Molecular docking, synthesis, kinetics study, structure–activity relationship and ADME analysis of morin analogous as *Helicobacter pylori* urease inhibitors

Ritu Kataria¹ and Anurag Khatkar²*

**Abstract**

**Background:** Urease are responsible for several pathogenic states in human as well as in animals and its inhibition is utmost urgent. Clinically used drugs are associated with many side effects; recently several researches have shown that flavonoids have good urease inhibition properties. Morin, a natural flavonoid has been investigated for urease inhibition studies which includes designing of library of morin analogues and their in-silico evaluation with the help of Schrodinger’s maestro package of molecular docking software against crystallographic complex of plant enzyme Jack bean urease (PDB ID: 3LA4) followed by synthesis and in vitro evaluation.

**Results:** Best thirteen derivatives of morin were selected on the basis of their interaction energy and dock score for synthesis and further investigated for in-vitro antioxidant, urease inhibitory and Anti-*H. Pylori* activity. In-vitro results revealed that a large number of synthesized compounds were found to possess excellent antioxidant and urease Inhibition properties.

**Conclusions:** Among the synthesized compounds, N-(2-chlorophenyl)-N-((4E)-2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-ylidene)thiourea (M2b) and N-(4-bromophenyl)-N-((4E)-2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-ylidene)thiourea (M2i) were found to be most potent urease inhibitor and antioxidant with IC₅₀ value 10.74 ± 0.018, 11.12 ± 0.033 and 7.37 ± 0.024, 7.73 ± 0.015 and 7.795 ± 0.003 µM. Derivative M2i exhibited good anti-*H. pylori* activity having MIC = 500 µg/ml and zone of inhibition 15.00 ± 0.50 mm as compared to standard AHA having MIC = 1000 µg/ml and zone of inhibition 9.00 ± 0.50 mm determined against *H. Pylori* bacterium (ATCC 43504, DSM 4867) by well diffusion technique. Furthermore, molecular docking study explained the binding pattern of synthesized ligand within active cavity of jack bean protein and drug similarity was explained by ADME studies by quikprop module of molecular docking software.

**Keywords:** Morin, Urease inhibition, Natural phenolic compounds, Antioxidant

© The Author(s) 2019. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Introduction

Urease (urea amidohydrolase; E.C. 3.5.1.5) is a nickel containing metalloenzyme brings catalytic hydrolysis of urea and leads to the formation of ammonia and carbamate which instinctively disintegrates, at normal functioning pH, to give another ammonia molecule and bicarbonate [1]. Its presence in soil was first reported by Rotini [2]. It increases rate of biochemical dissociation of urea by 10^{14} times [3]. Urease plays role of key enzyme for global nitrogen cycle and supplies nitrogen to plants for seed germination and for growth [4]. However, high urease action is responsible for release of unusually a lot of ammonia into climate which may prompt natural and monetary issues [1, 4]. Ureases have been found among plants, microscopic organisms like bacteria, algae, fungi and invertebrates [12]. Catalytic mechanism of plant and microbial originated urease is similar although they possesses structural differences, probably as they have similar pattern of amino acids and Ni^{2+} ions at active center which indicates its emergence from a common ancestry [4–6].

Urease are responsible for several pathogenic states in both human as well as in animals such as urinary and GIT infections, gastric cancer, stone formation in kidney, pyelonephritis, encrustation in catheter, ammonia encephalopathy, hepatic coma [7–11]. Urease is also a virulence factor found in pathogenic bacteria H. Pylori and is one of the main cause for its spreading in gastric environment by catalyzing the urea present there. Released ammonia thereby causes elevation in pH and makes a comfortable environment for pathogen H. Pylori to survive and spread colonies. The presence of excess of urease cause breakdown of fertilizer urea and ammonia released in high concentration in the climate and in addition plants get damaged due to toxicity of ammonia and elevation in soil pH, consequently posturing noteworthy ecological and financial troubles [10].

Morin (3, 5, 7, 2′, 4’-pentahydroxyflavone), a bioflavonoid found in fruits, vegetables and various medicinal herbs such as figs, mulberry, Osage orange (Maclura pomifera), sweet chestnut (Castanea sativa, Family-Fagaceae) guava (Psidium guajana L.) leaves, Brazilian wood (Chlorophora tinctoria), almond (Prunus dulcis, Family-Rosaceae), onion, seaweeds, apple, Chinese and Asian medicinal herb as well as in several beverages [12–18] was initially isolated from the Moraceae family [12]. It has been documented that morin is enriched with wide range of biological activities like anti-inflammatory activity [13], antiallergen [15], hepatoprotective [19], antihypertensive, antioxidant, nephroprotective, antidiabetic, anticlastogenic activities, cytoprotective effects, xanthine oxidase–hypoxanthine activity decreases the frequency of septic shock [14] and various types of cancers like liver cancer, breast cancer, colon cancer, oral tumor and have been used as an herbal medicine from long time [10–21].

Drugs used clinically for the treatment of ailments caused by urease producing bacteria includes bismuth complex, phosphoramidates, imidazole derivatives and hydroxamic acids are associated with numerous side effects like teratogenic effects shown by hydroxamic acid and rapid disintegration of phosphoramidates at low pH. Pharmaceutical industry is in continuous search for better safe and effective drug for treating such disease. Naturally occurring flavonoids have shown urease inhibitory as well as anti H. Pylori activity as reported by several researchers. Awllia Jalaluddin et al. [22] recently evaluated nine flavonoids for inhibition of jack bean urease and five of them were found to be good in activity having IC_{50} values 14.2 ± 0.3 to 132.9 ± 3.3 μM shown in Fig. 1 [23]. Similarity in pharmacophore of morin with these flavonoids i.e. 2-phenyl-4H-chromen-4-one and pharmacological enrichment of morin motivated the researchers to design more potent derivatives of it by using docking studies and then evaluation was done for antioxidant, urease inhibition and anti-H. Pylori activity by in-vitro studies.

Results and discussion

Chemistry

In the current study, morin derivatives (M2a–i, M3–6) were synthesized by following the procedure illustrated in Scheme 1 [24–26]. Synthetic scheme involve two-step process involving formation of substituted aryl thiourea by reaction of substituted anilines with ammonium thiocyanate in presence of hydrochloric acid. Morin derivatives were synthesized in second step by reaction with equimolar concentration of arylthiourea(M2a-i)/thiosemicarbazide(M3)/phenylthiosemicarbazide(M4)/phenyldiazine(M5)/benzylcarbazate(M6) in ethanol with glacial acetic acid as catalyst. Monitoring of reaction was confirmed by single spot in TLC under UV lamp. Evaluation of structure of novel derivatives was confirmed by thin layer chromatography and completion of reaction was confirmed by single spot in TLC under UV lamp. Evaluation of structure of novel derivatives was confirmed by spectroscopic methods such as IR, ^1^HNMR, ^13^CNMR, elemental analysis. Derivatisation was confirmed by downward shift of peak at 1680 (C=O)str to 1615–1625 (C=N)str. Appearance of a peak at 1539–1571 of N–C = S str was observed in compound M2a–M2i which further confirmed the formation of derivatives. Whereas in compounds M3, M4, M5 and M6 additional peak at 1033–1078 for N–Nstr was also observed which confirmed formation of M3–M6 compounds. ^1^HNMR signals were interpreted by their value of chemical shift for particular protons of synthesized derivatives, coupling constant and multiplicities of signals. For instance in compound M2a appearance of singlet at δ 12.68, 10.68,
9.7, 9.68 was noticed for OH, OH, NH and OH groups respectively and 7.53–6.30 for aromatic protons, whereas in $^{13}$C NMR signals at δ 187 (C–S) and 157 (C–N) confirmed formation of compound. Further and final confirmation process involves analyzing their mass spectrum for determination of molecular weight in which Q-ToF Micro instrument was used as ion source. Maximum number of the derivatives showed peak at $M^{+}$ (molecular ion peak), ($M^{+1}$), ($M^{+2}$) in positive chemical ionization and ($M^{+1}$), ($M^{+2}$), $M^{+}$ during negative chemical ionization mode. Elemental analysis of diosmin derivative was carried out in CHNS analyzer where C, H and N in percent were found within acceptable range.

**Biological activity**

The novel synthesized Morin derivatives were examined for *in vitro* urease inhibitory action by determining the ammonia concentration released during the reaction.

\[
\begin{align*}
\text{IC}_{50}=57.8\pm1.15 \text{ µM} \\
\text{IC}_{50}=132.2\pm3.3 \text{ µM} \\
\text{IC}_{50}=19.4\pm1.3 \text{ µM} \\
\text{IC}_{50}=17\pm0.02 \text{ µM}
\end{align*}
\]

Fig. 1 Reported flavonoids having same pharmacophore with morin possessing urease inhibitory activity.
by indophenol technique as described by Weatherburn [27] as well as for antioxidant nature [28, 29] by determining the ability of compounds to donate hydrogen and electrons using DPPH method. All the synthesized compounds were found to be potent inhibitors of jack bean urease and showed good antioxidant potential.
with IC$_{50}$ value ranging between 10.74–20.48 µM and 7.37–11.9 µM respectively. Among them compounds M2b, M2i and M2a displayed the excellent urease inhibition with IC$_{50}$ value 10.74 ± 0.018, 11.12 ± 0.033 and 12.71 ± 0.027 µM even two folds more active than standard drug thiourea and also displayed good antioxidant behavior with IC$_{50}$ value 7.37 ± 0.024, 7.73 ± 0.015 and 7.795 ± 0.003 µM against DPPH using ascorbic acid as standard as shown in Tables 1 and 2 and Figs. 2, 3 and 4.

Compounds M2b and M2i which were found potent in urease inhibition and antioxidant activity as well as in terms of docking score were tested further against antibacterial efficiency against *Helicobacter pylori* ATCC 43504, DSM 4867 using AHA as a standard and DMSO as control. MIC$_{50}$ values were calculated for compounds and the results revealed that M2i displayed its potency with good zone of inhibition i.e. 15.00 ± 0.00 mm as compared to standard 9.00 ± 0.50 mm and MIC$_{50}$ value was found to be comparable to Standard i.e. 500 µg/ml. It could be concluded that compound M2i indicated excellent antibacterial action against *H. Pylori*, antioxidant and urease inhibitory activity.

### Enzyme kinetics

Study of inhibitory effect of morin derivatives on jack bean urease was performed to check the inhibitory potential, kinetics studies and mechanism of inhibition in phosphate buffer and 1 mM EDTA at pH 8.2. Lineweaver–Burk plots (1/absorbance versus 1/urea) was constructed from kinetic data to determine the mechanism of enzyme inhibition by varying the concentration of substrate urea in the presence of different concentrations of most potent compound M2i. Inhibition constant (K$_i$) was determined as the intersection on x-axis of the plot of 1/Vmax and varying concentration of inhibitor obtained from Lineweaver-Burke plot and enzyme kinetic studies also confirm the mode of inhibition as shown in Fig. 5.

### Structure activity relationship

Studying results from antioxidant nature and urease inhibitory activities of newly synthesized morin derivatives, structure activity relationship can be derived (Fig. 6).

1. Incorporation of substituted arylthiourea group enhanced the urease inhibition as well as antioxidant behavior of morin manyfolds such as compounds (M2a–M2i) having IC$_{50}$ value for urease inhibition from 10.74 ± 0.018 to 20.48 ± 0.026 µM.
2. Attachment of bromo substituted arylthiourea increased the effectiveness of molecule (M2i) in inhibiting growth of pathogenic bacteria *H. Pylori* with MIC value 500 µg/ml and zone of inhibition 15.00 ± 0.00 mm as compared to standard acetohy-

### Table 1 In-vitro urease inhibition activity and IC$_{50}$ of synthesized morin derivatives

| Compound | IC$_{50}$ (µM)$^a$ | Compound | IC$_{50}$ (µM)$^a$ |
|----------|--------------------|----------|--------------------|
| M2a      | 12.71 ± 0.027      | M2i      | 11.12 ± 0.033      |
| M2b      | 10.74 ± 0.018      | M3       | 17.9 ± 0.007       |
| M2c      | 19.5 ± 0.005       | M4       | 18.96 ± 0.011      |
| M2d      | 20.41 ± 0.031      | M5       | 17.68 ± 0.07       |
| M2e      | 20.2 ± 0.001       | M6       | 17.66 ± 0.02       |
| M2f      | 19.06 ± 0.015      | Morin    | 21.77 ± 0.016      |
| M2g      | 20.48 ± 0.026      | Thiourea | 22.80 ± 0.011      |
| M2h      | 16.55 ± 0.011      |          |                    |

$a$ Values related for the evaluated compound absorption which provide 50% inhibition of Urease inhibition action, and are the mean SEM; statistical significance: p < 0.05 against the equivalent IC$_{50}$ values achieved against urease, as identified through ANOVA/Dunnett’s test.

### Table 2 In-vitro DPPH radical scavenging activities and IC$_{50}$ of synthesized morin derivatives (antioxidant activity)

| Compound | IC$_{50}$ (µM)$^a$ | Compound | IC$_{50}$ (µM)$^a$ |
|----------|--------------------|----------|--------------------|
| M2a      | 7.795 ± 0.003      | M2i      | 7.73 ± 0.015       |
| M2b      | 7.37 ± 0.024       | M3       | 11.9 ± 0.031       |
| M2c      | 10.18 ± 0.006      | M4       | 8.099 ± 0.018      |
| M2d      | 11.56 ± 0.013      | M5       | 8.78 ± 0.008       |
| M2e      | 8.744 ± 0.008      | M6       | 9.65 ± 0.022       |
| M2f      | 10.50 ± 0.036      | Morin    | 12.83 ± 0.028      |
| M2g      | 10.66 ± 0.019      | Ascorbic acid | 8.59 ± 0.004 |
| M2h      | 9.290 ± 0.007      |          |                    |

$a$ Values related for the evaluated compound absorption which provide 50% inhibition of Urease inhibition action, and are the mean SEM; statistical significance: p < 0.05 against the equivalent IC$_{50}$ values achieved against urease, as identified through ANOVA/Dunnett’s test.
droxamic acid having MIC 500µg/ml and zone of inhibition 9.00 ± 0.50 mm.

3. Substitution of thiosemicarbazide and phenylthiosemicarbazide groups in morin leads to improvement of urease inhibition activity to greater extent due to presence of thiourea groups in the structure.

4. Phenylhydrazine and benzylcarbazate addition improved activity of morin due to stronger interaction of compounds with enzyme through H-bond formation by imine group and pi–pi stacking interactions.

5. Presence of hydroxyl groups improved the antioxidant activity.

**Molecular docking study**

Newly designed ligands were studied for molecular simulation studies with the help of Schrodinger's maestro package of molecular docking software [30–34]. Molecules were docked into Jack bean urease crystallographic complex (PDB ID: 3LA4) by Induced fit docking (IFD) method. The predicted binding pattern revealed that synthesized ligand bind within catalytic cavity firmly via hydrogen bond formation, pi–pi stacking and hydrophobic interaction. Position and alignment of particular substituents on molecules was found to be responsible for perfect binding of ligand with enzyme. In the binding model of the most active compound (M2b) eight hydrogen bonds were noticed between residues Lys 709, Glu 718, Try 32, Val 744, Pro 717, Ash 730, Ser 421 with hydroxyl and NH groups of synthesized ligands. Hydrophobic interactions among ligand and residues Ala 16, Leu 13, Leu 839, Pro 717, Phe 712, Tyr 32, Val 744, Met 746, Val 36, Ala37 was noticed. The second most potent compound (M2i) was found to form six hydrogen bonds...
among Pro 717, Ser 421, Glu 742, Try 32, Ash 730 Lys 709 with hydroxyl and NH groups, a salt bridge formation was also observed between oxygen and Lys 716 residue. Ligand was found to interact with Phe 712, Pro 717, Tyr 32, Met 746, Val 744, Pro 743, Val 36, Ala 37, Leu 13, Ala 16, Leu 839 residues hydrophobically. Similarly six hydrogen bond formations was observed in inhibitor of third rank (M2a) between Glu 718, Ash 730, Lys 716, Val 744, Met 746 residues and hydroxyl, thiol and NH group of ligand. Docking studies revealed that hydrogen bond interactions fix the ligands firmly and tightly in the active site. In-silico studies and in-vitro results comply with each other, according to which, compounds M2b, M2i and M2a were found to be most potent ligands. Molecular docking parameters were shown in Table 3 and interaction pattern of ligands within the pocket were described in Table 4.

Hydrogen bond formations were considered as most important for perfect fitting of ligand within the enzyme. Each one of molecule demonstrated good docking score from $-7.117$ to $-10.977$ as compared to $-3.459$ and $-3.049$ of standard thiourea and Acetohydroxamic acid as well as excellent binding energy ranges from $-45.27$ to $-61.834$ kJ/mol as compared to $-21.156$ kJ/mol and $-17.454$ kJ/mol of standard thiourea and Acetohydroxamic acid. Docking studies concluded that designed ligands have excellent binding capability as compared to parent and standard compounds; a correlation has been set up in docking score and IC$_{50}$ values of synthesized ligands having $R^2$ value 0.7085 has been shown in Fig. 7 signified that molecule having greater the docking score have lesser value of IC$_{50}$ for urease inhibition.

**Admet studies**

In drug discovering the ADME profile of drug like molecules is very important and for this purpose Schrodinger’s maestro molecular modeling package Qikprop module was utilized. The Absorption, distribution,
metabolism and excretion details of the designed molecules are given in Table 5.

Blood brain barrier partition coefficient (QPlogBB), estimated IC<sub>50</sub> value for HERG K<sup>+</sup> channels obstruction (log HERG), permeation through skin estimation (QPlogKp), apparent Caco-2 cell permeability estimation in nm/sec (QPqcaco) and apparent MDCK cell permeability estimation in nm/sec (QPPMDCK), partition coefficient in octanol and water Log P, solubility in aqueous media log S, Lipinski’s rule of five. Results revealed that ADME parameters of each ligand within the bounds of satisfactory range without violating Lipinski’s rules.

ADMET calculations showed that synthesised ligands quietly obey rule of five without any considerable violations also many ligands possessed in range values of QPlog S, QPlogHERG, QPPCaco, QPlogBB, QPPMDCK, QPlogKp, QPlogKhsa, HBD, HBA, Log P, % HOA which made them ligands of choice for urease protein [35–39].

### Experimental

#### Materials used

Jack bean urease and morin was purchased from Sigma Aldrich and Himedia respectively. Analytical grade reagents and solvents were utilized for study and obtained locally. Progression of reaction was observed via. Thin layer chromatography and recrystallization of products was done in order to purify the compounds which were again checked for purity by thin layer chromatography (TLC) performed on plates covered with silica gel G. Measurement of melting point was done in open capillary tubes on a melting point apparatus and was uncorrected. The spectral data, IR and <sup>1</sup>H NMR, <sup>13</sup>CNMR were measured by standard procedures. Brucker 12060280, Germany Software: OPUS 7.2.1394 spectrophotometer in cm<sup>-1</sup> was used for recording IR spectra of derivatives and elemental analysis was done on Perkin–Elmer 2400 C, H, N analyzer. The <sup>1</sup>HNMR and <sup>13</sup>CNMR spectra were recorded in DMSO-d<sub>6</sub> on a Brucker DRX-300 FTNMR instrument.

#### Synthetic procedure

Morin derivatives (M2a–i, M3–6) synthesis was accomplished via. a simple and efficient way by two steps which have been outlined in Scheme 1. The synthesis of compounds M2a–M2i was started by formation of substituted aryl thiourea using different substituted anilines then derivatives of morin were prepared reacting synthesized substituted aryl thiourea/thiosemicarbazide/phenylthiosemicarbazide/phenylhydrazine/benzylcarbazate solution with equimolar concentration of morin in ethanol in presence of acetic acid as catalyst according to previously reported procedure with slight modification [24–26]. Characterisation of all the synthesized compounds was done by IR, <sup>1</sup>H NMR, <sup>13</sup>CNMR and elemental analysis and was found in full accordance with their depicted structures.

#### General synthetic procedure for preparation of derivatives of morin

**Step 1: synthesis of substituted aryl thiourea**

Substituted Aniline (0.32 mol) was taken in a 250 ml round bottom flask and, to this, conc. hydrochloric acid (32.19 mL, 0.32 mol) was added dropwise with continuous stirring. 100 mL of water was added after appearance of turbidity about after 20 min. followed by addition of ammonium thiocyanate solution (29.42 g, 0.38 mol).

### Table 3 Docking parameters of the designed derivatives

| Compound | Docking score | Binding energy | Glide hbond | Glide ecoul | Glide evdw |
|----------|---------------|----------------|-------------|------------|-----------|
| M2a      | -9.225        | -61.834        | -2.212      | -15.894    | -45.94    |
| M2b      | -10.977       | -59.062        | -2.364      | -13.986    | -45.076   |
| M2c      | -7.94         | -52.778        | -1.989      | -4.563     | -48.214   |
| M2d      | -7.117        | -48.622        | -1.073      | -4.498     | -44.124   |
| M2e      | -8.306        | -52.965        | -1.907      | -4.693     | -48.002   |
| M2f      | -7.634        | -52.788        | -1.34       | -5.445     | -47.343   |
| M2g      | -7.202        | -55.996        | -0.83       | -7.193     | -48.803   |
| M2h      | -8.576        | -48.949        | -1.65       | -4.621     | -44.329   |
| M2i      | -10.273       | -45.27         | -2.955      | -11.623    | -33.6447  |
| M3       | -8.266        | -47.885        | -1.293      | -12.371    | -35.514   |
| M4       | -8.12         | -54.757        | -1.81       | -10.458    | -44.298   |
| M5       | -8.306        | -49.449        | -1.69       | -7.449     | -42       |
| M6       | -8.536        | -59.562        | -1.424      | -12.371    | -47.323   |
| Thiourea | -3.459        | -21.156        | -1.484      | -8.152     | -13.004   |
| AHA      | -3.049        | -17.454        | -1.311      | -8.523     | -8.936    |

**Scheme 1:** General Synthetic Procedure for Preparation of Derivatives of Morin

1. **Step 1:** Synthesis of Substituted Aryl Thiourea

   - **M2a–i, M3–6** synthesis was accomplished via. a simple and efficient way by two steps which have been outlined in Scheme 1.

2. **Step 2:** Preparation of Morin Derivatives

   - Progression of reaction was observed via. Thin layer chromatography and recrystallization of products was done in order to purify the compounds which were again checked for purity by thin layer chromatography (TLC) performed on plates covered with silica gel G. Measurement of melting point was done in open capillary tubes on a melting point apparatus and was uncorrected. The spectral data, IR and <sup>1</sup>H NMR, <sup>13</sup>CNMR were measured by standard procedures.

3. **Step 3:** Characterisation of All the Synthesized Compounds

   - ADME calculations showed that synthesised ligands obey rule of five without any considerable violations also many ligands possessed in range values of QPlog S, QPlogHERG, QPPCaco, QPlogBB, QPPMDCK, QPlogKp, QPlogKhsa, HBD, HBA, Log P, % HOA which made them ligands of choice for urease protein [35–39].
### Table 4 Protein-ligand interaction details of synthesized derivatives

| Ligands | Interaction diagram | Interaction description |
|---------|---------------------|------------------------|
| **M2a** | ![Interaction Diagram](image) | Hydrogen bond interaction: Glu 718, Ash 730, Lys 716, Val 744, Met 746  
Hydrophobic interaction: Phe 712, Pro 717, Try 32, Val 36, Ala 37, Ala 16, Pro 743, Val 744, Met 746, Leu 839 |
| **M2b** | ![Interaction Diagram](image) | Hydrogen bond interaction: Lys 709, Glu 718, Tyr 32, Val 744, Pro 717, Ash 730, Ser 421  
Hydrophobic interaction: Pro 717, Phe 712, Tyr 32, Val 744, Val 36, Met 746, Ala 37, Ala 16, Leu 13, Leu 839 |
| **M2c** | ![Interaction Diagram](image) | Hydrogen bond interaction: Phe 712, Val 744, Lys 716, Leu 839  
Hydrophobic interaction: Phe 712, Phe 840, Leu 839, Phe 838, Tyr 837, Val 744, Pro 743, Ala 16, Leu 13, Ala 37, Val 36, Tyr 32 |
| **M2d** | ![Interaction Diagram](image) | Hydrogen bond interaction: Ash 730, Glu 742  
Hydrophobic interaction: Phe 712, Pro 717, Tyr 32, Val 36, Ala 37, Pro 743, Val 744, Met 746, Leu 839, Phe 838, Leu 13, Ala 16 |
| **M2e** | ![Interaction Diagram](image) | Hydrogen bond interaction: Val 744, Glu 742  
Hydrophobic interaction: Trp 728, Phe 712, Met 746, Val 744, Pro 743, Leu 839, Tyr 32, Leu 13, Ala 16, Val 36, Ala 37 |
| **M2f** | ![Interaction Diagram](image) | Hydrogen bond interaction: Glu 718, Glu 742  
Hydrophobic interaction: Trp 728, Met 746, Phe 712, Val 744, Tyr 32, Pro 743, Val 36, Ala 37, Tyr 837, Leu 839  
π–π stacking: Phe 712 |
### Table 4 (continued)

| Ligands | Interaction diagram | Interaction description |
|---------|---------------------|------------------------|
| M2g     | ![Image](M2g.png)   | Hydrogen bond interaction: Glu 742  
Hydrophobic interaction: Pro 717, Phe 838, Leu 839, Phe 840, Met 746, Val 744, Val 36, Pro 743, Leu 13  
π–cation interaction: Lys 716 |
| M2h     | ![Image](M2h.png)   | Hydrogen bond interaction: Ser 421, Val 744  
Hydrophobic interaction: Tyr 32, Leu 13, Val 36, Ala 37, Ala 16, Pro 717, Met 746, Val 744, Pro 743, Phe 838, Leu 839 |
| M2i     | ![Image](M2i.png)   | Hydrogen bond interaction: Pro 717, Ser 421, Glu 742, Tyr 32, Asn 730, Lys 709  
Hydrophobic interaction: Phe 712, Tyr 32, Met 746, Val 36, Val 744, Pro 743, Ala 37, Leu 13, Pro 717 |
| M3      | ![Image](M3.png)    | Hydrogen bond interaction: Asn 730, Val 744  
Hydrophobic interaction: Phe 712, Tyr 32, Pro 717, Val 744, Met 746, Leu 839 |
| M4      | ![Image](M4.png)    | Hydrogen bond interaction: Tyr 32, Lys 716, Ser 421, Met 746  
Hydrophobic interaction: Pro 717, Tyr 417, Tyr 32, Val 36, Val 744, Met 746, Pro 748, Trp 728  
π–π stacking: Trp 728  
π–cation interaction: Lys 716 |
reaction mixture was heated until the solution becomes turbid, after discontinuing the heating it was poured over ice cold water, and filtration of precipitates was done which were finally dried. Crude product obtained was recrystallized by ethanol.

**Step 2: synthesis of Schiff bases of morin**
Equimolar concentration of Morin (0.01 mmol) and substituted arylthiourea/thiosemicarbazide/phenylthiosemicarbazide/benzylcarbazate (0.01 mmol) were solubilized in ethanol (50 ml). Small amount of glacial acetic acid (1–2 ml) was added to the reaction mixture followed by refluxing for 5–6 h. Reaction completion was monitored by thin layer chromatography. Reaction mixture was concentrated; precipitates formed were filtered off and dried. Recrystallization of crude product was done by ethanol and compounds (M2a–i, M3–6) were obtained.

**N-((4E)-2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-ylidene)-N-phenylthiourea (M2a)**
- Rf = Toluene: Chloroform: methanol (4:4:1) = 0.64; Yield = 71.2%; m.p (246–248 °C); IR cm⁻¹ 3649 (O–H str), 3523 (N–H str), 1651 (C=C str), 1614 (C=N str), 1539 (N–C=S str), 1313 (C–O str), 1172 (C–N str); ¹H NMR (400 MHz, DMSO-d₆) δ 12.68 (s, 1H, OH), 10.68 (s, 1H, OH), 9.74 (s, 1H, NH), 9.68 (s, 1H, OH), 9.38 (s, 2H Ar–H), 7.53–7.25 (m, 5H, Ar–H), 7.24 (d, 1H, Ar–H), 7.18–6.90 (m, 2H, Ar–H), 6.14–6.30 (m, 2H, Ar–H); ¹³C NMR (400 MHz, CDCl₃) δ = 187.81, 166.55, 163.81, 159.55, 157.75, 157.20, 146.11, 139.95, 135.22, 129.13, 127.42–127.13, 123.12, 123.11–122.88, 112.83, 107.64, 104.87, 102.35–102.06, 99.77, 91.34; MS ES + (ToF): m/z 436.07 [M⁺]; CHNS: Calc (C₂₂H₁₆N₂O₆S): C, 60.54; H, 3.70; N, 6.42; S, 7.35; Found: C, 60.57; H, 3.69; N, 6.41; S, 7.33.
N-(2-chlorophenyl)-N-((4E)-2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-ylidene)thio urea (M2b) \( R_2 = \text{Toluene: Chloroform: methanol (4:4:1)} \): Yield = 66.6%; \( \text{mp} (202–204 \, ^\circ\text{C}) \); IR cm\(^{-1}\) 3651 (O–H str), 3591 (N–H str), 1652 (C=C str), 1621 (C=O str), 1541 (N=C=S str), 1316 (C=O str), 1174 (C–N str), 750 (C–Cl); \( ^1\text{H} \) NMR (400 MHz, DMSO-\( d_6 \)) \( \delta \) 13.01 (s, 1H, OH), 10.57 (s, 1H, OH), 9.57 (s, 1H, NH), 9.40 (s, 2H, OH), 7.66 (dd, 1H, OH), 7.59–7.33 (m, 3H, Ar–H), 7.05 (m, 1H, Ar–H), 6.62–6.55 (m, 2H, Ar–H), 6.43–6.34 (m, 2H, Ar–H); \( ^{13}\text{C} \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 138.57, 166.92, 163.11, 159.53, 157.93, 157.18, 142.31, 136.37, 135.13, 130.75–129.18, 128.20, 127.89–127.74, 127.28, 126.07, 124.72–124.43, 113.43, 107.63, 104.85, 101.20–101.07, 97.68, 91.51; MS ES + (ToF): m/z 470.02 [M\(^+\)]; CHNS: Calc (C\(_{22}\)H\(_{15}\)Cl\(_3\)O\(_5\)S) C: 56.11; H: 3.21; N: 5.95; S: 6.81; Found: C: 56.09; H: 3.22; N: 5.94; S: 6.80.

N-((4E)-2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-ylidene)-N-(3-nitrophenyl)thiourea (M2d) \( R_2 = \text{Toluene: Chloroform: methanol (4:4:1)} \): Yield = 71.9%; \( \text{mp} (240–242 \, ^\circ\text{C}) \); IR cm\(^{-1}\) 3690 (O–H str), 3549 (N–H str), 1698 (C=C str), 1614 (C=N str), 1541 (N=C=S str), 1507 (N/O) 1302 (C–O str), 1172 (C–N str); \( ^1\text{H} \) NMR (400 MHz, DMSO-\( d_6 \)) \( \delta \) 13.05 (s, 1H, OH), 10.48 (s, 1H, OH), 9.87 (s, 1H, OH), 9.65 (s, 1H, NH), 9.41 (s, 2H, OH), 8.54–8.48 (m, 1H, Ar–H), 7.96–7.86 (m, 2H, Ar–H), 7.55 (m, 1H, Ar–H), 7.34 (d, 1H, Ar–H), 6.60–6.52 (m, 2H, Ar–H), 6.54–6.36 (m, 2H, Ar–H); \( ^{13}\text{C} \) NMR \( ^{13}\text{C} \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 187.75, 166.57, 163.81, 159.94, 157.93, 157.19, 148.76, 145.11, 140.08, 135.18, 131.13, 128.33, 127.88–127.79, 117.85, 116.90, 112.43, 107.62, 104.85, 102.38–102.10, 99.67, 91.73; MS ES + (ToF): m/z 481.9 [M\(^+\)]; CHNS: Calc (C\(_{22}\)H\(_{15}\)N\(_3\)O\(_8\)S) C: 54.88; H: 3.14; N: 8.73; S: 6.66; Found: C: 54.85; H: 3.16; N: 8.72; S: 6.66.

### Table 5: Simulation studies of derivatives by Quikprop

| Synthesised Ligands | QPlogS | QPlog HERG | QPPCaco | QPlogBB | QPlogKp | HBD | HBA | LogP o/w | Rule of Five |
|---------------------|--------|------------|---------|---------|----------|-----|-----|---------|-------------|
| M2a                 | 5.333  | -6.948     | 85.491  | -1.979  | 9.89     | 5   | 7   | 2.629   | 1           |
| M2b                 | 5.373  | -6.405     | 96.414  | -1.705  | 9.83     | 5   | 7   | 2.9     | 1           |
| M2c                 | 5.573  | -6.912     | 9.439   | -3.243  | 10.254   | 5   | 8   | 1.937   | 2           |
| M2d                 | 5.267  | -6.734     | 8.44    | -3.256  | 9.974    | 5   | 8   | 1.713   | 2           |
| M2e                 | 5.366  | -6.611     | 10.739  | -3.046  | 10.589   | 5   | 8   | 1.933   | 2           |
| M2f                 | 6.14   | -6.647     | 19.107  | -2.648  | 10.422   | 5   | 8   | 2.661   | 3           |
| M2g                 | 5.831  | -6.414     | 13.503  | -2.778  | 11.635   | 5   | 8   | 2.395   | 3           |
| M2h                 | 4.782  | -6.086     | 109.727 | -1.611  | 10.606   | 5   | 7   | 2.657   | 1           |
| M2i                 | 5.646  | -6.361     | 88.46   | -1.695  | 9.959    | 5   | 7   | 3.004   | 2           |
| M3                  | 3.201  | -5.146     | 18.445  | -2.331  | 11.441   | 7   | 8   | 0.073   | 1           |
| M4                  | 4.438  | -6.88     | 54.098  | -2.341  | 10.667   | 7   | 8.25 | 1.869   | 1           |
| M5                  | 5.597  | -8.051     | 64.909  | -2.614  | 11.971   | 5   | 6.5 | 2.412   | 2           |
| M6                  | 5.323  | -7.262     | 20.721  | -3.082  | 10.963   | 5   | 8   | 2.057   | 1           |
N-(4-chloro-2-nitrophenyl)-N'-(4E)-2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-ylidene)thiosemicarbazide (M2) Rf = Toluene: Chloroform: methanol (4:4:1) = 0.61; Yield = 70.4%; m.p (204–206 °C); IR cm⁻¹ 3631 (O–H str), 3521 (N–H str), 1650 (C=O str), 1525 (C=N str), 1349 (C–O str), 1130 (C–N str), 1047 (O–H str), 990 (s, 1H, OH), 8.52 (d, 1H, OH), 8.07 (d, 1H, Ar–H), 7.81 (d, 1H, Ar–H), 7.29 (d, 1H, Ar–H), 6.67–6.55 (m, 2H, Ar–H), 6.41–6.32 (m, 2H, Ar–H); ¹³C NMR (400 MHz, CDCl₃) δ = 187.91, 166.57, 163.81, 159.94, 157.18, 157.93, 145.11, 140.54, 139.78, 135.18, 127.92–127.69, 126.10–125.82, 124.63, 123.27–123.01, 122.80, 114.23, 106.84, 102.38–102.07, 99.67, 91.74; MS ES + (ToF): m/z 515.98 [M⁺]; CHNS: Calc: (C₁₂H₁₁N₃O₆S): C, 51.20; H, 2.49; N, 11.19; S, 8.54; Found: C, 51.20; H, 2.49; N, 11.19; S, 8.54.

(1Z)-1-(3,5,7-trihydroxy-2-(2,4-dihydroxyphenyl)-4H-chromen-4-ylidene)thiosemicarbazide (M3) Rf = Toluene: Chloroform: methanol (4:4:1) = 0.68; Yield = 68.8%; m.p (204–206 °C); IR cm⁻¹ 3631 (O–H str), 3521 (N–H str), 1650 (C=O str), 1525 (C=N str), 1349 (C–O str), 1130 (C–N str), 1047 (O–H str), 990 (s, 1H, OH), 8.52 (d, 1H, OH), 8.07 (d, 1H, Ar–H), 7.81 (d, 1H, Ar–H), 7.29 (d, 1H, Ar–H), 6.67–6.55 (m, 2H, Ar–H), 6.41–6.32 (m, 2H, Ar–H); ¹³C NMR (400 MHz, CDCl₃) δ = 188.48, 166.57, 163.81, 159.94, 157.18, 157.93, 145.11, 140.54, 139.78, 135.18, 127.92–127.69, 126.10–125.82, 124.63, 123.27–123.01, 122.80, 114.23, 106.84, 102.38–102.07, 99.67, 91.74; MS ES + (ToF): m/z 515.98 [M⁺]; CHNS: Calc: (C₁₂H₁₁N₃O₆S): C, 51.20; H, 2.49; N, 11.19; S, 8.54; Found: C, 51.20; H, 2.49; N, 11.19; S, 8.54.

(4Z)-4-(3,5,7-trihydroxy-2-(2,4-dihydroxyphenyl)-4H-chromen-4-ylidene)-1-phenylthiosemicarbazide (M4) Rf = Toluene: Chloroform: methanol (4:4:1) = 0.72; Yield = 73.1%; m.p (198–200 °C); IR cm⁻¹ 3616 (O–H str), 3562 (N–H str), 1651 (C=O str), 1601 (C=N str), 1521 (C–O str), 1338 (C–N str), 1186 (C–O str), 1062 (N–N str); ¹¹H NMR (400 MHz, DMSO-d₆) δ 13.21 (1H, OH), 11.70 (1H, NH), 10.12 (1H, OH), 9.95 (s, 1H, OH), 9.88 (s, 1H, OH), 8.64 (s, 2H, NH₂), 7.59 (t, 1H, Ar–H), 6.59–6.51 (m, 2H, Ar–H), 6.43–6.27 (m, 2H, Ar–H); ¹³C NMR (400 MHz, CDCl₃) δ = 180.16, 163.73, 159.94, 157.11, 157.50, 154.25, 143.84, 131.07, 130.47, 127.92–127.71, 111.89, 107.62, 102.38–102.09, 101.83, 99.06, 91.12; MS ES + (ToF): m/z 375.05 [M⁺]; CHNS: Calc: (C₁₂H₁₁N₃O₆S): C, 51.20; H, 2.49; N, 11.19; S, 8.54; Found: C, 51.20; H, 2.49; N, 11.19; S, 8.54.
104.85, 102.38–102.07, 99.67, 91.74; MS ES + (ToF): m/z 451.08 [M⁺]; CHNS: Calc (C_{22}H_{17}N_{3}O_{6}S): C, 58.53; H, 3.80; N, 9.31; S, 7.10. Found: C, 58.56; H, 3.82; N, 9.30; S, 7.09.

(4Z)-2-(2,4-dihydroxyphenyl)-4-(2-phenylhydrazino)-4H-chromene-3,5,7-triol (M5) \( \text{R}=\text{Toluene}: \) Chloroform: methanol (4:4:1) \( \delta \) 12.24 (s, 1H, NH), 11.42 (s, 1H, OH), 11.10 (s, 1H, OH), 10.44 (s, 1H, NH), 9.86 (s, 1H, OH), 9.72 (s, 1H, OH), 7.50 – 7.42 (m, 2H), 7.33–7.24 (m, 2H, Ar–H). 1H NMR (400 MHz, CDCl₃) δ 14.19 (s, 1H, NH), 11.45 (s, 2H, OH), 11.14 (s, 1H, OH), 10.49 (s, 1H, OH), 9.88 (s, 1H, OH), 9.70 (s, 1H, OH), 7.39–7.24 (m, 6H, Ar–H), 6.57 (dd, 2H, Ar–H), 6.00–5.95 (m, 2H, Ar–H); \( ^{13} \text{C} \) NMR (400 MHz, CDCl₃) \( \delta \) 163.73, 159.82, 156.92, 155.74, 143.68, 143.64, 131.08, 130.85, 129.97–129.63, 119.95, 116.25–115.92, 111.89, 107.62, 102.38–102.09, 101.86, 99.06, 91.12. MS ES + (ToF): m/z 392.10 [M⁺]; CHNS: Calc (C_{21}H_{16}N_{2}O_{6}): C, 64.28; H, 4.11; N, 11.14. Found: C, 64.26; H, 4.12; N, 11.13.

Urease inhibitory assay

Urease enzyme inhibition investigation studies for all synthesized compounds were done by the method developed by Weatherburn [27] named Indophenol against Jack Bean Urease. Briefly, incubation of solution of Jack bean protein 25 μL was done at temperature of 30 °C for a period of 15 min with 55 μL of buffers solution having 100 mM urea and 5 μL of test solution in 96-well plates. 45 μL phenol solution composed phenol 1% w/v and solution of sodium nitroprusside 0.005% w/v and alkali reagent 70 μL composed of sodium hydroxide 0.5% w/v and active sodium hypochloride solution 0.1% were mixed to every well. The measurement of increase in absorbance at 625 nm was done after 50 min by help of a microplate reader (Molecular Device, USA). Readings were recorded in triplicate set in a final volume of 200 μL using thiourea as standard. Each assay was performed at pH 8.2 (0.01 M K₂HPO₄, 0.01 M LiCl₂, 1 mM EDTA). Calculation of Inhibition in percentage of synthesized derivatives was done by the formula given as:

\[
I\% = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100
\]

where, \( A_{\text{control}} \) is control absorbance; \( A_{\text{sample}} \) is test absorbance.
**Dpph assay for antioxidant activity**

DPPH method was adopted for measuring the antioxidant nature of the synthesized derivatives. According to this method synthesized compounds were allowed to react for 0.5 h at 37 °C with stable free radical, 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH). The DPPH was taken in a concentration of 300 μM. Decrease in value of absorption at 515 nm was measured after the period of incubation using a microplate reader using ascorbic acid as a standard. Molecule which can donate a proton to DPPH and cause its reduction act as an antioxidant. Following reduction deep violet coloured DPPH solution changes to yellow depending upon the nature of antioxidant compound, which brings a measurable decrease in value of absorption at 517 nm. The proton or electron donating capacity of the derivative was estimated from the fading of deep purple colored methanolic solution of 1, 1-diphenylpicrylhydrazyl (DPPH). Incubation of mixture was done at room temperature for 30 min and measurements were done at 517 against blank [28, 29]. Calculation of percentage inhibition was done by following formula:

\[ I\% = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100 \]

where, \( A_{\text{control}} \) is control absorbance; \( A_{\text{sample}} \) is test absorbance

**Anti-H. pylori activity**

Novel synthesized derivatives were investigated against *H. pylori* bacterium (ATCC 43504 DSM 4867, AHA as standard) for their antibacterial activity by using Well diffusion technique. The stock solution of compounds in DMSO (1000 μg/mL) were prepared. Cell suspension was prepared from culture grown on BHI broth. The cell suspensions of all the cultures were adjusted to \( 1-2 \times 10^8 \) cells/mL. *Helicobacter pylori* (100 µl) was inoculated by spread plate technique on agar plates (90 mm). Agar wells (5 mm) were made on *H. pylori* inoculated media and impregnated with 2500 µg of each sample and standard which were incubated @ 35 °C for 24–48 h with 5% CO₂ and observed for zone of inhibition around the well. MIC was determined against *Helicobacter pylori* by micro broth dilution technique as per NCCLS method [41–45].

**Kinetic parameters of urease activity**

Kinetic parameters i.e. Km, Vmax and Michaelis constant were calculated by verifying the concentration effect of substrate on the rate of reaction of urease enzyme in presence as well as in absence of synthesized compounds. Mechanism behavior of derivatives was studied by dissolving 1% of them in dimethyl sulfoxide and inhibition mode studied by Lineweaver–Burk plots was found to be non-competitive as Km was contant but Vmax changed. Urease enzyme kinetic studies of interaction with synthesized was done GraphPad Prism 7 software.

**Statistical analysis**

Output of statistical analysis has been depicted in mean ± SEM whereas statistical examination of data collected experimentally was done by one-way analysis of variance (ANOVA). Considerable difference revealed by ANOVA p < 0.05 was regarded significant. Evaluation of statistical data was done by Graph Pad Prism 5.0 Version for Windows (San Diego, CA, USA).

**Conclusion**

In conclusion, thirteen Morin derivatives have been synthesized successfully via. a simple two step reaction and evaluated for their DPPH- free radical scavenging activity and urease inhibitory activity against jack bean urease. Among the series M2b, M2i and M2a (IC₅₀ 110.74 ± 0.018, 11.12 ± 0.033 and 12.71 ± 0.027 μM even two folds more active than standard drug thiourea and also displayed good antioxidant behavior with IC₅₀ value 7.37 ± 0.024, 7.73 ± 0.015 and 7.795 ± 0.003 μM. Moreover these are found to be most potent by having excellent dock score 10.977, −10.273, −9.225 and binding energy −59.062, −45.27, −61.834 kJ/mol as compared to standard drugs −3.459, −3.049 and −21.156 kJ/mol and −17.454 kJ/mol of standard thiourea and Acetoxydroxamic acid. These top molecules were again further examined for anti-*H. Pylori* activity and M2i was found to be more potent as compared to standard drug AHA with MIC = 500 μg/ml and zone of inhibition 15.00 ± 0.00 mm as compared to standard having MIC = 1000 μg/ml and zone of inhibition 9.00 ± 0.50 mm. Derivative M2i can be the potential lead compound in future for treatment of pathologies caused by urease as well as against *H. Pylori* infection.

**Additional file**

Additional file 1. Supplementary file for spectral data.

**Abbreviations**

ADMET: absorption distribution metabolism excretion; MIC: minimum inhibitory concentration; TLC: thin layer chromatography; UV: ultra violet; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; SEM: standard error mean; ANOVA: analysis of variance; DMSO: dimethyl sulphoxide; AHA: acetohydroxamic acid; HOA: human oral absorption; RCSB: Research Collaboratory for Structural bioinformatics; NCCLS: National Committee for Clinical Laboratory Standards.
Authors' contributions
Both RK and AK have equal contributions. Both authors read and approved the final manuscript.

Author details
1 International Institute of Pharmaceutical Sciences, Sonepat, Haryana, India.
2 Laboratory for Preservation Technology and Enzyme Inhibition Studies, Department of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak, Haryana, India.

Acknowledgements
We are really thankful to Schrödinger team, especially Vnood Devraj Schrödinger, Inc. (New York, USA) for providing necessary help required to complete the computational work and Skanda life sciences Pvt. Ltd, Banglore for carrying out H. Pylori studies.

Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request (Additional file 1).

Funding
Not applicable.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 10 December 2018 Accepted: 20 March 2019
Published online: 01 April 2019

References
1. Mobley HLT, Hausinger RP (1989) Microbial ureases: significance, regulation and molecular characterization. Microbiol Rev 53(3):85–103
2. Rotmni OT (1935) Latrasformazione enzimatica dell’ urea nel tereno. Annali Laboratorio Ricercas Spallanzani 3:143–154
3. Tamaddon F, Ghasi S (2015) Urease: a highly biocompatible catalyst for switchable Biginelli reaction and synthesis of 1, 4-dihydropyridines from the in situ formed ammonia. Catal Commun 72:63–67
4. Mobley HLT, Island MD, Hausinger RP (1995) Molecular biology of microbial ureases. Microbiol Rev 59(3):451–480
5. Ha NC, Oh ST, Sung JY, Cha KA, Lee MH, Oh BH (2001) Supramolecular assembly and acid resistance of Helicobacter pylori urease. Nat Struct Biol 8:505–509
6. Jabri E, Carr MB, Hausinger RP, Karplus PA (1995) The crystal structure of urease from Klebsiella aerogenes. Science 268:998–1004
7. Jiang HJ, Choi MH, Shin WG, Kim KH, Chung YW, Kim KO, Kim HY (2008) Has peptic ulcer disease changed during the past ten years in Korea? A prospective multicenter study. Dig Dis Sci 53(15):1527–1531
8. Mobley HLT, Island MD, Hausinger RP (1995) Molecular biology of microbial ureases. Microbiol Rev 59(3):451–480
9. Collins CM, D’Orazio SE (1998) Bacterial ureases: structure, regulation of expression and role in pathogenesis. Mol Microbiol 9:907–913
10. Sujoy B, Aparna A (2013) Potential clinical significance of urease enzyme. Eur Sci J. https://doi.org/10.19044/esj.2013/v9n21p%253p1
11. Cuiir S, Benini S, Rypnievski WR (1999) Structural properties of the nickel ions in urease: novel insights into the catalytic and inhibition mechanisms. Coord Chem Rev 192:331–355
12. Li HW, Zou TB, Jia Q (2016) Anticancer effects of morin-7-sulphate sodium, a flavonoid derivative, in mouse melanoma cells. Biomed Pharmacother 84:909–916
13. Galvez J, Cielo G, Crespo ME (2001) Intestinal anti-inflammatory activity of morin on chronic experimental colitis in the rat. Aliment Pharmacol Ther 15(12):2027–2039
14. Fang SH, Hou YC, Chang WC, Hsu SL, Chao PD, Chiang BL (2003) Morin sulfates/glucuronides exert anti-inflammatory activity on activated macrophages and decreased the incidence of septic shock. Life Sci 74(6):743–756
15. Kim JW, Lee JH, Hwang BY (2009) Morin inhibits Fyn kinase in mast cells and IgE-mediated type I hypersensitivity response in vivo. Biochem Pharmacol 77(9):1506–1512
16. Dilshara MG, Jayasooriya RG, Lee S, Choi YH, Kim GY (2016) Morin downregulates nitric oxide and prostaglandin E 2 production in LPS-stimulated BV2 microglial cells by suppressing NF-κB activity and activating HO-1 induction. Environ Toxicol Pharmacol 44:62–68
17. Khazan M, Hdyat M (2014) The role of nitric oxide in health and diseases. Smetr 3(1):e20987
18. Paoli P, Ciri P (1830) Caselli A (2013) The insulin-mimetic effect of Morin: a promising molecule in diabetes treatment. Biocim Biophys Acta Subj Gen 43:3102–3111
19. Lee HS, Jung KH, Hong SW (2008) Morin protects acute liver damage by carbon tetrachloride (CCl4) in rat. Arch Pharmacal Res 31(9):1160–1165
20. Pal R, Chaudhary ML, Tiwari PC, Babu S, Pant KK (2015) Protective role of theophylline and their interaction with nitric oxide (NO) in adjuvant-induced rheumatoid arthritis in rats. Int Immunopharmacol 29(2):854–862
21. Mobley H, Hausinger RP (1989) Microbial ureases: significance, regulation, and molecular characterization. Microbiol Rev 53(1):85–108
22. Avilla Jalaluddin JA, Al-Ghamdi M, Huwaid E, Javaid S, Rasheed S, Iqbal Choudhary M (2016) Flavonoids as natural inhibitors of jack bean urease Enzyme. Lett Drug Des Discov 13(3):243–249
23. Aslam MAS, Mahmood SU, Shahid M, Saeed A, Iqbal J (2011) Synthesis, biological assay in vitro and molecular docking studies of new Schiff base derivatives as potential urease inhibitors. Eur J Med Chem 46(1):5473–5479
24. Ansari MM, Deshmukh SP, Khan R, Musaddiq M (2014) Synthesis antimicrobic and anticancer evaluation of 1-Aryl-S-(o-methoxyphenyl)-2-S-benzyl isothiobiuretius. Int J Med Chem. https://doi.org/10.1155/2014/352626
25. Mughal EU, Ayaz M, Hussain Z, Hasan A, Sadiq A, Riaz M, Malik A, Hussain S, Choudhary MI (2006) Synthesis and antibacterial activity of substituted flavones, 4-thioflavones and 4-iminoflavones. Bioorg Med Chem 14(14):4704–4711
26. Brodowska K, Sykula A, Garkibba E, Lodyga-Chrusci尔斯ka E, Sökja M (2016) Naringenin Schiff base: antioxidant activity, acid-base profile, and interactions with DNA. Transition Met Chem 41(2):179–189
27. Weatherburn MW (1967) Phenol-hypochlorite reaction for determination of ammonia. Anal Chem 39(6):971–974
28. Hanif M, Shaolb K, Saleem M, Hasam Rama N, Zaib S, Iqbal J (2012) Synthesis, urease inhibition, antioxidant, antibacterial, and molecular docking studies of 1, 3-oxadiazole derivatives. ISRN Pharmacol 13:2012
29. Choudhary MI, Begum A, Abbaskhan A, Musharraf SG, Ejaz A (2008) Two new antioxidant phenylpropanoids from Lindelofia stylosa. Chem Biodivers 5(2):2676–2683
30. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, Repasky MP, Knoll EH, Shelley M, Perry JK, Shaw DE (2004) Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J Med Chem 47(7):1739–1749
31. Halgren TA, Murphy RB, Friesner RA, Beard HS, Pollard WT, Repasky MP, Knoll EH, Shelley M, Perry JK, Shaw DE (2004) Glide: a new approach for rapid, accurate docking and scoring. Enrichment factors in database screening. J Med Chem 47(7):1750–1759
32. Glide, version 6.6, Schrödinger, LLC, New York, NY, 2015
33. Lee SK, Mbwambo ZH, Chung Luengyi L, Gamezi EJ, Mehta RG, Kinghorn AD, Pezzuto JM (1998) Evaluation of the antioxidant potential of natural products. Comb Chem High Throughput Screening 1(1):35–46
34. Genheden S, Ryde U (2015) The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities. Exp Op Drug Disc 10:449–461
35. Godschalk F, Genheden S, Söderhjelm P, Ryde U (2013) Comparison of MM/GBSA calculations based on explicit and implicit solvent simulations. Phys Chem Chem Phys 15:7731–7739
36. Sengupta D, Verma D, Naik PK (2008) Docking-MM/GB/SA and ADME screening of HIV-I NNRTI inhibitor: nevirapine and its analogues. Silico Biol 8:275–289
37. Cecchelli R, Berezowski V, Lundquist S, Culot M, Renftel M, Dehouck MP, Fenart L (2007) Modelling of the blood-brain barrier in drug discovery and development. Nat Rev Drug Discov 6:650–661
38. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2012) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 64:4–17
39. Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, Sanschagrin PC, Mainz DT (2006) Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. J Med Chem 49:177–196
40. Hanif M, Saleem M, Hussain MT, Rama NH, Zaib S, Adlam MA, Jones PG, Iqbal J (2012) Synthesis, urease inhibition, antioxidant and antibacterial studies of some 4-amino-5-aryl-3H-1,2,4-triazole-3-thiones and their 3,6-disubstituted 1,2,4-triazolo[3,4-b]1,3,4-thiadiazole derivatives. J Braz Chem Soc 23(5):854–860
41. Xiao ZP, Shi DH, Li HQ, Zhang LN, Xu C, Zhu HL (2007) Polyphenols based on isoflavones as inhibitors of Helicobacter pylori urease. Bioorg Med chem 15(11):3703–3710
42. Li HQ, Xiao ZP, Yan T, Lv PC, Zhu HL (2009) Amines and oximes derived from deoxybenzoins as Helicobacter pylori urease inhibitors. Eur J Med Chem 44(5):2246–2251
43. Xiao ZP, Wang XD, Peng ZY, Huang S, Yang P, Li QS, Zhou LH, Hu XJ, Wu LJ, Zhou Y, Zhu HL (2012) Molecular docking, kinetics study, and structure–activity relationship analysis of quercetin and its analogues as Helicobacter pylori urease inhibitors. J Agric Food Chem 60(42):10572–10577
44. Xiao ZP, Peng ZY, Dong JJ, He J, Ouyang H, Feng YT, Lu CL, Lin WQ, Wang JX, Xiang YP, Zhu HL (2013) Synthesis, structure–activity relationship analysis and kinetics study of reductive derivatives of flavonoids as Helicobacter pylori urease inhibitors. Eur J Med Chem 63:685–695
45. Numao N, Iwahori A, Hirota Y, Sasatum M, Kondo I, Onimura K, Sampe R, Yamane S, Itoh S, Katoh T, Kobayashi S (1997) Antibacterial activity of two alkylamines integrated an indane scaffold: mimicry of a complementary unit on magainin. Biol Pharm Bull 20(7):800–804