Classical and variant approaches to synthesis of N-Mannich bases of phenyl hydroxyl ketones, their characterization, urease inhibition and antioxidant activities

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Abstract: In continuation of our synthesis and investigation of bioactivities of Mannich bases, synthesized N - Mannich bases were evaluated for their urease inhibition and antioxidant properties. Characterization was achieved by Ultraviolet/visible (UV), Infrared (IR), Nuclear magnetic resonance (^{1}HNMR) and Mass spectrophotometries. Elemental analysis, optical rotation (OR) and circular dichroism (CD) measurement also confirmed and provided information about the structure of these compounds. Synthesis of novel 2-hydroxy-1,2,3-triphenyl-3-(phenylamino) propan-1-one, 2-hydroxy-1,2-diphenyl-3-(phenylamino) propan-1-one, 2-hydroxy-3-(2-hydroxyethylamino)-1,2,3-triphenylpropan-1-one and 2-hydroxy-1,2,3-triphenyl-3-(p-tolylamino)propan-1-one are newly reported along with 3-(2-(3,4-dimethoxyphenyl)ethylideneamino)-3-(4-hydroxyphenyl)-1-(2,4,6-trimethoxy phenyl) propan-1-one prepared by a variant approach involving use of tertiary amine. Jack bean Urease was used for Urease inhibition assay while *in vitro* antioxidant screening of the compounds was carried out using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). IC_{50} values obtained showed that the compounds showed significant activities as urease inhibitors and antioxidants when compared with Thiourea, Ascorbic acid and Butylated hydroxyanisole (BHA) used as standards in the two assays. Kinetics of inhibition of *H. pylori* urease by 1-Phenylaminomethyl-naphthalen-2-ol (GK1) and 2-(3-Phenylaminopropionyloxy)-benzoic acid (GK5) indicated that enzyme activity decreased as concentration of test compound increased. The IC_{50} value of 14.5 ± 0.71 mM (GK1) and 14.3± 0.32 (GK5) indicated better activity when compared to thiourea (21.6 ± 0.12) used as standard.

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Introduction

The etiology of many diseases has been traced down to oxidation reactions while secretion of urease by *Helicobacter pylori* has been the cause several infections like gastric and urinary tract syndromes. Therefore, control of oxidation reactions and enzymes that may damage cells will go a long way in ameliorating a number of disease conditions. Our group has been involved in the synthesis of novel Mannich bases and investigation of their biological activities with the aim of discovering and developing novel intervention agents (Oloyede et al., 2011; Oloyede et al., 2014a-c; Oloyede et al., 2015). There exist various organic synthetic reactions to produce target compounds. Mannich reaction is one of such organic reaction and is an amino alkylation of an acidic proton placed next to a carbonyl functional group by formaldehyde and a primary or secondary amine or ammonia. The final product is a β-amino-carbonyl compound also known as Mannich base (Hayashi, et al., 2003; Horibe et al., 2008; Hatano et al., 2010). Classical Mannich reaction involves mainly the use of aldehyde, a primary or secondary amine and an enolisable carbonyl compound as CH-acidic substrate with a general impression that tertiary amine could not or may not react so easily in this type of reaction because the mechanism of the Mannich reaction starts with the formation of an iminium ion from the amine and the formaldehyde (Tanaka et al., 2008; Guo and Zhao, 2013; Pullar, et al., 2013). The compound with the carbonyl functional group tautomerizes to the enol form, and attack the iminium ion. It is an example of nucleophilic addition of an amine to a carbonyl group followed by dehydration to the Schiff base. The Schiff base is an electrophile which reacts with a compound containing an acidic proton (which is, or had become an enol) in the second step in the electrophilic addition. Mannich reaction is also a condensation reaction (Thomas, et al., 1990; Xu, et al., 2004; Selva, 2013). But modern variants to this reaction mechanism have been hypothesized (Michael et al., 1998; Weng, 2014; Leonte, et al., 2015).

In addition to this, structural activity relationship has shown that many Mannich bases have bioactivity as cytostatics anti- cancer, anti-HIV, anti - Parkinson, antitumour, antimicrobial, immunological response modifier, analgesic, anticonvulsant amongst others (Ali and Sharharyar, 2007, Shivananda et al., 2011; Kumar, 2013; Vijey, 2013). Many have also been reported to have antioxidant activities (Oloyede et al., 2014 b and c; Oloyede et al., 2015). Many novel drugs which are able to combat oxidation have emanated through search for novel antioxidants. Another active area of research in pharmacology and biochemistry is the discovery and improvement of enzyme inhibitors. Natural enzyme inhibitors can be poisons and can be used as defenses against predators. The potency (the concentration needed to inhibit the enzyme) and specificity (lack of binding to other proteins) determines a medicinal enzyme inhibitor. This is so because potency and a high specificity ensure that a drug will have low toxicity and little side effects. Many enzyme inhibitors occur naturally and are involved in the regulation of metabolism. Proteins (protease and nucleases) are cellular enzyme inhibitors that specifically bind to and inhibit an enzyme target. This helps in the control of enzymes that may damage a cell (Jung et al., 1995; Wittekindt et al., 2010; Zahid et al, 2014).

The aim of this research therefore is to synthesize and characterize new Mannich bases by both classical and variant approaches as well as to investigate their antioxidant and urease inhibition activities.

Materials And Method

Chemicals and Reagents

The following BDH chemicals and reagents: formaldehyde, chloroform, dichloromethane, ethyl acetate, n-hexane, methanol, hydrochloric acid, ammonia solution, conc. tetraoxosulphate (VI) acid, conc. hydrochloric acid, ammonia solution, sodium potassium tartarate, potassium chloride, glacial acetic acid, benzaldehyde, aniline, chloroform, methanol, ethanol, p-toluidine crystals, benzoin, ethanolamine, hexane, carbontetrachloride, and diethylether were used. Those that were general purpose chemicals were distilled prior to use. Dimethylsulphoxide (M & B, England), and silica gel 30 - 260 microns (Merck, Germany) and 2, 2 - diphenyl-1-picrylhydrazyl (DPPH), and butylatedhydroxylanisole (BHA) were obtained from Sigma Chemical Co (St Louis, MO). urease (Jack bean), were purchased from Sigma-Aldrich, Germany. P-nitro-phenyl-α-D-glucopyranoside (p-NPG), sodium carbonate (Na_2CO_3), sodium dihydrogen phosphate and di-sodium hydrogen phosphate were purchased from Merck, Germany.

General experimental procedures

Typical reflux set up involving a condenser and a Heidolph MR3004 Heater with magnetic stirrer was used. All weighing were done on Analytical balance KERN ALS 220-4 KERN & SOHN, Germany. All reactions were monitored with Thin layer chromatograpy (TLC) at room temperature. Analysis of synthesized compounds (0.2 mg of sample in 5 mL methanol) was carried out using Precoated TLC Aluminium sheets Silica gel 60 F_{254} (20 cm × 20 cm, 0.2 mm thick; Merck, Germany). Silica gel (230–400 mesh) was used for column chromatography (CC). Visualization of the TLC plates was carried out under UV at 254 and 366 nm and by spraying with vanillin sulphuric acid and ceric sulfate reagent solution with heating. The melting points of all crystalline solids were determined by a Buchi M-560 melting point apparatus and are uncorrected. The UV/Visible absorption of 0.01% w/v of the samples was obtained in methanol on Evoltion 300 Thermo Scientific UV – visible spectrophotometer. The Infra red (IR) spectra were recorded on FT-IR-8900 Fourier Transform IR Spectrophotometer Shimadzu IR spectrometer. Optical rotations were recorded on JASCO P-2000 polarimeter, Polatronic D Schhmidt and Haensch, Germany at 589 nm, using 1 mL solution in a cell of path length 10 mm. Tolerance level was ±0.007. JASCO J-810 Spectropolarimeter was used for Circular dichroism (CD) measurement with the following conditions concentration 0.5 mg/mL, cell path length: 0.5 mm, buffer concentration: 3 mM metal ions used as stabilizer was kept to minimum. Both ID and 2D Nuclear magnetic Resonance (NMR) ^1H was recorded on AVANCE AV-400 spectrometer operating at 400 MHz for ^1H, respectively. The chemical shift values (δ) are reported in ppm and the coupling constants (J) are in Hz. The EIMS, HREIMS were recorded on JEOL MS 600H-1 with a data system showing mass to charge (m/z). Elemental analysis was carried out using Perkin Elmer Series11 CHNS/O Analyser 2400.

Preparation of Mannich Bases

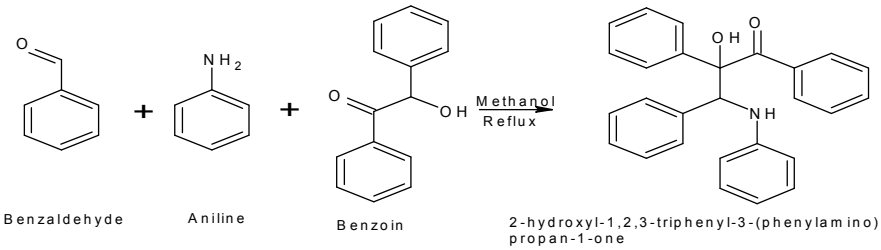
All preparations had as starting materials non-toxic medicinally active compounds. The preparation of the Mannich bases GK1-GK9 (1-Phenylaminomethyl-naphthalen-2-ol (GK1), (2-{ [2 – hydroxy ethyl) amino] methyl}phenyl) phenyl peroxyanhydride (GK2), 1-Phenyl-3-(phenylamino) propan-1-one (GK3), Phenyl (2-[phenyl amino) methyl] phenyl) peroxyanhydride (GK4), 2-(3-Phenylaminopropionyloxy)-benzoic acid (GK5), 3-Phenylamino-1-(2, 4, 6-trimethoxy-phenyl)-propan-1-one (GK6), 4-(3-oxo-1,3- diphenylpropylamino) benzoic acid (GK7), 3-(p-tolylamino)-N-phenylpropanamide (GK8) and N-phenyl-3-(phenylamino) propanamide (GK9) had been previously reported by Oloyede et al., 2014a-c;

Oloyede et al., 2015). The preparation of Mannich bases: 2-hydroxy-1,2,3-triphenyl-3-(phenylamino) propan-1-one (GK10), 2-hydroxy-1,2-diphenyl-3-(phenylamino) propan-1-one (GK11), 2-hydroxy-3-(2-hydroxyethylamino)-1,2,3-triphenylpropan-1-one (GK12) and 2-hydroxy-1,2,3-triphenyl-3-(p-tolylamino)propan-1-one (GK13) are hereby described. Benzoin was the lead compound in all preparations and was based on procedure used for the synthesis of Mannich bases previously described in literature but with little modification (Muthumani et al, 2010; Kumar et al., 2013; Oloyede et al., 2015; Annapurma and Jalapothi, 2016). Many variant Mannich synthesis involving the use of a tertiary amine were employed but only GK14 (3-(2-(3,4-dimethoxyphenyl)ethylideneamino)-3-(4-hydroxyphenyl)-1-(2,4,6-trimethoxyphenyl)propan-1-one) was obtained when 3,4-dimethoxyphenyl acetonitrile was used as the lead compound.

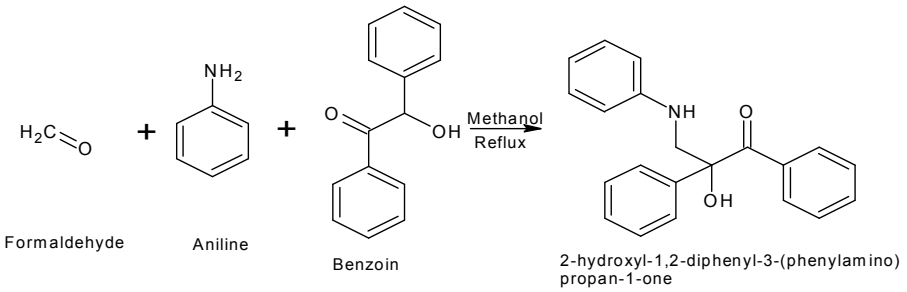
General Procedure for Preparation (GK 10 - GK14)

Equimolar mixture of reagents was used. Benzoin (0.05 M, 2.65 g in 10 mL methanol) was the lead reagent, benzaldehyde and aniline (GK10), formaldehyde and aniline (GK11), benzaldehyde and ethanolamine (GK12), benzaldehyde and p-toluidine (GK13) with the addition of 0.5 mL NaOH in 30 mL of methanol were mixed together and refluxed for 20 min at room temperature. The reaction mixture was later refluxed at 64.7^{0}C in a basic medium using a paraffin bath on magnetic stirrer with temperature control for 5-6 hrs. GK14 was prepared by refluxing 4-hydoxybenzaldehyde (0.05M), 0.05M 1-(2,4,6-trimethoxyphenyl) ethanone in 30 mL of methanol for 30 min at room temperature, 0.05M of 3,4-dimethoxyphenyl acetonitrile containing 0.5 mL of NaOH in 30 mL of methanol was added to the mixture and refluxed for 8hrs at 68^{0}C. The solution was cooled for 24hrs at 0^{0}C and crystals formed were filtered under pressure using a suction pump, washed and recrystallized with warm ethanol. All reactions were monitored using Thin Layer Chromatography (TLC).

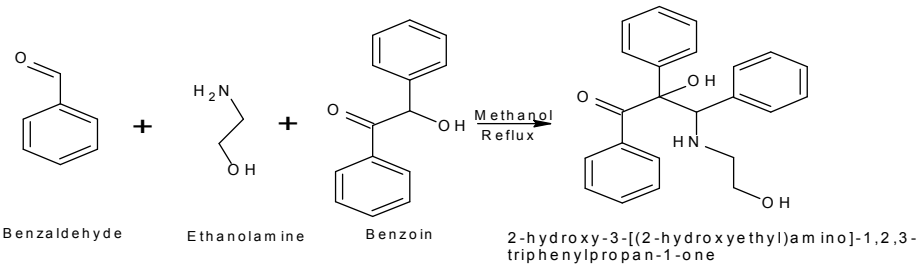
The equation for the reaction is as follows:



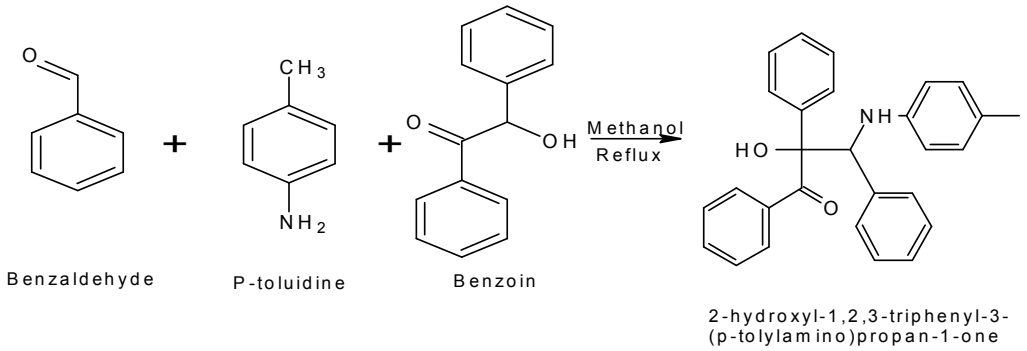
Scheme 1: Preparation of 2-hydroxy-1,2,3-triphenyl-3-(phenylamino)propan-1-one (GK10)



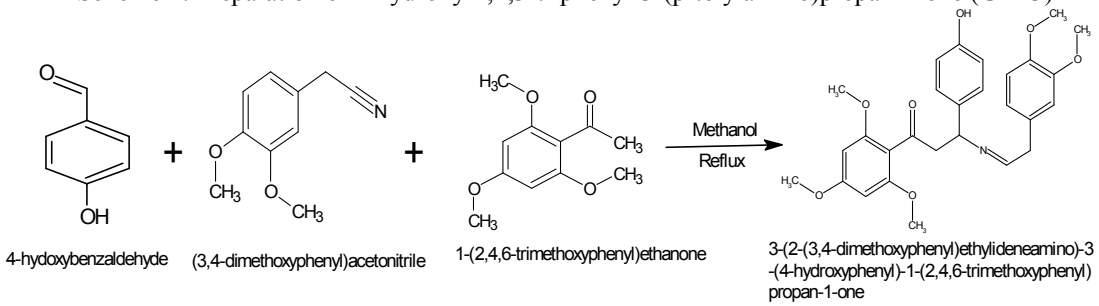
Scheme 2: Preparation of 2-hydroxy-1,2-diphenyl-3- (phenylamino)propan-1-one (GK11)



Scheme 3: Preparation of 2-hydroxy-3-[(2-hydroxyethylamino)]-1,2,3-triphenylpropan-1-one (GK12)



Scheme 4: Preparation of 2-hydroxy-1,2,3-triphenyl-3-(p-tolylamino)propan-1-one (GK13)



Scheme 5: Preparation of 3-(2-(3,4-dimethoxyphenyl)ethylideneamino)-3-(4-hydroxyphenyl)-1-(2,4,6-trimethoxyphenyl)propan-1-one (GK14)

Urease inhibition assay

The indophenol method as described by Ferheen et al., 2009 and Wittekindt et al., 2010 was used. This involved determination of urease activity by measuring ammonia production. Synthesized compounds were prepared in different concentrations, ranging from 25-500 µg. 5 µL (1 mM concentration) of each prepared solution was mixed with 25 µL Jack bean Urease enzyme solution. The mixture was incubated at 30 ^{o}C. Aliquots were taken after 15 minutes and transferred immediately to assay mixtures containing 100 mM urea in 55 µL of buffer and again incubated for 30 min in 96 well plates. After which 45 µL each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µL of alkali reagent (0.5% w/v NaOH and 0.1 % active chloride NaOCl) were added to each well. Increase in absorbance at 630 nm was measured after 50 min, using a microplate reader (Spectramax plus 384 Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 µL. The results (change in absorbance per min) were processed by using SoftMax Pro software (Molecular Device, USA). All the assays were performed at pH 8.2 (0.01 M $K_2HPO_4.3H_2O$, 1 mM EDTA and 0.01 M $LiCl_2$). Thiourea was used as the standard inhibitor of urease. The percent inhibition was computed according to: % Inhibition = Test activity of control - test activity of sample x 100/Test activity of control. Median inhibitory concentrations were determined by plotting percent inhibition vs the logarithms of final toxicant concentrations. The concentration giving 50% inhibition (ICs_0) was derived from least squares linear regression (Ferheen et al., 2009).

Antioxidant activity using 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH)

DPPH (2,2-diphenyl-1-picryl-hydrazyl) method was used. A solution of 0.3 mM DPPH was prepared in methanol and various concentrations of each compound (62.5 µg - 500 µg) was prepared. Each compound (5 µL) was mixed with 95 µl of DPPH solution. The mixture was dispersed in 96 well plates and incubated at 37° C for 30 min. Absorbance measurement was carried out at 515 nm and percent radical scavenging activity was determined in comparison with control. BHA was used as standard. Percentage inhibition and Inhibition concentration at 50% (IC_{50}) were calculated (Oloyede, et al, 2015 and 2017).

Statistical Analysis

All experimental data were analyzed statistically by one-way analysis of variance, using SPSS software package (version 12.0; SPSS, Inc., Chicago, Illinois, USA). Absorbance measurements were done in triplicate and results are expressed in terms of mean ± standard deviation (Values of $P \leq 0.05$ were considered to be significant). Percentage Inhibition was calculated as % Inhibition = $\{(A_{DPPH} - A_S)/A_{DPPH}\} \times 100$ where A_{DPPH} = Absorbance of DPPH and A_S = absorbance of sample and IC_{50} values were calculated using

GraphPad Prism 5 version 5.01 (Graph pad software, Inc., La Jolla, CA, USA.) software and EZ-Fit™ Enzyme Inhibition Analysis by Perrella Scientific Inc. USA was used to calculate enzyme kinetics for the most significant urease inhibitor.

Results

Full spectroscopic data confirming GK10-GK14 are hereby presented while additional information on the structures of GK1-GK9 is also presented. However, previous data regarding GK1-GK9 can be obtained in Oloyede et al, 14 a-c and Oloyede et al, 2015. We also present the results of urease inhibition and antioxidant properties of all the synthesized compounds.

1-Phenylaminomethyl-naphthalen-2-ol (GK1): $[\alpha]_D^{25}$ (MeOH):0.000 CD curve $[\theta]_{nm}$, mdeg 219 (max) +0.362071. ^{1}H NMR (400 MHz, DMSO-d6, δ ppm), 4.74 (s, 2H,-CH_2-), 6.63 (s, 1H, NH -*Ar*), 6.82-7.33 (s, 5H, CH–*Ar*), 7.51-8.05 (m, 6H, CH-Naphthalene), 10.10(OH- *Ar*).

(2-{ [2 – hydroxy ethyl) amino] methyl}phenyl) phenyl peroxyanhydride (GK2): $[\alpha]_D^{25}$ (MeOH) -0.0112; CD curve $[\theta]_{nm}$, mdeg: 225 (max) +5.4641, 242 (min) -1.907021. ^{1}H NMR (400 MHz, DMSO-d6, δ ppm), 2.71 (m, 2H, CH_2), 3.48 (m, 2H, CH_2), 3.78 (d, 2H, CH_2), 4.75 (s, 1H, OH-alcohol), 2.13 (NH - amine) 7.19-8.23 (m, 9H, CH-*Ar*).

1-Phenyl-3-(phenylamino) propan-1-one (GK3): $[\alpha]_D^{25}$ (MeOH) 0.000, CD curve $[\theta]_{nm}$, mdeg: 215 (max) +0.890145. ^{1}H NMR (400 MHz, DMSO-d6, δ ppm), 3.23 (dd, 2H, -CH_2-), 3.38 (m, 2H, -CH_2-), 5.85 (m, 1H, NH-*Ar*), 6.55-7.34 (s, 5H, CH-*Ar*), 7.55-7.99 (d, 5H, CH-*Ar*).

Phenyl (2-[phenyl amino) methyl] phenyl) peroxyanhydride (GK4): $[\alpha]_D^{25}$ (MeOH) 0.000 CD curve $[\theta]_{nm}$, mdeg 221 (max), +0.740805. ^{1}H NMR (400 MHz, DMSO-d6, δ ppm): 4.36 (dd, 2H, N-CH_2-), 6.79 (t, 1H, NH-*Ar*), 6.53-6.85 (d, 5H, CH-*Ar*), 7.23-7.78 (s, 4H, CH-*Ar*), 7.88-8.25 (d, 5H, CH-*Ar*).

2-(3-Phenylaminopropionyloxy)-benzoic acid (GK5): $[\alpha]_D^{25}$ (MeOH) -0.00786; CD curve $[\theta]_{nm}$, mdeg: 316 (max) +6.38303, 292 (min) +0.640780, 268 (max) +1.630026, 240 (min) +0.236263 ^{1}H NMR (400 MHz, DMSO-d6, δ ppm), 2.58 (t, 2H, -CH_2-), 3.40 (m, 2H, -CH_2-), 5.79 (t, 1H, NH), 6.59-7.26 (s, 5H, CH-*Ar*), 7.78-8.21 (s, 4H, CH-*Ar*), 12.06 (OH-carboxylic).

3-Phenylamino-1-(2, 4, 6-trimethoxy-phenyl)-propan-1-one (GK6): $[\alpha]_D^{25}$ (MeOH) +0.00165; CD curve $[\theta]_{nm}$, mdeg: 304 (max) +5.74518, 274 (min) -2.28328, 244 (max) +11.062, 222 (min) +4.33861. ^{1}H NMR (400 MHz, DMSO-d6, δ ppm), 3.19 (t, 2H, -CH_2-), 3.34 (dd, 2H, N-CH_2-), 3.85 (s, 9H, 3x-CH_3), 5.78 (s,1H, NH), 6.19 (s, 2H, CH-*Ar*), 6.61-7.25 (s,5H, CH-*Ar*).

4-(3-oxo-1,3- diphenylpropylamino) benzoic acid (GK7): $[\alpha]_D^{25}$ (MeOH) -0.02236; CD curve $[\theta]_{nm}$, mdeg: 288 (max) +5.97447, 254 (min) +1.44154, 226 (max) +5.34426. ^{1}H NMR (400 MHz, DMSO-d6, δ ppm), 3.19 (t, 2H, -CH_2-), 4.02 (s, 1H, NH), 4.41 (m, 1H, CH-*tert*), 6.80-6. 87 (s, 4H, CH-*Ar*) 7.29-7.45 (s,5H, CH-*Ar*) 7.59-7.98 (s,5H, CH-*Ar*) 12.89 (s,1H, OH-*carboxylic*).

3-(p-tolylamino)-N-phenylpropanamide (GK8): $[\alpha]_D^{25}$ (MeOH) -0.0138; CD curve $[\theta]_{nm}$, mdeg: 256 (max) +8.93437, 232 (min) +4.12008, 215 (max) +11.56030. ^{1}H NMR (400 MHz, DMSO-d6, δ ppm), 2.29 (m 3H, CH_3), 2.68 (t, 2H, -CH_2-), 3.60 (m, 2H, -CH_2-), 5.89 (t, 1H, NH-*Ar*), 6.49-7.05 (s, 4H, CH-*Ar*), 7.18-7.67 (s,5H, CH-*Ar*), 10.09 (s,1H, NH-*sec* amide).

N-phenyl-3-(phenylamino) propanamide (GK9): $[\alpha]_D^{25}$ (MeOH) -0.00936; CD curve $[\theta]_{nm}$, mdeg: 270 (min) -10.5286, 216 (max) +7.57111. ^{1}H NMR (400 MHz, DMSO-d6, δ ppm): 2.68 (t, 2H, -CH_2-), 3.58 (m, 2H, -CH_2-), 5.89 (t, 1H, NH-*Ar*), 6.59-7.26 (s, 5H, CH-*Ar*), 7.18-7.67 (s, 5H, CH-*Ar*), 10.09 (s, 1H, NH-*sec* amide).

2-hydroxy-1, 2, 3-triphenyl-3-(phenylamino) propan-1-one (GK10): colourless crystals; yield: 20% (on dry weight basis); $[\alpha]_D^{25}$ (MeOH) -0.0643; CD curve $[\theta]_{nm}$, mdeg: 236 (max) +12.3842, 226 (min) +11.2057. M.pt: 131-133^{o}C. R*f* 0.3 (Silica gel F254, hexane: chloroform, 1:3). UV nm (EtOH, λmax nm): 258.00 (0.1), 310.00 (3.4), 335.00 (2.5), 329.00 (4.7). IR (KBr) υ cm^{-1}: 3403.07 (O-H stretch), 3064.42 (N-H stretch), 2929.97 (C-H aliphatic stretch), 1675.38 (C=O stretch), 1587.52 (C=C Aromatic), 1490.90 (C-H aliphatic bend), 1389.47 (C-N amine). ^{1}H NMR (400 MHz, DMSO-d6, δ ppm), 3. 98 (d, 1H, NH-*Ar*), 4. 68 (s, 1H, -CH-), 6.98 (s, 1H, OH-Alcohol), 6.89-7.26 (s, 5H, CH-*Ar*), 7.28-7.41 (s, 5H, CH-*Ar*), 7.36-7.38 (s, 5H, CH-*Ar*), 7.55-7.98 (s, 5H, CH-*Ar*). EI-MS [M+] = 393.170, Molecular weight (calc) measured for $C_{27}H_{23}NO_2$: 393. 477. Analysis (%) Found: C (81.97%), H (5.91%), N (3.52%), calculated: C (82.42%), H (5.89%), N (3.56%). Soluble in methanol, ethanol, chloroform, acetone, diethyl ether, ethylacetate, sparingly soluble in hexane, n-butanol, tetrahydrofuran, dimethylsulphoxide 1,4-dioxan. Insoluble in water, hydrochloric acid, sulphuric acid and carbon tetrachloride.

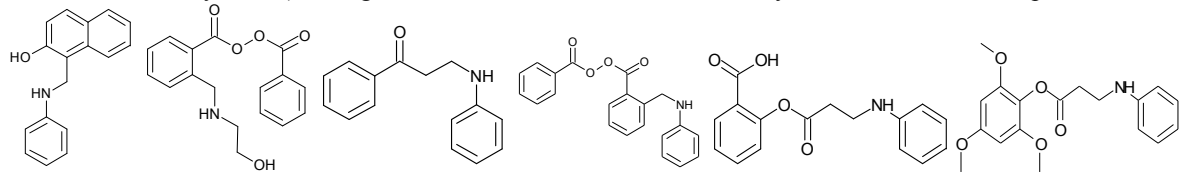
2-hydroxy-1,2-diphenyl-3-(phenylamino) propan-1-one (GK11): colourless crystals; yield: 23.1% (on dry weight basis); $[\alpha]_D^{25}$ (MeOH) -0.0118; CD curve $[\theta]_{nm}$, mdeg: 226 (max) +14.8963, 210 (min) +9.94334. M.pt: 133-135^{o}C. R*f* 0.32 (Silica gel F_{254}, Hexane: Chloroform, 1:3). UV (EtOH, λmax nm): 319.00 (2.9), 343.00 (2.9), 299.00 (3.0), 259.00 (3.2).

IR (KBr) υ cm^{-1}: 3402.40 (O-H stretch), 3058.82 (N-H stretch), 2924.36 (C-H aliphatic stretch), 1674.70 (C=O stretch), 1586.83 (C=C Aromatic), 1488.11 (C-H aliphatic bend), 1389.33 (C-N amine). ^{1}H NMR (400 MHz, DMSO-d6, δ ppm), 3. 78 (s, 2H, -CH_2-), 6.90 (s, 1H, OH-Alcohol), 8.01 (s, NH-*Ar*), 6.59-7.26 (s, 5H, CH-*Ar*), 7.36-7.38 (s, 5H, CH-*Ar*), 7.55-7.98 (s, 5H, CH-*Ar*). EI-MS [M+] = 317.141, Molecular weight (calc) measured for $C_{21}H_{19}NO_2$: 317. 381. Analysis (%) Found: C (78.99%), H (6.34%), N (4.72%) calculated = C (79.47%), H (6.03%), N (4.41%). Soluble in methanol, ethanol, chloroform, ethylacetate, acetone, sparingly soluble in 1,4-dioxan, diethylether. Insoluble in water, hydrochloric acid, sulphuric acid, carbon tetrachloride, acetonitrile, dimethyl sulphoxide, tetrahydrofuran, dimethyl formamide, n- butanol, and 2- propanol.

2-hydroxy-3-(2-hydroxyethylamino)-1, 2, 3-triphenylpropan-1-one (GK12) colourless crystals; yield: 60.8% (on dry weight basis); $[\alpha]_D^{25}$ (MeOH) -0.0274; CD curve $[\theta]_{nm}$, mdeg: 214 (max) +0.102063, 211 (min) +0.01030. M.pt: 132.5^{o}C 133-135^{o}C. R*f* 0.28 (Silica gel F_{254}, Hexane: Chloroform, 1:3). UV (EtOH, λmax nm): 202.00 (0.097), 224.00 (0.427), 233.00 (0.292), 243.00 (0.244), 249.00 (0.079), 255.00 (0.058), 267.00 (0.657). IR (KBr) υ cm^{-1}: 3382 (O-H stretch), 3058.00 (N-H stretch 2^o amine)), 1674(C=O stretch), 1586 (C=C stretch), 2925(C-H aliphatic stretch). ^{1}H NMR (400 MHz, DMSO-d6, δ ppm), 2.12 (m, 1H, NH-amine), 2.78 (m, 2H, -CH_2-), 3.68 (m, 2H, -CH_2-), 4.78 (s, 1H, OH-alcohol), 4. 89 (s, 1H, -CH-*tert.*), 6. 96 (s, 1H, OH-alcohol), 7.29-7.42 (s, 5H, CH-*Ar*), 7.36 -7.38 (s, 5H, CH-*Ar*), 7.55-7.98 (s, 5H, CH-*Ar*). EI-MS [M^+]= 361.167 Molecular weight (calc) measured for $C_{23}H_{23}NO_3$: 361.433. Analysis (%) Found: C (75.94%) H (6.39%) N (3.81%), calculated: C (76.43%), H (6.41%) N (3.88%). Soluble in methanol, ethanol, chloroform, ethylacetate, acetone, sparingly soluble in 1,4-dioxan, diethylether, insoluble in water, diethyl ether, hexane, carbon tetrachloride, hydrochloric acid, sulphuric acid, acetonitrile, dimethyl sulphoxide, tetrahydrofuran, dimethyl formamide, n- butanol, and 2- propanol.

2-hydroxy-1,2,3-triphenyl-3-(p-tolylamino)propan-1-one (GK13): colourless crystals; yield: 57% (on dry weight basis); $[\alpha]_D^{25}$ (MeOH) -0.00413; CD curve $[\theta]_{nm}$, mdeg: 291 (max) 0.1081, 266 (max) -6.12415, 222 (max) +24.6133. M.pt: 133.8-134.5^{o}C. R*f* 0.22 (Silica gel F_{254}, hexane: chloroform, 1:3). UV nm (EtOH, λmax nm): 194.00 (0.675), 202.00 (5.596), 207.00 (6.632), 218.00 (0.164), 225.00 (1.829), 228.00 (0.754), 238.00 (6.899), 249.00 (6.730), 262.00 (1.954), 266.00 (0.862). IR (KBr) υ cm^{-1}: 3382 (O-H stretch), 3058.00 (N-H stretch 2^o amine), 1674 (C=O stretch), 1586 (C=C stretch), 2924(C-H stretch). ^{1}H NMR (400 MHz, DMSO-d6, δ ppm), 2.30 (s, 3H, CH_3), 3. 98 (d, 1H, NH-*Ar*), 4. 68 (s, 1H, -CH- *tert*), 6.98 (s, 1H, OH-Alcohol), 6.49-7.06 (s, 4H, CH-*Ar*), 7.28-7.41 (s, 5H, CH-*Ar*), 7.36-7.38 (s, 5H, CH-*Ar*), 7.55-7.98 (s, 5H, CH-*Ar*). EI-MS [M^+] = 407.188 Molecular weight (calc) for $C_{28}H_{25}NO_2$: 407.500. Analysis (%) Found: C (82.42%), H (6.23%), N (3.47%). calculated = C (82.53%), H (6.18%), N (3.44%).Soluble in methanol, ethanol, chloroform, acetone, diethyl ether, ethylacetate, sparingly soluble in hexane, n-butanol, tetrahydrofuran, dimethylsulphoxide 1,4-dioxan, insoluble in water, diethyl ether, hexane, carbon tetrachloride, hydrochloric acid and sulphuric acid.

3-(2-(3,4-dimethoxyphenyl)ethylideneamino)-3-(4-hydroxyphenyl)-1-(2,4,6-trimethoxy phenyl) propan-1-one (GK14): brownish-black crystals; yield: 77% (on dry weight basis); $[\alpha]_D^{25}$ (MeOH) -0.0138; CD curve $[\theta]_{nm}$, mdeg: 300 (min) -4.35599, 282 (max) +9.46374, 262 (min) -2.86746, 242 (max) +9.37244, 220 (min) -3.4884. M.pt: 187.5- 188.1^{o}C R*f* 0.6 (Silica gel F_{254}, hexane: EtOAC, 1:4). UV nm (EtOH, λmax nm): 207.00 (0.714), 222.00 (0.762), 283.00 (0.900). IR (KBr) υ cm^{-1}: 3166.0 (O-H stretch), 2880.2 (-C-H stretch CH_3), 2841.2 (-C-H stretch CH2), 2749.6 - 2494.5 (=C-H Aromatic), 20161.1 (C=N), 1672.9 (C=O stretch), 1598.7 - 1453.9 (C=C aromatic stretch), 1285.1 - 1121.7 (C-O stretch), 976.7 - 602.0 (=C-H bend), ^{1}H NMR (400 MHz, DMSO-d6, δ ppm): 3.81 (s, 15H, 5 x CH_3, methoxy), 2.57 (s, 2H, -CH_2-, methylene), 3.12 (d, 2H, -CH_2-, methylene), 3. 28 (t, 1H, -CH-, *tert*), 6.24-6.68 (s, 4H, 4x CH-*Ar*), 6.71-6.86 (s, 3H, 3x CH-*Ar*), 6.94-7.16 (s, 2H, 2x CH-*Ar*), 7.81 (dd, 1H, CH-*aldimine*), 9.51 (s, 1H, OH-Alcohol). EI-MS [M^+]= 493. 102; (calc) for $C_{28}H_{31}NO_7$: 493.548. Analysis (%) Found: C (68.01%), H (5.97%), N (2.94%), calculated: C (68.14%) H (6.33%), N (2.84%). Soluble in methanol, ethanol, chloroform, acetone, diethyl ether, ethylacetate, sparingly soluble in hexane, n-butanol, tetrahydrofuran, dimethylsulphoxide 1,4-dioxan, insoluble in water, diethyl ether, hexane, carbon tetrachloride, hydrochloric acid and sulphuric acid.



GK1 GK2 GK3 GK4 GK5 GK6

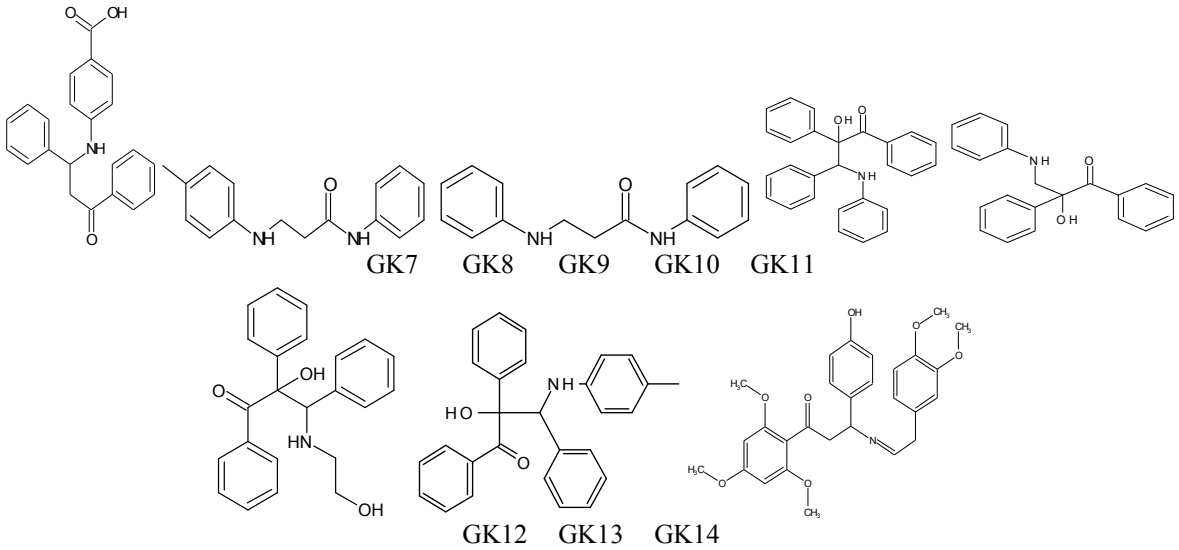


Figure 1: Structures of Synthesized compounds GK1-GK14

Table 1: IC_{50} (µM) values of compounds in the Antioxidant and Urease inhibitory assays

S/N	Compound	Antioxidant IC_{50} (µM)	Urease Inhibition IC_{50} (µM)
1.	GK1	87.5 ± 0.45	14.5± 0.66
2.	GK2	28.6 ± 0.43	Nil
3.	GK3	56.3 ± 0.21	17.9 ± 0.45
4.	GK4	17.7 ± 0.36	18.0 ± 0.83
5.	GK5	> 200	14.3± 0.32
6.	GK6	71.4 ± 0.19	22.2 ± 0.51
7.	GK7	57.4 ± 0.36	Nil
8.	GK8	> 200	> 200
9.	GK9	Nil	15.5 ± 0.31
10.	GK10	Nil	38.7 ± 0.49
11.	GK11	Nil	43.5 ± 0.75
12.	GK12	> 200	20.3 ± 0.41
13.	GK13	> 200	18.5 ± 0.65
14.	GK14	15.7 ± 0.22	98.3
15.	BHA	44.2 ± 0.09	-
16.	Thiourea	-	21.6 ± 0.12

* IC_{50} (µM); inhibition at 50 % concentration for all compounds in the antioxidant and urease inhibition assays.

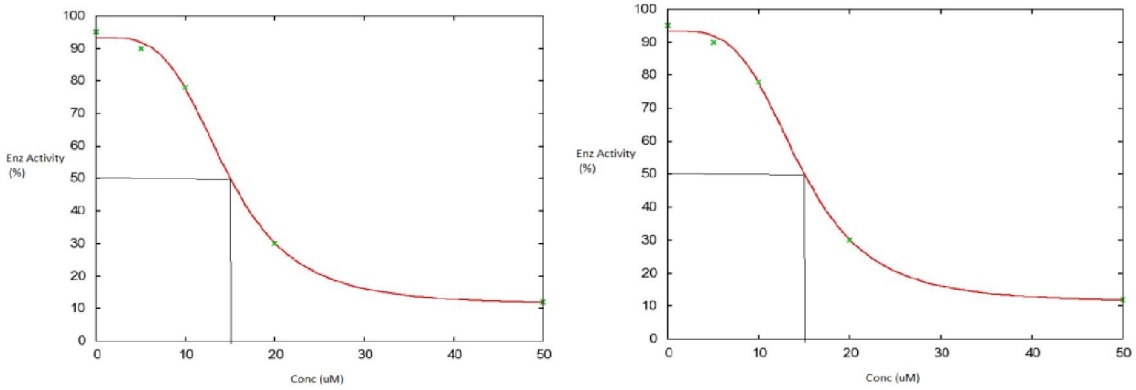
Table 2: Percentage Inhibition in the antioxidant screening using DPPH (GK10-GK14)*

Conc. *(mg/mL)*	GK10	GK11	GK12	GK13	GK14	Ascorbic acid	BHA
1.0	23.53	23.53	17.88	19.46	91.62	91.71	90.88
0.50	18.91	17.44	20.67	17.55	92.76	92.55	91.34
0.25	17.02	16.18	22.35	19.55	95.29	92.36	91.49
0.125	14.08	15.34	25.70	20.86	96.24	93.60	91.65
0.0625	12.82	13.45	36.69	35.10	98.12	94.57	92.18

*Antioxidant activity showing percentage inhibition of Mannich bases when compared with standards: ascorbic and butylated hydroxylanisole (BHA) at 517 nm. Absorbance of DPPH at 517 nm = 0.983.

Inhibition at 50 % concentration (IC_{50} (µM)) for all compounds (Table 1) in the antioxidant inhibition assays gave results indicating that GK 14 (15.7 ± 0.22), GK4 (17.7 ± 0.36), GK2 (28.6 ± 0.43) showed better activity as free radical scavengers in the DPPH assay when activity was compared to BHA (44.2 ± 0.09). IC_{50} (µM) of 15.7 ± 0.22 for GK 14 and 98.12% (Percentage inhibition) at 0.0625 mg/mL (Table 2) also confirmed the antioxidant potential of GK14 as a free radical scavenger. This inhibition percent 98.12% was better than that of ascorbic acid (94.57%) and BHA (92.18%) at the lowest concentration at 0.0625 mg/mL.

GK1 (14.5 ± 0.71 mM), GK5 (14.3± 0.32), GK3 (17.9 ± 0.45), GK4 (18.0 ± 0.83), GK9 (15.5 ± 0.31), GK12 (20.3 ± 0.41) and GK13 (18.5 ± 0.65) showed good activity in the urease inhibition assay when activity was compared to thiourea with IC_{50} (µM) of 21.6 ± 0.12. For all these compounds, as the concentration of test compound is increased, enzyme activity decreased (Table 1 and Figure 2).



GK1 **GK5**

Figure 2: Kinetics of inhibition of *H. pylori* urease by GK1 and GK5

Enzyme activity decreased as concentration of test compound increased (Figure 2). The linear function for this relation is a good-enough approximation (□2 = 0.857, 0.854) for GK1 and GK5 respectively. The obtained IC_{50} value was 14.5 ± 0.71 mM (GK1) and 14.3± 0.32 (GK5) where the IC_{50} indicated the test compound concentration descend the activity of urease to 50%. These values are lower than that of thiourea (21.6 ± 0.12) used as standard indicating better activity. Approximately for both compunds, At V_{max} 32.4808 ± 32.23 (99.21%), K_M has a value of -3.25874 ±2.145 (65.84%), Minimum 11.1447 ± 2.891 (25.94%) and Maximum 93.3558 ±2.037, IC_{50} 14.5926 ±0.7195 (4.931%), and Hill coeff. of 3.80253 ± 0.4779 (12.57%). GK3 (17.9 ± 0.45), GK4 (18.0 ± 0.83), GK9 (15.5 ± 0.31), GK12 (20.3 ± 0.41) and GK13 (18.5 ± 0.65) also showed good activity in the urease inhibition assay when compared to thiourea.

Discussion

The newly synthesized bases 2-hydroxyl-1,2-3triphenyl-3-(phenylamino) propan-1-one (GK10) and 2-hydroxyl-1,2-diphenyl-3(phenylamino)propan-1-one (GK11), 2-hydroxy-3-(2-hydroxyethylamino)-1,2,3-triphenylpropan-1-one (GK12), 2-hydroxy-1,2,3-triphenyl-3-(p-tolylamino) propan-1-one (GK13) and 3-(2-(3,4-dimethoxyphenyl)ethylideneamino)-3-(4-hydroxyphenyl)-1-(2,4,6-trimethoxy phenyl) propan-1-one (GK14) obtained as pure crystals were soluble in polar organic solvents. The IR data confirmed the presence of functional groups with absorption peaks (Vmax) in the range of at 3166.0 - 3403.07 cm^{-1} assigned to O-H stretching frequency, 3058.00 - 3064.42 cm^{-1} assigned to N-H stretch, 2924.36 - 2929.97 cm^{-1} was due to aliphatic C-H stretching frequency, 1674.70 - 1675.38 cm^{-1} was due to C=O stretching frequency, aromatic C=C stretch was observed at 1488.11 - 1587.52 cm^{-1} was assigned to C-H bending frequency and the functional group C-N was seen in the range of 1389.47cm^{-1}. Bands at 3300-3500 typical of N-H stretch of Mannich bases (primary amines have two bands; secondary have one band, often very weak) is missing in GK14. However, band at 20161.1 cm^{-1} confirmed the presence of C=N. The bands from UV-Visible absorption spectra observed at wavelength above 202 - 287 revealed that the compounds are highly conjugated and suggests the presence of π- π* and n- π* transitions respectively. Proton NMR of the compounds (^{1}H NMR) generally

showed signals at around δ (ppm) 2.12-3.98 due to aromatic N-H characterisctic of Mannich bases, 4. 68 was signal due to methine -CH-, the OH signal was observed as a singlet in the region 6.98 while the aromatic CH was observed at 6.89-7.41 depending on the chromophore. However, GK 14 displayed distinct signals at δ (ppm) 3.81 assignable to the 15 hydrogen singlet of the five methoxy moiety and aromatic CH signals at 6.24-7.16. The aldimine CH signal was observed at δ (ppm) 7.81 while singlet observed at 9.51 was the OH functional group.

Circular dichroism (CD) gives information about the secondary structure of compounds indicating the presence of one or more chiral chromophores (light-absorbing groups) and is the difference in the absorption of left-handed circularly polarised light (L-CPL) and right-handed circularly polarised light (R-CPL) and is measured as a graph of wavelength (nm) against CD (mdeg). GK14 has more chromophores than all the other compounds, followed by GK6, 5, 7, 8, 13, 9, 11, 10, 2, 12 in that order. GK1, 3 and 4 showed weak light absorbing properties. Optical rotation (OR) measures difference in symmetry and non superimposability on the compound's mirror image (chirality). The compounds were laevorotatory except GK6 which was dextrorotatory, while GK1, 3 and 4 were not optically active.

Compounds containing reactive functional (electrophilic) groups such as alkenes, hydroxyls, aldehydes, nitrogen mustards often covalently modify an enzyme through an irreversible inhibition by reacting with amino acid side chains. Reversible inhibitors on the other hand attach to enzymes with non-covalent interactions such as ionic and hydrogen bonds or hydrophobic interaction (Irwin and Segel, 1993; Holmes et al., 2002; Hostettmann, et al., 2006; Walsh et al., 2011). Multiple weak bonds between the inhibitor and the active site combine to produce strong and specific binding. In contrast to substrates and irreversible inhibitors, reversible inhibitors (competitive, uncompetitive, non-competitive and mixed competitive), often classified according to the effect of varying the concentration of the enzyme's substrate on the inhibitor, generally do not undergo chemical reactions when bound to the enzyme and can be easily removed by dilution or dialysis (Stone and Morrison, 1986; Szedlacsek and Duggleby,1995; Walsh, 2012). GK1 show mixed reversible inhibition whereby the inhibitor can bind to the enzyme at the same time as the enzyme's substrate (ES) but with different affinities ($K_i \neq K_i'$).. and both affecting each other. Mixed-type inhibitors interfere with substrate binding (increase K_m) and hamper catalysis in the ES complex (decrease V_{max}).

Antioxidants scavenge free radicals or have the capacity to inhibit the oxidation of other molecules. Antioxidant screening is relevant in recent times because oxidation is a chemical reaction that transfer electron or produce free radicals causing chains of reactions thereby damaging cells when they are in excess. They play major role in causing diseases such as cancer, stroke, diabetes, Parkinson's disease and other neurodegenerative diseases (Ajila and Oloyede, 2012; Onocha et al., 2015).

Conclusion

Novel Mannich bases 2-hydroxy-1,2,3-triphenyl-3-(phenylamino) propan-1-one, 2-hydroxy-1,2-diphenyl-3-(phenylamino) propan-1-one, 2-hydroxy-3-(2-hydroxyethylamino)-1,2,3-triphenylpropan-1-one, and 2-hydroxy-1,2,3-triphenyl-3-(p-tolylamino)propan-1-one were prepared using the classical method. A variant approach using a tertiary amine gave 3-(2-(3,4-dimethoxyphenyl)ethylideneamino)-3-(4-hydroxyphenyl)-1-(2,4,6-trimethoxyphenyl)propan-1-one which showed the highest activity in the antioxidant screening when activity was compared with ascorbic acid and butylated hydroxylanisole. Kinetics of urease inhibitin also gave result indicating better activity than thiourea for many of the Mannich bases.

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Declaration of Interest

Authors declare that no conflict of interest is associated with this work.

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