 4.21 (m, 1H), 3.85 – 3.74 (m, 2H), 3.70 (q, *J* = 5.0 Hz, 4H), 3.42 (dtd, *J* = 17.1, 9.4, 7.9, 4.8 Hz, 1H), 3.33 (dp, *J* = 17.0, 5.9, 5.4 Hz, 1H), 2.85 – 2.63 (m, 5H), 2.53 (s, 1H), 2.49 (dd, *J* = 14.7, 9.9 Hz, 1H), 1.94 (ddq, *J* = 16.4, 10.6, 5.4, 4.8 Hz, 2H) ^{13}C NMR (151 MHz, $CDCl_3$) **δ** 143.83, 130.89, 129.23, 129.04, 128.94, 128.83, 127.02, 126.87, 122.63, 117.79, 117.55, 116.58, 112.43, 112.28, 112.17, 112.10, 110.70, 77.26, 76.84, 75.78.

Anti-oxidant Potential

DPPH free radical scavenging assay

Free radical scavenging potential of compounds was evaluated by monitoring the discoloration of the stable purple color of 2, 2-diphenyl-1-picrylhydrazyl free radical to a yellow colored 2, 2-diphenyl-1-picrylhydrazyl molecule. The highest % scavenging was shown by 3a with 39% scavenging followed by 21%, 18%, 15%, and 11% by 3c, 3d, 3e, 3b respectively as shown in Figure 2.

Total antioxidant capacity

The formation of the green-colored phosphomolybdenum complex was used for the evaluation of the Total Antioxidant Capacity of test samples. The antioxidant capacity was determined in µg equivalent of ascorbic acid per mg of compounds (µg AAE/mg compound). Significant Antioxidant capacity among synthesized compounds was exhibited by the Mannich base with Diphenylamine 3d i.e. 91± 0.16 µg AAE/mg. It was preceded by 3a, 3e, 3b, 3c i.e. 55± 0.24, 51± 0.22, 31± 0.31, 26 ± 0.18, respectively.

Total Reducing Power

The reducing power was figured out in synthetic compounds in terms of ascorbic acid equivalent per mg compound (µg AAE/mg). The calculated results showed minor reducing power of synthesized compounds. The reducing power of 3a, 3b, 3c, 3d, 3e 5.4, 5.12, 4.23, 2.90, 4.03 µg AAE/mg as shown in Figure 2.

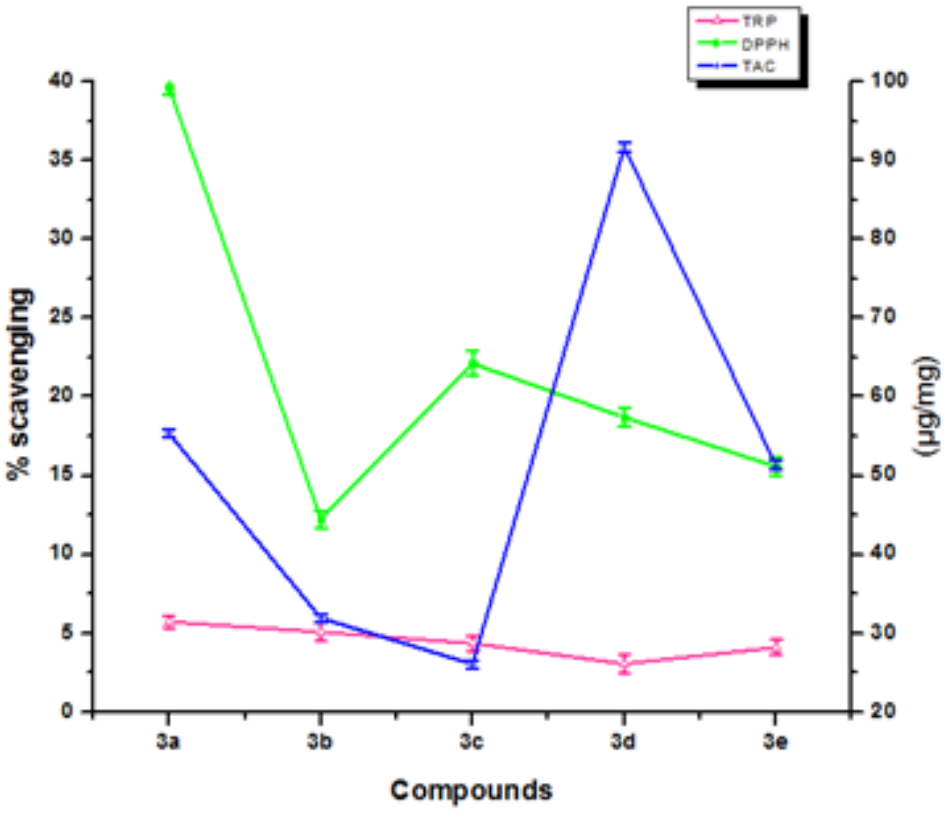

FIGURE 2 - Graphical description of DPPH scavenging potential, TRP, TAC of synthesized compounds. Values given are expressed as mean of triplicate ± standard deviation.

Enzyme inhibition assays

α-Amylase inhibition assay

α-Amylase Inhibition assay was used to evaluate the antidiabetic activity of synthesized compounds. Mannich bases with Piperidine showed the highest 31% inhibition of alpha amylase. It was followed by 3e,3c, 3d, 3a i.e. 28%, 26%, 24%, 15% inhibition as shown in Figure 3.

α-Glucosidase inhibition assay

α-glucosidase inhibition assay was used to further confirm the antidiabetic activity of synthesized compounds. Mannich bases with Piperazine showed the highest alpha-glucosidase activity with an IC_{50} value of 66.66µg/ml. It was preceded by 3b, 3d, 3e, 3c i.e. 41%, 38 %, 36%, 22% inhibition respectively as given in Figure 3.

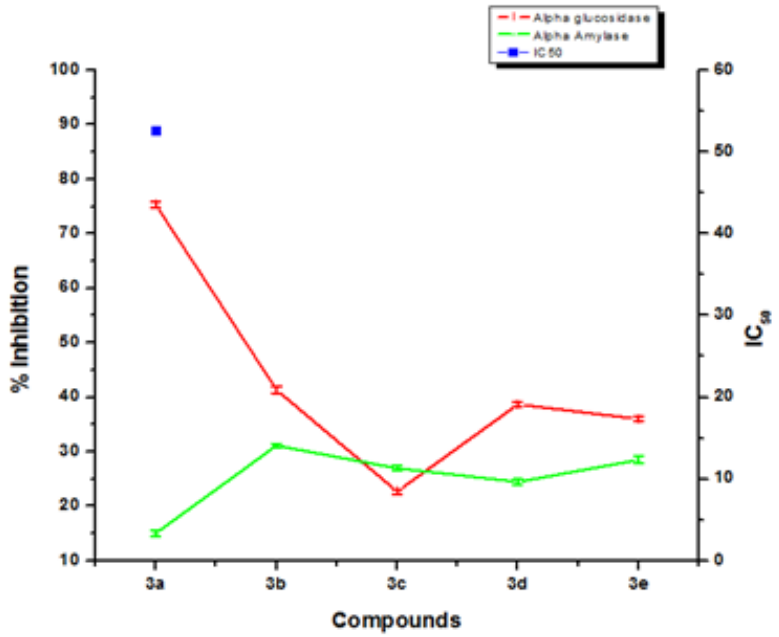

FIGURE 3 - Graphical description of α-glucosidase and α-amylase inhibition potential of synthesized compounds. Values given are expressed as mean of triplicate ± standard deviation.

Protein kinase inhibition assay

Evaluations of all the Mannich bases were done for the protein kinase inhibition potential. Compounds 3a, 3b, 3c, 3d, 3e showed minor inhibition of hyphae formation at given concentration i.e. 100 µg/disc with a bald zone of inhibitions 8 mm, 10 mm, 10 mm, 9 mm, 9 mm while no clear zone was found. The nontoxic effect of DMSO (negative control) was confirmed by the absence of ZOI.

Antimicrobial activity of synthesized compounds

Antibacterial and Antifungal evaluation of the compound was done. The disc diffusion method was used for antibacterial and antifungal evaluation five synthesized Mannich bases as mentioned in section 2. Five different strains of bacteria were used including *Staphylococcus aureus*, *Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli.* For anti-fungal evaluation following strains were used *Mucor sp, F.solani, A.niger, A.Flavus, A.fumigatus.* But none of the compounds were found active against any strain.

Cytotoxicity potential (Brine shrimp lethality assay)

Artemia salina nauplii were used for the preliminary assessment of cytotoxicity. Significant activity was shown by all the compounds as given in Table I.

TABLE I - Brine shrimp lethality potential of synthesized compounds

Samples	% Mortality (concentration: µg/ml)				LC_{50} µg/ml
	200	100	50	25	
3a	100	80	70	40	17.64
3b	90	70	60	30	51.47
3c	80	60	50	30	74.32
3d	100	90	70	40	10.17
3e	80	60	40	20	93.57

Computational Pharmacokinetics Studies (Dry Lab Section)

All the synthesized compounds were found in compliance with the Lipinski's rule of five as shown in Table II. Therefore, all the synthesized compounds can be used as orally active agents.

TABLE II - Simulated pharmacokinetic properties

Compound	% Abs	TPSA^2(A^0)	n-rotb	MW	MV	miLog Po/w	n-OHNH	n-ON	N violation
Rule				<500		<5	<5	<10	≤1
3a	78.82	87.46	4	372	318.	-0.11	2	7	0
3b	82	75.43	4	371	322.	1.51	1	6	0
3c	79	84.67	4	373	315	0.44	1	7	0
3d	82	75.43	6	455	392	3.99	1	6	0
3e	82	75.43	8	387	350	2.35	1	6	0

Docking Studies

Homology modeling:

In this study alpha-glucosidase from *Saccharomyces cerevisiae* (baker's yeast) was used for the *in-vitro* assay of compounds to determine the antidiabetic potential of synthesized compounds. Until today the X-ray crystallographic structure of Saccharomyces alpha-glucosidase is not determined. A 3D structure of S. cerevisiae alpha-glucosidase was modeled by using the Swiss model. Uniprot was used to get the sequence of the alpha-glucosidase in FASTA format. A series of protein structures were given to be used as a template. Among them, 3D structure of isomaltase from *S.cervisiae* (PDB 3AJ7) was selected as a template sequence (72.6% similarity). Ramachandran plot was used to validate constructed model as shown in Figure 4. Rampage was used for analysis, which shows the following results.

Number of residues in favored region (~98.0% expected): 561 (97.6%)
Number of residues in allowed region (~2.0% expected): 14 (2.4%)
Number of residues in outlier region: 0 (0.0%)

Blind docking was performed with the help of Auto dock vina. Blind docking is a terminology used when the whole enzyme is selected rather than specific receptor sites. Acarbose was posed against the enzyme and its binding mode and type of interaction were examined. The docking results of compounds showed that they bind to the same site as that of standard drug

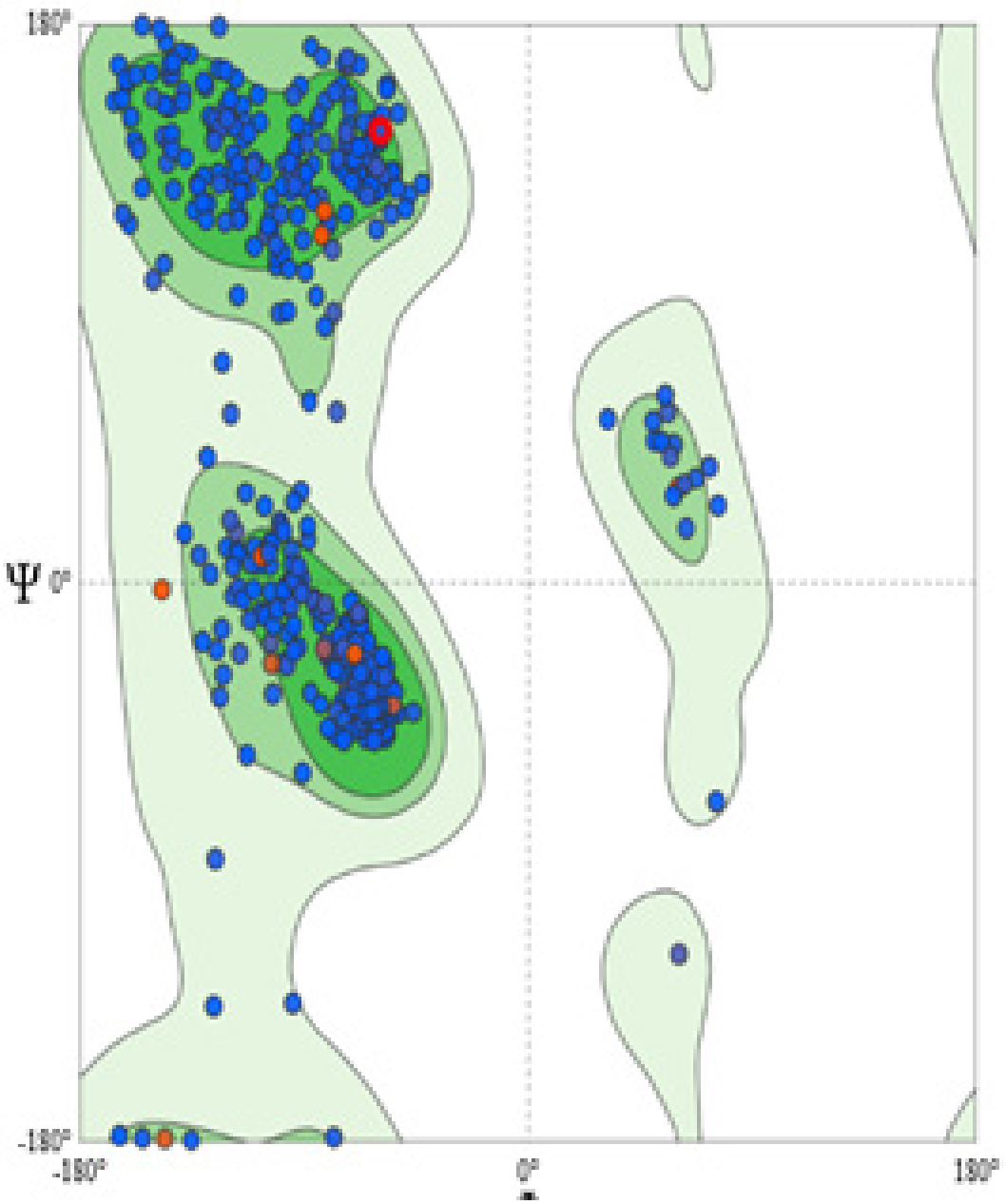

FIGURE 4 - Ramachandran plot generated by Swiss model for homology modelled α-glucosidase.

Docking

Compound 3a, which was found most active against alpha-glucosidase was forming two hydrogen bonds. The nitrogen atom of piperazine was forming a hydrogen bond with ASN 241 while the Oxygen atom of sulfonic acid formed a hydrogen bond with ARG 439. Pi-Pi-T-shaped interaction was also observed between the aromatic ring and PHE 157. Figure 5 showing these interactions as explained above.

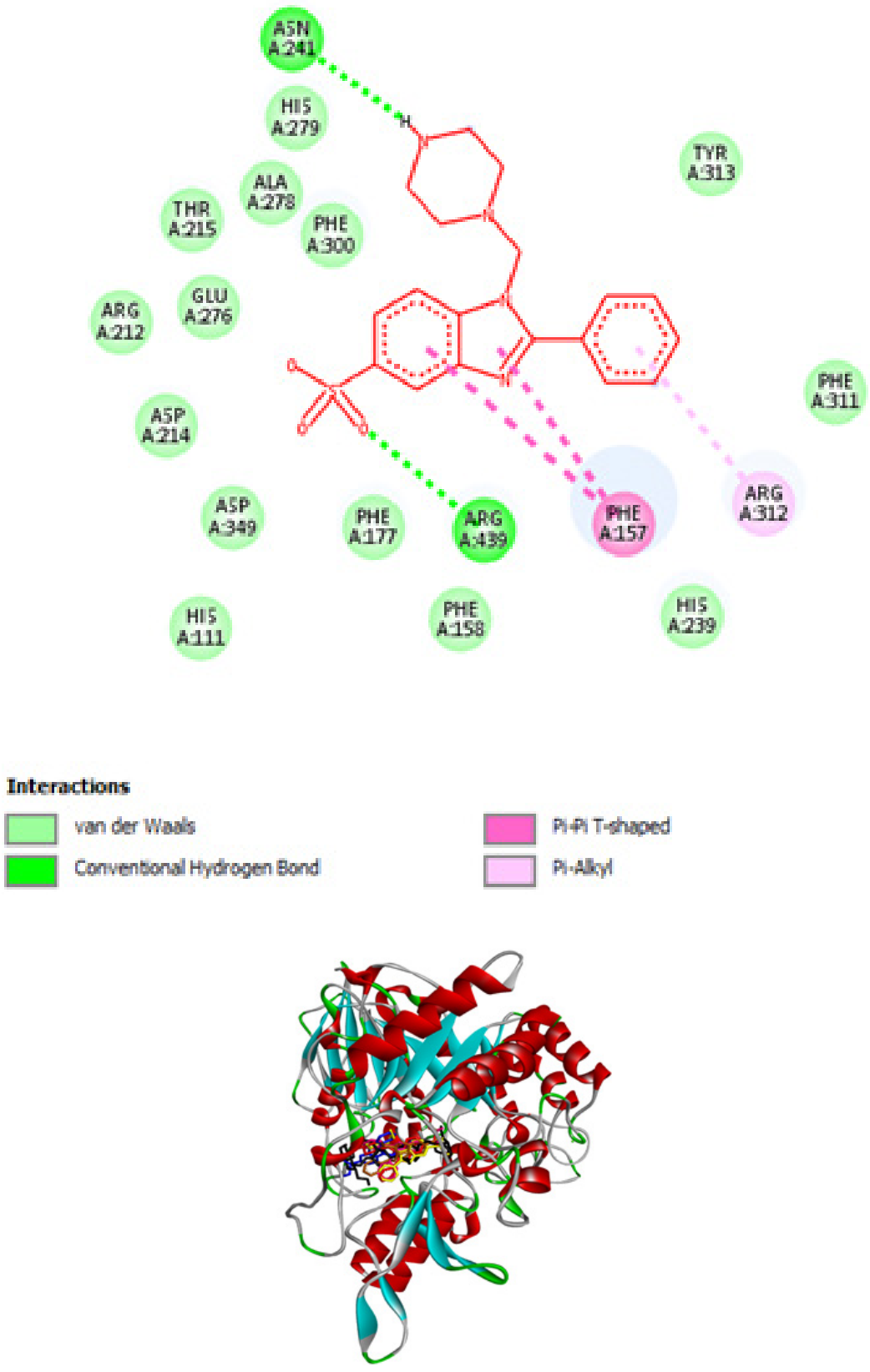

FIGURE 5 - (a) 2d representation of interaction between most active ligand and amino acid residues (b) superposed model of all the ligands 3a (red), 3b (pink), 3c (blue), 3d (brown), 3e (yellow) with Acarbose (black).

DISCUSSION

A new series of Mannich bases were synthesized with 2-Phenyl-5-Benzimidazole Sulfonic acid by clubbing formaldehyde and different secondary amines such as Piperazine, Piperidine, morpholine, Dipropylamine, Diphenylamine. Benzimidazole gained considerable interest in the past decades, especially in the field of medicinal chemistry, as Benzimidazole ring system is a key element in biologically active compounds. Five mannich bases were synthesized with Piperazine, Piperidine, Morpholine, Diphenylamine, Dipropyl amine. They were simply prepared by reflux condensation of 2-Phenyl-5-Benzimidazole Sulfonic acid with secondary amine. The procedure followed from the article mentioned in section 2 (Hamama et al., 2011). Compounds were purified and recrystallized from ethanol. Docking is used to predict the interaction of the ligand with a target. Docking studies help us suggesting that the ligand molecule can become a new drug candidate in the future or not (Cho et al., 2005). Interactions are in form of hydrogen bonds, vander wall forces etc. (Ayyappan et al., 2015). The structures of all five compounds were docked with α-glucosidase enzyme by using autodock vina. Autodock vina program was used through PyRx (Dallakyan, Olson, 2015). Results were positive showing good binding affinity as compared to an appropriate standard drug. Acarbose was used as a standard drug for α-glucosidase inhibition. The ligand order of affinity was found against α-glucosidase compound II > compound IV > compound I > compound V > compound III > Ascorbic acid. Data from computational studies show that synthesized Mannich bases are pharmacologically active. In the end, different *in vitro* assays were performed for the assessment of biological activities, which is an important part of research work to know how much the compounds are active practically. It gives information about toxicity and paved the way for further research work to be carried out on compounds to completely explore the pharmacological potential of compounds.

Antimicrobial Activity

Previous studies have shown that the mannich bases have increased antimicrobial activities and reduced toxicity. The mannich bases of sulfonamides and Tetracycline have increased activity and reduced toxicity than their parent compounds (Joshi, Manikpuri, Tiwari, 2007). Considering the importance of Mannich bases and benzimidazole we were expecting the synthesized compounds to be active against different microbial strains. Therefore we subjected synthesized compounds to different antibacterial and antifungal activities against five different bacterial strains including *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Escherichia coli* and five fungal strains including *Mucor sp, F.solani, A.niger, A.Flavus, A.fumigatus*. But the result reveals that the newly synthesized compounds have no significant activity against any of the strains as compared to standard cefixime and Roxithromycin.

Antioxidant Assay

Different diseases like cancer, hypertension, diabetes, ischemia, and asthma are caused by the disturbance in the balance between oxidant and Antioxidant and this imbalance is known as oxidative stress. Available synthetic anti-oxidants like butylated hydroxyanisole and butylated hydroxytoluene are associated with asthma, cancer risk and behavioral effects in children. Therefore, there is a need for synthetic antioxidants with reduced toxicity. We were expecting newly synthesized Mannich bases that might act as antioxidants as shown by previous studies. A single antioxidant activity could not fully reveal the antioxidant profile, therefore, three different *in vitro* assays, testing the antioxidants potential with three different mechanisms were employed to get the antioxidant profile of synthesized Mannich bases. A test assay, which employs phosphomolybdenum was utilized to explore the antioxidant potential of synthesized compounds. Compound 3a among all synthesized compounds showed the highest antioxidant activity. Another *in vitro* assays performed to determine the antioxidant potential of synthesized compounds include TAC and TRP. All compounds showed average to excellent antioxidant potential. Compound 3d showed significant Total Antioxidant with a value of 91.54 ± 0.16 µg AAE/mg. while in the case of Total reducing

power none of the compounds was found with significant activity. The highest value obtained was 5.7 µg AAE/mg of compound 3a.

Cytotoxicity Potential

It is thought Brine shrimp larvae behave like mammalian carcinoma cells and the cytotoxic effects of tested samples may depict their potential anticancer and antitumor activities (Shahwar et al., 2012). Previously benzimidazole based Anticancer drugs have been approved for clinical use for example Bendamustine while several other benzimidazole derivatives had been reported with significant anticancer activities. All the compounds were subjected to brine shrimp lethality assay to know about the possible cytotoxicity. Compound 3d was found most cytotoxic with LC_{50} value of 10.17 µg/ml, which is followed by 3a, 3b, 3c, 3e with LC_{50} value of 17.64 µg/ml, 51.47 µg/ml, 74.32 µg/ml, 93.57 µg/ml, respectively.

Enzyme Inhibition Potential

Previously benzimidazole derivatives had been synthesized and tested against α-amylase and α-glucosidase inhibition potential, which showed that Benzimidazole can act as potent inhibitors of these enzymes. 2-Aryl benzimidazole derivatives have been synthesized, which exhibit significant α-amylase inhibition (Adegboye et al., 2018). During the current assay, α-amylase inhibition assay of all four compounds was evaluated. None of the compounds showed significant activity. Compound 3d showed maximum activity with 31% ± 0.38 inhibition. In the case of the α-glucosidase enzyme compound, 3a showed maximum activity with an IC_{50} value of 66.66 µg/ml. In the case of protein kinase inhibition assay, no significant activity was found.

CONCLUSION

In this study, Mannich base derivatives of 2-Phenyl-5-Benzimidazole sulfonic acid were successfully synthesized and characterized. The structure of these bases was subjected to *in silico* studies for binding with α-glucosidase enzyme from *Saccharomyces cerevisae*, which manifest good results. The synthesized were screened for biological potential against different enzymes. Compound 3a was found with moderate inhibiting activity against the α-glucosidase enzyme. Antimicrobial assay reveals that none of the compounds is active against strains of bacteria and fungi used in this study.

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CONFLICT OF INTEREST

No conflict of interest among authors.

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