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Synthesis and dynamics studies of barbituric acid derivatives as urease inhibitors

Assem Barakat^{1,2*}, Abdullah Mohammed Al-Majid^{1†}, Gehad Lotfy^{3†}, Fiza Arshad^{4†}, Sammer Yousuf^{4†}, M. Iqbal Choudhary^{5†}, Sajda Ashraf^{5†} and Zaheer Ul-Haq^{5†}

Abstract

Background: Discovery of potent inhibitors of urease (jack bean) enzyme is the first step in the development of drugs against diseases caused by ureolytic enzyme.

Results: Thirty-two derivatives of barbituric acid as zwitterionic adducts of diethyl ammonium salts were synthesized. All synthesized compounds (**4a–z** and **5a–s**) were screened for their in vitro inhibition potential against urease enzyme (jack bean urease). The compounds **4i** ($IC_{50} = 17.6 \pm 0.23 \mu\text{M}$) and **5l** ($IC_{50} = 17.2 \pm 0.44 \mu\text{M}$) were found to be the most active members of the series, and showed several fold more urease inhibition activity than the standard compound thiourea ($IC_{50} = 21.2 \pm 1.3 \mu\text{M}$). Whereas, compounds **4a–b**, **4d–e**, **4g–h**, **4j–4r**, **4x**, **4z**, **5b**, **5e**, **5k**, **5n–5q** having IC_{50} values in the range of $22.7 \pm 0.20 \mu\text{M}$ – $43.8 \pm 0.33 \mu\text{M}$, were also found as potent urease inhibitors. Furthermore, Molecular Dynamics simulation and molecular docking studies were carried out to analyze the binding mode of barbituric acid derivatives using MOE. During MD simulation enol form is found to be more stable over its keto form due to their coordination with catalytic Nickel ion of Urease. Additionally, structural–activity relationship using automated docking method was applied where the compounds with high biological activity are deeply buried within the binding pocket of urease. As multiple hydrophilic crucial interactions with Ala169, KCX219, Asp362 and Ala366 stabilize the compound within the binding site, thus contributing greater activity.

Conclusions: This research study is useful for the discovery of economically, efficient viable new drug against infectious diseases.

Keywords: Barbituric acid, Zwitterions, Urease enzyme, Urolithiasis, MD simulation and molecular docking

Background

Urease is a nickel containing enzyme produced by plants, fungi, algae, and bacteria. It is involved in nitrogen turnover and in crop fertilization, as well as in human and animal pathologies. Urease catalyse the hydrolysis of urea in its ammonia and carbon dioxide. Beside its medical, ecological and economical significances as urease has historical significances as it was the first enzyme to be crystallised in 1926 by Sumner [1–3]. Since its discovery in plants [4], *Canavalia ensiformis* (*Fabaceae*) urease has

been exhaustively investigated [5]. Its activity is strictly dependent on nickel ions (Ni^{2+}) [6]. The first X-ray diffraction based structure of a urease was reported by Jabri and coworkers in 1995 from *Klebsiella aerogenes* [7]. Later on, other structures for ureases from *Bacillus pasteurii* [8], *Helicobacter pylori* [9] and *C. ensiformis* [10] were reported. The elucidation of the urease structure from a legume (jack bean) was crucial to better understand the requirements for ureolytic activity of this class of enzymes in different organisms [10] were reported.

Urease enzyme is a virulence factor in certain human and animal ailments. It contributes to the development of kidney stones, pyelonephritis, peptic ulcers leading to gastric cancers, and other diseases [11]. It also causes the pathogenesis of hepatic coma urolithiasis, hepatic encephalopathy, pyelonephritis, ammonia and urinary

*Correspondence: ambarakat@ksu.edu.sa

[†]Assem Barakat, Abdullah Mohammed Al-Majid, Gehad Lotfy, Fiza Arshad, Sammer Yousuf, M. Iqbal Choudhary, Sajda Ashraf and Zaheer Ul-Haq contributed equally

¹ Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

Full list of author information is available at the end of the article

catheter encrustation [12, 13]. The gastric cancer [14, 15] is the fourth most common cancer and the second most common cause of cancer-related deaths worldwide [16]. It is often resulted from pathologies due to *Helicobacter pylori*. Urease lets bacteria to persist at the low pH of the stomach during colonization and lead to pathogenesis of gastric and peptic ulcers which in the long run may cause cancer [17]. The treatment of infection caused by ureolytic bacteria with antimicrobials, however, often proved to be unsuccessful [13]. The barbiturates possessed a wide range of pharmacological applications, such as anti-convulsant, sedative, anxiolytic, urease inhibition [18], antifungal [19], antimicrobial [20, 21], antitumor, antiviral [13, 22] anti tuberculosis [23], mushroom tyrosinase inhibition [24], radio-sensitization [25], anti-inflammatory, anticancer [26], anesthetic [27], diaminopimelate aminotransferase inhibition [28], and anti-proliferative activities [29].

Based on the therapeutic and pharmacological significances of urease inhibition, our research group is involved in the search of simple but biologically interesting molecules that are easy to synthesize in just fewer steps with high yields. This type of chemistry is easily adopted by the pharmaceutical industry for commercialization. Previously, our research group reported zwitterionic adduct derived from barbituric acid as NO scavenger [30]. In view of these studies; the combined use of green synthetic technology for the high yield production of novel pharmacophoric barbituric acid derivatives and their systematic evaluation of biological activities as urease inhibition is discussed in this paper.

Methods

General

All chemicals were purchased from Sigma-Aldrich, Fluka etc., and were used without further purification, unless otherwise stated. All melting points were measured on a Gallenkamp melting point apparatus in open glass capillaries and are uncorrected. IR Spectra were measured as KBr pellets on a Nicolet 6700 FT-IR spectrophotometer. The NMR spectra were recorded on a Varian Mercury Jeol-400 NMR spectrometer. $^1\text{H-NMR}$ (400 MHz), and $^{13}\text{C-NMR}$ (100 MHz) were run in deuterated chloroform (CDCl_3). Chemical shifts (δ) are referred in terms of *ppm* and *J*-coupling constants are given in *Hz*. Mass spectra were recorded on a Jeol JMS-600 H. Elemental analysis was carried out on Elmer 2400 Elemental Analyzer in CHN mode

Synthesis of 4 and 5 (GP1)

A mixture of **1** (3 mmol) and aldehyde **2** (1.5 mmol), as well as Et_2NH (1.5 mmol, 155 μL) were placed in 3 mL

of degassed H_2O . The reaction mixture was kept at rt up to 5 h under stirring. After completion of the reaction, monitored by TLC, the solid product was filtered, washed with ether (3 \times 20 mL) and dried to obtain pure products **4** and **5**.

4-(bis(6-Hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl)benzaldehyde diethylaminium salt **4a**

4a, as colorless crystal (1.5 g, 2.76 mmol, 92 %). IR (cm^{-1}): 3450, 3000, 2872, 1670, 1582, 1510, 1466, 1384, 1339; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) 17.58 (s, 1H, OH), 9.90(s, 1H, CHO), 7.73 (d, 2H, $J = 8.0$ Hz, Ph), 7.29 (d, 2H, $J = 8.0$ Hz, Ph), 5.93(s, 1H, benzyl-H), 3.33 (s, 12H, 4 CH_3), 3.06 (q, 4H, $J = 7.3$ Hz, CH_2CH_3), 1.27 (t, 6H, $J = 7.3$ Hz, CH_2CH_3); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): $\delta = 192.2, 165.3, 164.4, 151.7, 150.3, 134.3, 129.9, 127.3, 91.7, 42.2, 35.1, 29.0, 28.7, 11.5$; Anal. for $\text{C}_{24}\text{H}_{31}\text{N}_5\text{O}_7$; Calcd: C, 57.48; H, 6.23; N, 13.96; Found: C, 57.50; H, 6.25; N, 14.00; LC/MS (ESI): $m/z = 501.53$ [M] $^+$.

5,5'-(3-Tolylmethylene)

bis(1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione) diethylaminium salt **4b**

4b; rose-colored crystalline materials. m.p.: 135 $^\circ\text{C}$; (97 %, 1.41 g, 2.91 mmol). IR (KBr, cm^{-1}): 3455, 3201, 2988, 1693, 1667, 1611, 1573, 1443; $^1\text{H-NMR}$ (400 MHz, CDCl_3): $\delta 17.62$ (s, 1H, OH), 7.10 (t, 1H, $J = 7.3$ Hz, Ph), 6.92 (d, 1H, $J = 7.3$ Hz, Ph), 6.88 (d, 1H, $J = 7.3$ Hz, Ph), 5.82(s, 1H, benzyl-H), 3.32 (s, 12H, 4 CH_3), 3.01 (q, 4H, $J = 7.3$ Hz, CH_2CH_3), 2.25 (s, 3H, CH_3), 1.26 (t, 6H, $J = 7.3$ Hz, CH_2CH_3); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): $\delta = 165.3, 164.4, 151.8, 141.7, 137.4, 127.9, 127.1, 126.4, 123.6, 92.1, 42.0, 34.4, 28.9, 28.6, 21.8, 11.4$; Anal. for $\text{C}_{24}\text{H}_{35}\text{N}_5\text{O}_6$; Calcd: C, 59.12; H, 6.82; N, 14.36; Found: C, 59.13; H, 6.81; N, 14.35; LC/MS (ESI): $m/z = 487$ [M] $^+$.

5,5'-(4-Nitrophenyl)methylene)

bis(1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione) diethylaminium salt **4c**

4c; a yellow powder; m.p.: 195 $^\circ\text{C}$; (87 %, 1.35 g, 2.61 mmol); IR (KBr, cm^{-1}): 3453, 3205, 2987, 2904, 1675, 1608, 1576, 1511, 1438, 1343, 1254; $^1\text{H-NMR}$ (400 MHz, CDCl_3): $\delta 17.58$ (s, 1H, OH), 8.08 (d, 2H, $J = 8.8$ Hz, Ph), 7.29 (d, 2H, $J = 8.8$ Hz, Ph), 5.95(s, 1H, benzyl-H), 3.34 (s, 12H, 4 CH_3), 3.07 (q, 4H, $J = 7.3$ Hz, CH_2CH_3), 1.29 (t, 6H, $J = 7.3$ Hz, CH_2CH_3); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): $\delta = 165.2, 164.4, 151.6, 150.8, 146.1, 127.5, 123.5, 91.4, 42.2, 34.9, 28.9, 28.7, 11.5$; Anal. for $\text{C}_{23}\text{H}_{30}\text{N}_6\text{O}_8$; Calcd: C, 53.28; H, 5.83; N, 16.21; Found: C, 53.29; H, 5.85; N, 16.23; LC/MS (ESI): $m/z = 518$ [M] $^+$.

A suitable crystal for X-ray diffraction analysis was obtained from DCM/ Et_2O after 24 h. CCDC-1001798

contains the supplementary crystallographic data for this compound (Additional file 1).

**5,5'-((4-Methoxyphenyl)methylene)
bis(1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione)
diethylammonium salt 4d**

4d; rose-colored crystalline materials; m.p.: 160 °C; (90 %, 1.35 g, 2.7 mmol). IR (KBr, cm^{-1}): 3445, 3195, 2977, 2836, 1689, 1664, 1613, 1504, 1447, 1378, 1242; $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 17.67 (s, 1H, OH), 7.01 (d, 2H, $J = 8.8$ Hz, Ph), 6.75 (d, 2H, $J = 8.8$ Hz, Ph), 5.79 (s, 1H, benzyl-H), 3.33 (s, 12H, 4 CH_3), 2.99 (q, 4H, $J = 7.3$ Hz, CH_2CH_3), 1.26 (t, 6H, $J = 7.3$ Hz, CH_2CH_3); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): $\delta = 165.3, 164.3, 157.4, 151.7, 133.6, 132.0, 127.4, 114.3, 92.1, 55.6, 42.1, 33.8, 28.9, 11.5$; Anal. for $\text{C}_{24}\text{H}_{33}\text{N}_5\text{O}_7$; Calcd: C, 57.25; H, 6.61; N, 13.91; Found: C, 57.26; H, 6.61; N, 13.90; LC/MS (ESI): $m/z = 503[\text{M}]^+$.

**5,5'-((3-Bromophenyl)methylene)
bis(1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione)
diethylammonium salt 4e**

4e; colorless crystalline materials; m.p.: 169 °C; (92 %, 1.5 g, 2.76 mmol). IR (KBr, cm^{-1}): 3450, 3120, 2982, 1694, 1667, 1615, 1577, 1445, 1250; $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 17.63 (s, 1H, OH), 7.22 (d, 1H, $J = 7.3$ Hz, Ph), 7.19 (s, 1H, Ph), 7.07 (d, 1H, $J = 7.3$ Hz, Ph), 7.05 (d, 1H, $J = 7.3$ Hz, Ph), 5.84 (s, 1H, benzyl-H), 3.34 (s, 6H, 2 CH_3), 3.32 (s, 6H, 2 CH_3), 3.02 (q, 4H, $J = 7.3$ Hz, CH_2CH_3), 1.27 (t, 6H, $J = 7.3$ Hz, CH_2CH_3); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): $\delta = 165.2, 164.4, 151.7, 144.7, 129.7, 129.6, 128.7, 125.3, 91.5, 42.1, 34.4, 28.9, 28.7, 11.5$; Anal. for $\text{C}_{23}\text{H}_{30}\text{BrN}_5\text{O}_6$; Calcd: C, 50.01; H, 5.47; Br, 14.46; N, 12.68; Found: C, 50.03; H, 5.48; Br, 14.47; N, 12.71; LC/MS (ESI): $m/z = 552[\text{M}]^+$.

A suitable crystal for X-ray diffraction analysis was obtained from DCM/ Et_2O after 24 h. CCDC-1001799 contains the supplementary crystallographic data for this compound.

**5,5'-((4-hydroxyphenyl)methylene)
bis(6-hydroxy-1,3-dimethylpyrimidine-2,4(1H,3H)-dione)
diethylammonium salt 4f**

4f; a yellow powder; m.p.: 180 °C; (88 %, 1.3 g, 2.64 mmol); IR (KBr, cm^{-1}): 3458, 3200, 2980, 2904, 1677, 1620, 1572, 1511, 1438, 1343, 1254; $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 17.62 (s, 1H, OH), 7.31 (d, 2H, $J = 8.8$ Hz, Ph), 6.99 (d, 2H, $J = 8.8$ Hz, Ph), 5.79 (s, 1H, benzyl-H), 3.33 (s, 12H, 4 CH_3), 3.03 (q, 4H, $J = 7.3$ Hz, CH_2CH_3), 1.27 (t, 6H, $J = 7.3$ Hz, CH_2CH_3); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): $\delta = 165.3, 164.4, 151.7, 141.1, 131.2, 128.5, 119.3, 91.7, 42.1, 34.2, 28.9, 28.7, 11.5$; Anal. for $\text{C}_{23}\text{H}_{31}\text{N}_5\text{O}_7$; Calcd:

C, 56.43; H, 6.38; N, 14.31; Found: C, 56.44; H, 6.36; N, 14.30; LC/MS (ESI): $m/z = 489.52[\text{M}]^+$.

**5,5'-(*p*-Tolylmethylene)
bis(1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione)
diethylammonium salt 4g**

4g; colorless needle materials; m.p.: 152 °C; (97 %, 1.41 g, 2.91 mmol). IR (KBr, cm^{-1}): 3455, 3210, 2984, 2820, 1560, 1449, 1359; $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 17.64 (s, 1H, OH), 6.99–6.96 (m, 4H, Ph), 5.80 (s, 1H, benzyl-H), 3.32 (s, 12H, 4 CH_3), 3.03 (q, 4H, $J = 7.3$ Hz, CH_2CH_3), 2.25 (s, 3H, CH_3), 1.28 (t, 6H, $J = 7.3$ Hz, CH_2CH_3); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): $\delta = 165.3, 164.3, 151.8, 138.6, 134.8, 128.9, 126.3, 92.1, 42.0, 34.2, 28.9, 28.6, 21.0, 11.4$; Anal. for $\text{C}_{24}\text{H}_{35}\text{N}_5\text{O}_6$; Calcd: C, 59.12; H, 6.82; N, 14.36; Found: C, 59.13; H, 6.81; N, 14.35; LC/MS (ESI): $m/z = 487[\text{M}]^+$.

A suitable crystal for X-ray diffraction analysis was obtained from DCM/ Et_2O after 24 h. CCDC-957025 contains the supplementary crystallographic data for this compound.

**5,5'-(*N*-Naphthalen-2-ylmethylene)
bis(1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione)
diethylammonium salt 4h**

4h; beige powder; m.p.: 146 °C; (94 %, 1.47 g, 2.82 mmol). IR (KBr, cm^{-1}): 3454, 3200, 2967, 1668, 1585, 1438, 1250; $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 17.33 (s, 1H, OH), 8.10 (d, 2H, $J = 8.8$ Hz, naphthyl-H), 7.99 (d, 2H, $J = 8.8$ Hz, naphthyl-H), 7.92 (d, 2H, $J = 8.8$ Hz, naphthyl-H), 7.90 (d, 2H, $J = 8.8$ Hz, naphthyl-H), 7.84 (d, 2H, $J = 8.8$ Hz, naphthyl-H), 7.68–7.38 (m, 3H, naphthyl-H), 6.37 (s, 1H, benzyl-H), 3.39 (s, 12H, 4 CH_3), 3.01 (q, 4H, $J = 7.3$ Hz, CH_2CH_3), 1.30 (t, 6H, $J = 7.3$ Hz, CH_2CH_3); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): $\delta = 164.9, 151.7, 136.8, 135.3, 134.3, 131.5, 129.1, 128.5, 127.0, 125.2, 124.9, 123.8, 93.2, 41.8, 33.2, 28.8, 11.4$; Anal. for $\text{C}_{27}\text{H}_{33}\text{N}_5\text{O}_6$; Calcd: C, 61.94; H, 6.35; N, 13.38; Found: C, 61.95; H, 6.34; N, 13.40; LC/MS (ESI): $m/z = 523[\text{M}]^+$.

**5,5'-(*p*-Tolylmethylene)
bis(6-hydroxypyrimidine-2,4(1H,3H)-dione)
diethylammonium salt 4i**

4i; white powder; m.p.: 205 °C; (95 %, 1.22 g, 2.85 mmol); IR (KBr, cm^{-1}): 3459, 3120, 2978, 2811, 1689, 1612, 1325, 1252; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO}-d_6$): δ 17.18 (s, 1H, OH), 10.09 (bs, 4H, NH), 6.93 (m, 4H, Ph), 5.90 (s, 1H, benzyl-H), 2.79 (q, 4H, $J = 7.3$ Hz, CH_2CH_3), 2.20 (s, 3H, CH_3), 1.07 (t, 6H, $J = 7.3$ Hz, CH_2CH_3); $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO}-d_6$): $\delta = 164.8, 164.1, 151.3, 142.1, 133.5, 128.5, 127.1, 91.6, 42.6, 30.6, 21.1, 13.0$; Anal. for $\text{C}_{20}\text{H}_{25}\text{N}_5\text{O}_6$; Calcd: C, 55.68; H, 5.84; N, 16.23; Found: C, 55.67; H, 5.83; N, 16.22; LC/MS (ESI): $m/z = 431[\text{M}]^+$.

5,5'-((4-Chlorophenyl)methylene)**bis(6-hydroxypyrimidine-2,4(1H,3H)-dione) diethylammonium salt 4j**

4j; a white powder; m.p.: 221 °C; (95 %, 1.28 g, 2.85 mmol); IR (KBr, cm^{-1}): 3435, 3185, 2978, 2830, 1677, 1548, 1448, 1345, 1250; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ 17.17 (s, 1H, OH), 10.00 (bs, 4H, NH), 7.18 (m, 4H, Ph), 5.93 (s, 1H, benzyl-H), 2.88 (q, 4H, $J = 7.3$ Hz, CH_2CH_3), 1.12 (t, 6H, $J = 7.3$ Hz, CH_2CH_3); $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): $\delta = 164.7, 164.0, 151.2, 144.6, 133.5, 129.9, 129.1, 127.8, 91.3, 42.1, 30.7, 11.8$; Anal. for $\text{C}_{19}\text{H}_{22}\text{ClN}_5\text{O}_6$; Calcd C, 50.50; H, 4.91; Cl, 7.85; N, 15.50; Found: C, 50.51; H, 4.90; Cl, 7.83; N, 15.51; LC/MS (ESI): $m/z = 451[\text{M}]^+$.

5,5'-((4-Methoxyphenyl)methylene)**bis(6-hydroxypyrimidine-2,4(1H,3H)-dione) diethylammonium salt 4k**

4k; a beige powder; m.p.: 195 °C; (91 %, 1.22 g, 2.73 mmol); IR (KBr, cm^{-1}): 3449, 3190, 2991, 2835, 1688, 1592, 1505, 1383, 1247; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ 17.26 (s, 1H, OH), 9.99 (bs, 4H, NH), 6.92 (d, 2H, $J = 8.0$ Hz, Ph), 6.72 (d, 2H, $J = 8.0$ Hz, Ph), 5.88 (s, 1H, benzyl-H), 2.90 (q, 4H, $J = 7.3$ Hz, CH_2CH_3), 1.14 (t, 6H, $J = 7.3$ Hz, CH_2CH_3); $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): $\delta = 164.6, 164.0, 157.0, 151.2, 137.2, 132.4, 115.1, 91.7, 55.4, 42.1, 30.7, 11.6$; Anal. for $\text{C}_{20}\text{H}_{25}\text{N}_5\text{O}_7$; Calcd C, 53.69; H, 5.63; N, 15.65; Found: C, 53.69; H, 5.63; N, 15.66; LC/MS (ESI): $m/z = 447[\text{M}]^+$.

5,5'-((Naphthalen-2-yl)methylene)**bis(6-hydroxypyrimidine-2,4(1H,3H)-dione) diethylammonium salt 4l**

4l; a beige powder, m.p.: 192 °C; (93 %, 1.3 g, 2.79 mmol); IR (KBr, cm^{-1}): 3459, 3208, 2994, 1677, 1579, 1448, 1386, 1354; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ 16.92 (s, 1H, OH), 10.41 (bs, 4H, NH), 8.13 (d, 1H, $J = 8.8$ Hz, naphthyl), 7.81 (d, 1H, $J = 8.8$ Hz, naphthyl), 7.63 (d, 1H, $J = 8.8$ Hz, naphthyl), 7.38–7.32 (m, 4H, naphthyl), 6.46 (s, 1H, benzyl-H), 2.79 (q, 4H, $J = 7.3$ Hz, CH_2CH_3), 1.08 (t, 6H, $J = 7.3$ Hz, CH_2CH_3); $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): $\delta = 164.9, 151.1, 141.5, 135.8, 134.0, 132.4, 129.3, 128.7, 126.0, 125.8, 125.5, 125.2, 124.9, 123.8, 92.3, 42.5, 29.7, 12.7$; Anal. for $\text{C}_{23}\text{H}_{25}\text{N}_5\text{O}_6$; Calcd C, 59.09; H, 5.39; N, 14.98; Found: C, 59.12; H, 5.40; N, 15.01; LC/MS (ESI): $m/z = 467[\text{M}]^+$.

5-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(phenyl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylammonium salt 4m

4m; colorless crystalline material; m.p.: 159 °C; (98 %, 671 mg, 1.47 mmol). IR (KBr, cm^{-1}): 3150, 2959, 1667, 1617, 1585, 1422, 1256, 1227; $^1\text{H-NMR}$ (400 MHz, CDCl_3):

δ 15.28 (s, 1H, OH), 7.17–7.04 (m, 5H, Ph), 5.85 (s, 1H, benzyl-H), 3.29 (s, 12H, 4 CH_3), 2.96 (q, 4H, $J = 7.3$ Hz, CH_2CH_3), 2.42 (d, 2H, $J = 5.1$ Hz, CH_2), 2.29 (m, 2H, CH_2), 1.24 (t, 6H, $J = 7.3$ Hz, CH_2CH_3), 1.14 (s, 3H, CH_3), 1.05 (s, 3H, CH_3); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): $\delta = 192.5, 180.8, 152.5, 142.5, 128.0, 126.7, 125.1, 116.3, 90.9, 51.4, 45.9, 42.2, 33.0, 31.5, 29.6, 28.4, 27.6, 11.4$; Anal. for $\text{C}_{25}\text{H}_{35}\text{N}_3\text{O}_5$; calcd: C, 65.62; H, 7.71; N, 9.18; Found: C, 65.61; H, 7.73; N, 9.20; LC/MS (ESI): $m/z = 457[\text{M}]^+$.

A suitable crystal for X-ray diffraction analysis was obtained from $\text{CHCl}_3/\text{Et}_2\text{O}$ after 24 h. CCDC- 933624 contains the supplementary crystallographic data for this compound.

5-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(p-tolyl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylammonium salt 4n

4n; oily material (97 %; 685 mg, 1.45 mmol). IR (KBr, cm^{-1}): 3150, 2954, 2867, 1675, 1580, 1508, 1447, 1380, 1256, 1145; $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 15.25 (s, 1H, OH), 7.00–6.93 (m, 4H, Ph), 5.84 (s, 1H, benzyl-H), 3.28 (s, 12H, 4 CH_3), 2.90 (q, 4H, $J = 7.3$ Hz, CH_2CH_3), 2.30 (d, 4H, $J = 5.1$ Hz, CH_2), 2.22 (s, 3H, CH_3), 1.20 (t, 6H, $J = 7.3$ Hz, CH_2CH_3), 1.16 (s, 3H, CH_3), 1.04 (s, 3H, CH_3); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): $\delta = 196.5, 180.1, 152.8, 140.5, 134.2, 129.8, 128.7, 126.8, 126.7, 115.6, 91.0, 51.4, 45.9, 42.5, 32.6, 31.5, 29.6, 28.4, 27.6, 20.9, 11.9$; Anal. for $\text{C}_{26}\text{H}_{37}\text{N}_3\text{O}_5$; calcd: C, 66.22; H, 7.91; N, 8.91; Found: C, 66.24; H, 7.92; N, 8.87; LC/MS (ESI): $m/z = 471[\text{M}]^+$.

5-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(4-methoxyphenyl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylammonium salt 4o

4o; an oily material (92 %; 672 mg, 1.38 mmol). IR (KBr, cm^{-1}): 3047, 2953, 2866, 2499, 1679, 1577, 1510, 1427, 1373, 1255, 1214; $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 15.26 (s, 1H, OH), 6.98 (d, 2H, $J = 8.0$ Hz, Ph), 6.72 (d, 2H, $J = 8.0$ Hz, Ph), 5.69 (s, 1H, benzyl-H), 3.71 (s, 3H, CH_3), 3.29 (s, 12H, 4 CH_3), 2.87 (q, 4H, $J = 7.3$ Hz, CH_2CH_3), 2.31 (d, 4H, $J = 5.1$ Hz, CH_2), 1.19 (t, 6H, $J = 7.3$ Hz, CH_2CH_3), 1.12 (s, 3H, CH_3), 1.03 (s, 3H, CH_3); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): $\delta = 195.1, 187.2, 157.1, 134.5, 133.9, 127.8, 127.6, 115.6, 113.4, 55.2, 42.6, 31.5, 31.1, 27.9, 12.2$; Anal. for $\text{C}_{26}\text{H}_{37}\text{N}_3\text{O}_6$; calcd: C, 64.05; H, 7.65; N, 8.62; Found: C, 64.11; H, 7.64; N, 8.59; LC/MS (ESI): $m/z = 487[\text{M}]^+$.

5-((4-Chlorophenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylammonium salt 4p

4p; oily material (97 %; 715 mg, 1.45 mmol). IR (KBr, cm^{-1}): 3151, 2955, 2868, 2497, 1675, 1580, 1481, 1444,

1379, 1258, 1206; ^1H NMR (400 MHz, CDCl_3): δ 15.02 (s, 1H, OH), 7.12–6.95(m, 4H, Ph), 5.87 (s, 1H, benzyl-H), 3.30 (s, 12H, 4 CH_3), 2.90(q, 4H, $J = 7.3$ Hz, CH_2CH_3), 2.38 (s, 4H, CH_2), 1.20(t, 6H, $J = 7.3$ Hz, CH_2CH_3), 1.16(s, 3H, CH_3), 1.04(s, 3H, CH_3); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 198.1, 181.0, 152.5, 141.5, 130.6, 128.3, 128.2, 128.0, 127.9, 115.2, 90.7, 65.9, 49.8, 42.3, 32.4, 31.5, 31.2, 29.6, 28.4, 27.6, 15.3, 11.4$; Anal. for $\text{C}_{25}\text{H}_{34}\text{ClN}_3\text{O}_5$; calcd: C, 61.03; H, 6.97; Cl, 7.21; N, 8.54; Found: C, 61.06; H, 7.00; Cl, 7.18; N, 8.57; LC/MS (ESI): $m/z = 492$ [M] $^+$.

5-((4-Bromophenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylaminium salt 4q

4q; an oily material (95 %, 761 mg, 1.42 mmol). IR (KBr, cm^{-1}): 3155, 2955, 2867, 2500, 1674, 1579, 1430, 1376, 1204; ^1H NMR (400 MHz, CDCl_3): δ 15.20 (s, 1H, OH), 7.34 (d, 2H, $J = 8.0$ Hz, Ph), 6.98 (d, 2H, $J = 8.0$ Hz, Ph), 5.79 (s, 1H, benzyl-H), 3.27 (s, 12H, 4 CH_3), 2.99(q, 4H, $J = 7.3$ Hz, CH_2CH_3), 2.40 (d, 2H, $J = 5.1$ Hz, CH_2), 2.28(m, 2H, CH_2), 1.29(t, 6H, $J = 7.3$ Hz, CH_2CH_3), 1.18(s, 3H, CH_3), 1.04(s, 3H, CH_3); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 199.1, 191.2, 164.8, 152.4, 142.8, 132.5, 131.0, 129.9, 128.7, 128.6, 118.9, 115.9, 90.6, 51.2, 45.8, 42.3, 32.7, 31.5, 29.5, 28.5, 28.3, 27.6, 11.4$; Anal. for $\text{C}_{25}\text{H}_{34}\text{BrN}_3\text{O}_5$; calcd: C, 55.97; H, 6.39; Br, 14.89; N, 7.83; Found: C, 56.00; H, 6.40; Br, 14.86; N, 7.82; LC/MS (ESI): $m/z = 536$ [M] $^+$.

5-((3-Bromophenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylaminium salt 4r

4r; oily material (93 %, 745 mg, 1.39 mmol). IR (KBr, cm^{-1}): 3050, 2955, 2868, 2500, 1675, 1581, 1444, 1378, 1255, 1205; ^1H NMR (400 MHz, CDCl_3): δ 15.63 (s, 1H, OH), 7.22 (d, 1H, $J = 7.3$ Hz, Ph), 7.19 (s, 1H, Ph), 7.07 (d, 1H, $J = 7.3$ Hz, Ph), 7.05 (d, 1H, $J = 7.3$ Hz, Ph), 5.84 (s, 1H, benzyl-H), 3.34(s, 6H, 2 CH_3), 3.32(s, 6H, 2 CH_3), 2.98(q, 4H, $J = 7.3$ Hz, CH_2CH_3), 2.31 (d, 4H, $J = 5.1$ Hz, CH_2), 1.24(t, 6H, $J = 7.3$ Hz, CH_2CH_3), 1.12(s, 3H, CH_3), 1.03(s, 3H, CH_3); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 190.8, 186.4, 165.2, 164.4, 151.7, 144.7, 129.7, 129.6, 128.7, 125.3, 91.5, 42.1, 34.4, 28.9, 28.7, 11.5$; Anal. for $\text{C}_{25}\text{H}_{34}\text{BrN}_3\text{O}_5$; calcd: C, 55.97; H, 6.39; Br, 14.89; N, 7.83; Found: C, 56.01; H, 6.41; Br, 14.86; N, 7.84; LC/MS (ESI): $m/z = 536$ [M] $^+$.

5-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(1-nitrophenyl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylaminium salt 4s

4s; a beige material; m.p: 146 $^\circ\text{C}$; (92 %, 690 mg, 1.37 mmol). IR (KBr, cm^{-1}): 3054, 2953, 2865, 2500, 1673, 1580, 1510, 1427, 1373, 1255, 1214; ^1H NMR (400 MHz, CDCl_3): δ 15.33 (s, 1H, OH), 7.01–7.35 (m, 3H, Ph), 5.65 (s, 1H, benzyl-H), 3.70 (s, 12H, 4 CH_3), 2.89(q, 4H,

$J = 7.3$ Hz, CH_2CH_3), 2.30(d, 4H, $J = 14.7$ Hz, CH_2), 1.15(t, 6H, $J = 7.3$ Hz, CH_2CH_3), 1.10(s, 3H, CH_3), 1.00(s, 3H, CH_3); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 161.6, 153.2, 145.5, 141.6, 129.1, 128.2, 127.8, 125.8, 88.5, 49.1, 41.9, 27.5, 11.5$; Anal. for $\text{C}_{25}\text{H}_{34}\text{N}_4\text{O}_7$; calcd: C, 59.75; H, 6.82; N, 11.15; Found: C, 59.72; H, 6.80; N, 11.17; LC/MS (ESI): $m/z = 502$ [M] $^+$.

5-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(4-(dimethylamino)phenyl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylaminium salt 4t

4t; a beige material; m.p: 165 $^\circ\text{C}$; (73 %, 550 mg, 1.1 mmol). IR (KBr, cm^{-1}): 3055, 2950, 2865, 2500, 1669, 1580, 1510, 1427, 1373, 1255, 1214; ^1H NMR (400 MHz, CDCl_3): δ 15.33 (s, 1H, OH), 7.02 (d, 2H, $J = 8.0$ Hz, Ph), 6.75 (d, 2H, $J = 8.8$ Hz, Ph), 5.69 (s, 1H, benzyl-H), 3.70 (s, 12H, 4 CH_3), 3.01 (s, 6H, $\text{N}(\text{CH}_3)_2$), 2.89(q, 4H, $J = 7.3$ Hz, CH_2CH_3), 2.31(d, 4H, $J = 14.7$ Hz, CH_2), 1.15(t, 6H, $J = 7.3$ Hz, CH_2CH_3), 1.12(s, 3H, CH_3), 1.00(s, 3H, CH_3); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 161.6, 153.2, 145.5, 141.6, 129.1, 128.2, 127.8, 125.8, 88.5, 49.1, 41.9, 41.8, 27.5, 11.5$; Anal. for $\text{C}_{27}\text{H}_{39}\text{N}_4\text{O}_5$; calcd: C, 64.91; H, 7.87; N, 11.21; Found: C, 64.90; H, 7.87; N, 11.23; LC/MS (ESI): $m/z = 499.29$ [M] $^+$.

5-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(4-hydroxyphenyl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylaminium salt 4v

4v; a white solid material; m.p: 162 $^\circ\text{C}$; (91 %, 645 mg, 1.36 mmol). IR (KBr, cm^{-1}): 23097, 2939, 2884, 2828, 2498, 1747, 1574, 1530, 1506, 1466, 1384, 1241; ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 14.52 (s, 1H, OH), 8.50 (brs, 1H, OH), 6.76(d, 2H, $J = 8.0$ Hz, Ph), 6.50(d, 2H, $J = 8.0$ Hz, Ph), 6.04(s, 1H, benzyl-H), 3.07 (s, 12H, 2 CH_3), 3.14(q, 4H, $J = 7.3$ Hz, CH_2CH_3), 2.92 (q, 4H, $J = 13.9$ Hz, CH_2), 2.06 (s, 4H, CH_2), 1.12(t, 6H, $J = 7.3$ Hz, CH_2CH_3), 0.98(s, 3H, CH_3); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): $\delta = 198.0, 188.5, 154.1, 136.6, 128.3, 115.3, 114.3, 90.1, 50.9, 45.5, 42.1, 31.6, 30.7, 29.7, 11.7$; Anal. for $\text{C}_{25}\text{H}_{35}\text{N}_3\text{O}_6$; calcd: C, 63.41; H, 7.45; N, 8.87; Found: C, 63.40; H, 7.43; N, 8.85; LC/MS (ESI): $m/z = 473$ [M] $^+$.

4-((6-hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)benzaldehyde diethylaminium salt 4x

4x; as solid (1.26 g, 90 %). IR (cm^{-1}): 3156, 2950, 2872, 1678, 1590, 1508, 1375, 1256, 1232, 1167; ^1H -NMR (CDCl_3 , 400 MHz): 14.16 (s, 1H, OH), 9.80 (s, 1H, CHO), 8.01 (brs, 2H, NH), 6.98 (d, 2H, $J = 7.3$ Hz, Ph), 6.75 (d, 2H, $J = 7.3$ Hz, Ph), 5.61(s, 1H, benzyl-H), 3.73

(s, 6H, CH₃), 2.92 (q, 4H, *J* = 7.3 Hz, CH₂CH₃), 2.31 (m, 4H, 2CH₂), 1.26 (t, 6H, *J* = 7.3 Hz, CH₂CH₃), 1.05 (s, 3H, CH₃), 1.00 (s, 3H, CH₃); ¹³C-NMR (100 MHz, CDCl₃): δ = 193.0, 188.1, 165.0, 157.2, 127.8, 115.7, 113.8, 91.6, 55.2, 48.8, 48.6, 42.4, 31.5, 29.4, 27.7, 11.7; Anal. for C₂₆H₃₅N₃O₆; Calcd: C, 64.31; H, 7.27; N, 8.65; Found: C, 64.30; H, 7.26; N, 8.63; LC/MS (ESI): *m/z* = 485.57 [M]⁺.

5-((2,4-Dichlorophenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylamminium salt 4w

4w; a beige solid material; m.p: 164 °C; (90 %, 710 mg, 1.35 mmol). IR (KBr, cm⁻¹): 3059, 2995, 2867, 2114, 1741, 1658, 1591, 1463, 1429, 1370, 1341, 1256, 1201. ¹H-NMR (400 MHz, CDCl₃): δ 14.80 (s, 1H, OH), 7.29 (d, 1H, *J* = 8.0 Hz, Ph), 7.19 (s, 1H, Ph), 7.12 (d, 2H, *J* = 8.0 Hz, Ph), 5.76 (s, 1H, benzyl-H), 3.28 (s, 12H, 4CH₃), 3.07 (q, 4H, *J* = 7.3 Hz, CH₂CH₃), 2.37 (s, 2H, CH₂), 2.27 (d, 2H, *J* = 5.1 Hz, CH₂), 1.34 (t, 6H, *J* = 7.3 Hz, CH₂CH₃), 1.04 (s, 3H, CH₃), 1.01 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 199.1, 165.4, 164.4, 152.5, 139.8, 133.6, 131.7, 131.2, 129.3, 126.4, 115.7, 89.8, 51.2, 45.7, 41.9, 32.4, 31.2, 28.3, 28.2, 11.3; Anal. for C₂₅H₃₃Cl₂N₃O₅; calcd: C, 57.04; H, 6.32; Cl, 13.47; N, 7.98; Found: C, 57.09; H, 6.31; Cl, 13.44; N, 8.01; LC/MS (ESI): *m/z* = 526 [M]⁺.

5-((2,6-Dichlorophenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylamminium salt 4y

4y an oily material (89 %, 702 mg, 1.33 mmol). IR (KBr, cm⁻¹): 3048, 2955, 2869, 2728, 2494, 1676, 1575, 1428, 1372, 1238, 1196; ¹H NMR (400 MHz, CDCl₃): δ 14.80 (s, 1H, OH), 7.36 (d, 2H, *J* = 8.0 Hz, Ph), 7.29 (t, 1H, *J* = 8.0 Hz, Ph), 7.12 (d, 2H, *J* = 8.0 Hz, Ph), 5.98 (s, 1H, benzyl-H), 3.26 (s, 12H, 4CH₃), 2.92 (q, 4H, *J* = 7.3 Hz, CH₂CH₃), 2.37 (s, 2H, CH₂), 2.27 (d, 2H, *J* = 5.1 Hz, CH₂), 1.24 (t, 6H, *J* = 7.3 Hz, CH₂CH₃), 1.094 (s, 3H, CH₃), 1.04 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 192.8, 188.9, 165.3, 164.3, 152.5, 149.7, 137.4, 131.5, 129.8, 126.5, 124.2, 115.5, 114.7, 89.9, 53.5, 41.4, 31.9, 28.7, 28.2, 11.4; Anal. for C₂₅H₃₃Cl₂N₃O₅; calcd: C, 57.04; H, 6.32; Cl, 13.47; N, 7.98; Found: C, 57.08; H, 6.30; Cl, 13.45; N, 8.00; LC/MS (ESI): *m/z* = 526 [M]⁺.

5-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(naphthalen-2-yl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylamminium salt 4z

4z; a white solid material; m.p: 170 °C; (94 %, 715 mg, 1.41 mmol). IR (KBr, cm⁻¹): 2994, 2948, 2866, 2506, 1742, 1651, 1603, 1570, 1526, 1473, 1431, 1362, 1245; ¹H NMR (400 MHz, CDCl₃): δ 14.26 (s, 1H, OH), 7.46–7.22 (m, 7H, naphthyl), 6.20 (s, 1H, benzyl-H), 3.26 (s, 6H, 2CH₃), 3.23 (s, 6H, 2CH₃), 3.14 (q, 4H, *J* = 7.3 Hz,

CH₂CH₃), 2.41 (q, 4H, *J* = 5.1 Hz, CH₂), 2.23 (s, 2H, CH₂), 1.37 (t, 6H, *J* = 7.3 Hz, CH₂CH₃), 1.07 (s, 3H, CH₃), 1.01 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 199.0, 180.5, 165.3, 164.3, 152.5, 149.7, 136.8, 131.5, 129.9, 126.5, 124.2, 115.5, 114.7, 89.9, 50.9, 45.5, 41.7, 31.3, 30.7, 28.2, 11.1; Anal. for C₂₉H₃₇N₃O₅; calcd: C, 68.62; H, 7.35; N, 8.28; Found: C, 68.65; H, 7.34; N, 8.30; LC/MS (ESI): *m/z* = 507 [M]⁺.

2-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(phenyl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate diethylamminium salt 5a

5a; as solid (1.26 g, 95 %). IR (cm⁻¹): 2955 (s), 1586 (s), 1382 (s), 776 (s), 576 (s), 480 (s); ¹H-NMR (CDCl₃, 400 MHz) δ 13.91 (s, OH), 8.25 (bs, 1H, NH₂), 7.01–7.21 (m, 5H, ArH), 5.74 (s, 1H, PhCH), 2.84 (q, *J* = 6.6 Hz, 4H, NHCH₂CH₃), 2.31 (s, 8H, CH₂ + COCH₂), 1.18 (t, *J* = 6.6 Hz, 6H, NHCH₂CH₃), 0.95–1.14 (m, 12H, CH₃); ¹³C-NMR (CDCl₃, 100 MHz): δ 199.1, 179.3, 142.4, 128.0, 126.8, 125.2, 115.5, 50.6, 45.9, 42.3, 34.2, 32.0, 11.4; Anal. Calcd. for C₂₇H₃₇NO₄: C, 73.36; H, 8.98; N, 3.07; O, 14.57; Found: C, 73.43; H, 8.90; N, 3.17; O, 14.49; LC/MS (ESI): *m/z* = 441.29 [M]⁺.

2-((4-Chlorophenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate diethylamminium salt 5b

3c; as solid (92 %, 1.31 g). IR (cm⁻¹): 2956 (s), 1706 (s), 1573 (s), 1486 (s), 1382 (s), 1263 (s), 732 (s), 605 (s), 485 (s); ¹H-NMR (CDCl₃, 400 MHz) δ 13.59 (s, OH), 8.51 (bs, 2H, NH₂), 6.89–7.21 (m, 4H, ArH), 5.70 (s, 1H, PhCH), 2.90 (q, *J* = 7.3 Hz, 4H, NHCH₂CH₃), 2.30 (s, 8H, CH₂ + COCH₂), 1.21 (t, *J* = 7.3 Hz, 6H, NH₂CH₂CH₃), 0.91–1.16 (m, 12H, CH₃); ¹³C-NMR (CDCl₃, 100 MHz): δ 197.3, 188.6, 139.5, 130.8, 128.3, 128.1, 115.2, 49.7, 44.9, 42.2, 34.3, 33.1, 31.5, 11.3; Anal. Calcd. for C₂₇H₃₈ClNO₄: C, 68.23; H, 8.19; N, 2.97; O, 13.34; Found: C, 68.12; H, 8.05; N, 2.90; O, 13.44; LC/MS (ESI): *m/z* = 475.25 [M]⁺.

2-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(p-tolyl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate diethylamminium salt 5c

5c; as solid (93 %, 1.2 g). IR (cm⁻¹): 2957 (s), 1571 (s), 1483 (s), 1383 (s), 1267 (s), 739 (s), 488 (s); ¹H-NMR (CDCl₃, 400 MHz) δ 13.73 (s, OH), 7.83 (bs, 2H, NH₂), 6.91–7.05 (m, 4H, ArH), 5.73 (s, 1H, PhCH), 2.84 (q, *J* = 7.3 Hz, 4H, NHCH₂CH₃), 2.31 (s, 8H, CH₂ + COCH₂), 2.23 (s, 3H, PhCH₃), 1.18 (t, *J* = 7.3 Hz, 6H, NH₂CH₂CH₃), 0.94–1.16 (m, 12H, CH₃), ¹³C-NMR (CDCl₃, 100 MHz): δ 195.8, 187.3, 144.4, 134.0, 128.6, 126.8, 115.6, 51.8, 46.1, 42.7, 34.9, 32.7, 31.4, 20.9, 12.4; Anal. Calcd. for C₂₈H₄₁NO₄: C, 73.79; H, 9.14; N, 3.09; O, 13.91; Found: C, 73.81; H, 9.07; N, 3.07; O, 14.05; LC/MS (ESI): *m/z* = 455.30 [M]⁺.

**2-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)
(*m*-tolyl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate
diethylamminium salt **5d****

5d; as solid (91 %, 1.24 g). IR (cm⁻¹): 2952 (s), 1572 (s), 1483 (s), 1381 (s), 1227 (s), 1143 (s), 787 (s), 463 (s); ¹H-NMR (CDCl₃, 400 MHz) δ 13.78 (s, OH), 7.85 (bs, 2H, NH₂), 6.88–7.03 (m, 4H, ArH), 5.71 (s, 1H, PhCH), 2.91 (q, *J* = 7.4 Hz, 4H, NHCH₂CH₃), 2.38 (s, 8H, CH₂ + COCH₂), 2.28 (s, 3H, PhCH₃), 1.16 (t, *J* = 7.4 Hz, 6H, NH₂CH₂CH₃), 0.91–1.12 (m, 12H, CH₃); ¹³C-NMR (CDCl₃, 100 MHz): δ 195.9, 187.5, 144.7, 134.1, 128.4, 126.9, 115.8, 51.9, 46.3, 42.6, 34.8, 32.8, 31.2, 20.6, 12.3; Anal. Calcd. for C₂₈H₄₁NO₄: C, 73.85; H, 9.09; N, 3.13; O, 13.79; Found: C, 73.81; H, 9.07; N, 3.07; O, 14.05; LC/MS (ESI): *m/z* = 455.30 [M]⁺.

**2-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)
(4-methoxyphenyl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate
diethylamminium salt **5e****

5e; as solid (89 %, 1.26 g). IR (cm⁻¹): 3121 (s), 1668 (s), 1614 (s), 1578 (s), 1446 (s), 778 (s), 608 (s), 457 (s); ¹H-NMR (CDCl₃, 400 MHz) δ 14.67 (s, OH), 8.22 (bs, 2H, NH₂), 6.97 (d, *J* = 7.4 Hz, 2H, ArH), 6.72 (d, *J* = 7.4 Hz, 2H, ArH), 5.72 (s, 1H, PhCH), 3.72 (s, 3H, OCH₃), 2.85 (q, *J* = 7.4 Hz, 4H, NHCH₂CH₃), 2.30 (s, 8H, CH₂ + COCH₂), 1.20 (t, *J* = 7.4 Hz, 6H, NH₂CH₂CH₃), 0.96–1.16 (m, 12H, CH₃); ¹³C-NMR (CDCl₃, 100 MHz): δ 194.1, 187.5, 157.6, 133.1, 127.8, 115.7, 113.4, 55.2, 50.7, 45.3, 42.5, 34.1, 31.5, 31.1, 11.9; Anal. Calcd. for C₂₈H₄₁NO₅: C, 71.19; H, 8.79; N, 3.05; O, 17.11; Found: C, 71.31; H, 8.76; N, 2.97; O, 16.96; LC/MS (ESI): *m/z* = 471.30 [M]⁺.

**2-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)
(4-nitrophenyl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate
diethylamminium salt **5f****

5f; as solid (90 %, 1.26 g). IR (cm⁻¹): 2872 (s), 1582 (s), 1510 (s), 1466 (s), 1384 (s), 1339 (s), 757 (s), 487 (s); ¹H-NMR (CDCl₃, 400 MHz) δ 15.12 (s, OH), 8.32 (bs, 2H, NH₂), 8.01 (m, *J* = 8.8 Hz, 2H, ArH), 7.21 (d, *J* = 8.8 Hz, 2H, ArH), 5.92 (s, 1H, PhCH), 2.94 (q, *J* = 7.3 Hz, 4H, NHCH₂CH₃), 2.29 (s, 8H, CH₂ + COCH₂), 1.21 (t, *J* = 7.3 Hz, 6H, NH₂CH₂CH₃), 0.91–1.06 (m, 12H, CH₃); ¹³C-NMR (CDCl₃, 100 MHz): δ 194.9, 186.8, 151.9, 145.5, 127.7, 123.2, 114.8, 50.3, 42.5, 45.2, 34.1, 32.2, 31.6, 11.4; Anal. Calcd. for C₂₇H₃₈N₂O₆: C, 66.74; H, 7.98; N, 5.55; O, 19.91; Found: C, 66.64; H, 7.87; N, 5.76; O, 19.73; LC/MS (ESI): *m/z* = 468.27 [M]⁺.

**2-((2,6-Dichlorophenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate
diethylamminium salt **5g****

5g; as solid (91 %, 1.39 g). IR (cm⁻¹): 2953 (s), 2869 (s), 1711 (s), 1575 (s), 1497 (s), 1367 (s), 1220 (s), 776 (s), 448

(s); ¹H-NMR (CDCl₃, 400 MHz) δ 14.78 (s, OH), 8.71 (bs, 2H, NH₂), 7.24 (s, *J* = 14.4 Hz, 1H, ArH), 7.16 (m, 1H, ArH), 6.95 (d, *J* = 14.4 Hz, 1H, ArH), 5.89 (s, 1H, PhCH), 2.90 (q, *J* = 7.4 Hz, 4H, NHCH₂CH₃), 2.19 (bs, 8H, CH₂ + COCH₂), 1.17 (t, *J* = 7.4 Hz, 6H, NH₂CH₂CH₃), 0.88–1.03 (bs, 12H, CH₃); ¹³C-NMR (DMSO-*d*₆, 100 MHz): δ 198.3, 189.1, 139.1, 134.9, 128.2, 125.9, 114.2, 51.1, 47.6, 42.5, 34.3, 31.8, 30.3, 11.9; Anal. Calcd. for C₂₇H₃₇Cl₂NO₄: C, 63.46; H, 7.55; N, 2.43; O, 12.91; Found: C, 63.52; H, 7.31; N, 2.74; O, 12.54; LC/MS (ESI): *m/z* = 509.21 [M]⁺.

**2-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)
(3-nitrophenyl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate
diethylamminium salt **5h****

5h; as solid (90 %, 1.26 g). IR (cm⁻¹): 2872 (s), 1582 (s), 1510 (s), 1466 (s), 1384 (s), 1339 (s), 757 (s), 487 (s); ¹H-NMR (CDCl₃, 400 MHz) δ 15.12 (s, OH), 8.32 (bs, 2H, NH₂), 8.01 (m, *J* = 8.8 Hz, 2H, ArH), 7.21 (d, *J* = 8.80 Hz, 2H, ArH), 5.92 (s, 1H, PhCH), 2.94 (q, *J* = 7.3 Hz, 4H, NHCH₂CH₃), 2.29 (s, 8H, CH₂ + COCH₂), 1.21 (t, *J* = 7.3 Hz, 6H, NH₂CH₂CH₃), 0.91–1.06 (m, 12H, CH₃); ¹³C-NMR (CDCl₃, 100 MHz): δ 194.9, 186.8, 151.9, 145.5, 127.7, 123.2, 114.8, 50.3, 45.2, 42.5, 34.1, 32.2, 31.6, 11.4; Anal. Calcd. for C₂₇H₃₈N₂O₆: C, 66.74; H, 7.98; N, 5.55; O, 19.91; Found: C, 66.64; H, 7.87; N, 5.76; O, 19.73; LC/MS (ESI): *m/z* = 468.27 [M]⁺.

**2-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)
(2-nitrophenyl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate
diethylamminium salt **5i****

5i; as solid (87 %, 1.22 g). IR (cm⁻¹): 3096 (s), 2938 (s), 2869 (s), 1580 (s), 1539 (s), 1506 (s), 1384 (s), 1241 (s), 1033 (s), 778 (s), 604 (s), 524 (s); ¹H-NMR (CDCl₃, 400 MHz) δ 14.27 (s, OH), 8.74 (bs, 2H, NH₂), 7.10 (m, 4H, ArH), 6.22 (s, 1H, PhCH), 2.03 (q, *J* = 7.3 Hz, 4H, NHCH₂CH₃), 2.20 (bs, 8H, CH₂ + COCH₂), 1.29 (t, *J* = 7.3 Hz, 6H, NH₂CH₂CH₃), 0.99 (bs, 12H, CH₃); ¹³C-NMR (CDCl₃, 100 MHz): δ 198.9, 181.9, 149.7, 137.4, 131.3, 130.2, 125.9, 124.1, 114.5, 49.9, 44.8, 42.0, 33.6, 31.4, 29.4, 11.2; Anal. Calcd. for C₂₇H₃₈N₂O₆: C, 66.94; H, 7.87; N, 5.43; O, 19.96; Found: C, 66.64; H, 7.87; N, 5.76; O, 19.73; LC/MS (ESI): *m/z* = 468.27 [M]⁺.

**2-((4-Formylphenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate
diethylamminium salt **5j****

5j; as solid (75 %, 1.01 g). IR (cm⁻¹): 3150 (s), 1586 (s), 1519 (s), 1469 (s), 1381 (s), 1339 (s), 779 (s), 495 (s); ¹H-NMR (DMSO-*d*₆, 400 MHz) δ 16.45 (s, OH), 8.39 (bs, 2H, NH₂), 6.78 (m, *J* = 8.04 Hz, 2H, ArH), 6.49 (d, *J* = 8.04 Hz, 2H, ArH), 6.08 (s, 1H, PhCH), 3.00 (s, 6H, N(CH₃)₂), 2.89 (q, *J* = 7.32 Hz, 4H, NHCH₂CH₃),

2.10 (s, 8H, CH₂ + COCH₃), 1.15 (t, *J* = 7.32 Hz, 6H, NH₂CH₂CH₃), 0.88–1.01 (m, 12H, CH₃); ¹³C-NMR (DMSO-*d*₆, 100 MHz): δ 196.1, 183.6, 154.1, 136.1, 128.3, 115.3, 114.3, 50.9, 45.6, 42.0, 41.7, 34.2, 31.9, 29.8, 11.8; Anal. Calcd. for C₂₈H₃₉NO₅; C, 71.61; H, 8.37; N, 2.98; Found: C, 71.61; H, 8.37; N, 2.98; LC/MS (ESI): *m/z* = 69.28 [M]⁺.

2-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(4-hydroxyphenyl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate diethylammonium salt 5k

5k; as solid (88 %, 1.01 g). IR (cm⁻¹): 3157 (s), 1584 (s), 1519 (s), 1469 (s), 1381 (s), 1339 (s), 779 (s), 495 (s); ¹H-NMR (DMSO-*d*₆, 400 MHz) δ 16.41 (s, OH), 8.32 (bs, 2H, NH₂), 6.75 (m, *J* = 8.0 Hz, 2H, ArH), 6.45 (d, *J* = 8.0 Hz, 2H, ArH), 6.04 (s, 1H, PhCH), 2.88 (q, *J* = 7.3 Hz, 4H, NHCH₂CH₃), 2.50 (s, 1H, PhOH), 2.06 (s, 8H, CH₂ + COCH₃), 1.12 (t, *J* = 7.32 Hz, 6H, NH₂CH₂CH₃), 0.85–0.97 (m, 12H, CH₃); ¹³C-NMR (DMSO-*d*₆, 100 MHz): δ 196.1, 183.6, 154.1, 136.1, 128.3, 115.3, 114.3, 50.9, 45.6, 42.0, 34.2, 31.9, 29.8, 11.8; Anal. Calcd. for C₂₇H₃₉NO₅; C, 70.74; H, 8.89; N, 3.13; O, 17.61; Found: C, 70.87; H, 8.59; N, 3.06; O, 17.48; LC/MS (ESI): *m/z* = 383.19 [M]⁺.

4-((6-Hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(6-hydroxy-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl)benzaldehyde diethylammonium salt 5l

5l; as white solid (88 %, 1.20 g). IR (cm⁻¹): 3455, 3305, 3000, 2910, 1677, 1582, 1510, 1466, 1384, 1339; ¹H-NMR (CDCl₃, 400 MHz) 17.30 (s, 1H, OH), 9.90 (s, 1H, CHO), 8.23 (brs, 2H, NH), 7.56 (d, 2H, *J* = 8.0 Hz, Ph), 7.11 (d, 2H, *J* = 8.0 Hz, Ph), 5.85 (s, 1H, benzyl-H), 3.34 (s, 12H, 4CH₃), 3.03 (q, 4H, *J* = 7.3 Hz, CH₂CH₃), 1.25 (t, 6H, *J* = 7.3 Hz, CH₂CH₃); ¹³C-NMR (100 MHz, CDCl₃): δ = 192.1, 165.2, 164.1, 151.2, 150.0, 134.1, 129.5, 127.5, 91.6, 42.2, 35.1, 29.0, 28.7, 11.5; Anal. for C₂₂H₂₇N₅O₇; Calcd: C, 55.81; H, 5.75; N, 14.79; Found: C, 55.83; H, 5.76; N, 14.81; LC/MS (ESI): *m/z* = 473.48 [M]⁺.

5-((4-Chlorophenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylammonium salt 5m

5m; an oily product (90 %, 625 mg, 1.35 mmol). IR (KBr, cm⁻¹): 3049, 2954, 2865, 2499, 1738, 1699, 1590, 1483, 1375, 1292, 1258, 1225, 1205; ¹H NMR (400 MHz, CDCl₃): δ 13.32 (s, 1H, OH), 8.83 (brs, 2H, NH), 7.27 (d, 2H, *J* = 8.0 Hz, Ph), 7.00 (d, 2H, *J* = 8.0 Hz, Ph), 5.89 (s, 1H, benzyl-H), 2.88 (q, 4H, *J* = 7.3 Hz, CH₂CH₃), 2.31 (d, 4H, *J* = 5.1 Hz, CH₂), 1.19 (t, 6H, *J* = 7.3 Hz, CH₂CH₃), 1.09 (s, 3H, CH₃), 1.03 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 190.9, 141.0, 134.8, 131.0, 129.5, 128.3, 115.3, 91.1, 47.1, 42.7, 31.6, 31.5, 29.1, 28.2, 27.8, 11.3; Anal.

for C₂₃H₃₀ClN₃O₅; calcd: C, 59.54; H, 6.52; Cl, 7.64; N, 9.06; Found: C, 59.57; H, 6.51; Cl, 7.60; N, 9.02; LC/MS (ESI): *m/z* = 463 [M]⁺.

5-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(phenyl)methyl)-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylammonium salt 5n

5n; a white solid material; m.p: 215 °C; (93 %, 598 mg, 1.39 mmol). IR (KBr, cm⁻¹): 3027, 2948, 2867, 2156, 1683, 1593, 1451, 1374, 1291, 1257, 1141; ¹H-NMR (400 MHz, CDCl₃): δ 12.26 (s, 1H, OH), 9.31 (brs, 2H, NH), 7.12 (m, 5H, Ph), 5.52 (s, 1H, benzyl-H), 2.99 (q, 4H, *J* = 7.3 Hz, CH₂CH₃), 2.45 (d, 4H, *J* = 5.1 Hz, CH₂), 1.24 (t, 6H, *J* = 7.3 Hz, CH₂CH₃), 1.09 (s, 3H, CH₃), 1.03 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 198.5, 180.8, 152.5, 142.5, 128.0, 126.7, 125.1, 116.3, 90.9, 51.4, 45.9, 42.2, 33.0, 28.4, 27.6, 11.3; Anal. for C₂₃H₃₁N₃O₅; calcd: C, 64.32; H, 7.27; N, 9.78; Found: C, 64.29; H, 7.29; N, 9.80; LC/MS (ESI): *m/z* = 429 [M]⁺.

5-((4-Bromophenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylammonium salt 5o

5o; a white solid material; m.p: 208 °C; (89 %, 678 mg, 1.33 mmol); IR (KBr, cm⁻¹): 3093, 2939, 2885, 2829, 2551, 1746, 1686, 1576, 1506, 1466, 1416, 1268, 1241; ¹H NMR (400 MHz, CDCl₃): δ 13.31 (s, 1H, OH), 8.67 (brs, 2H, NH), 7.05 (m, 4H, Ph), 5.79 (s, 1H, benzyl-H), 2.79 (q, 4H, *J* = 7.3 Hz, CH₂CH₃), 2.35 (d, 4H, *J* = 5.1 Hz, CH₂), 1.21 (t, 6H, *J* = 7.3 Hz, CH₂CH₃), 1.11 (s, 3H, CH₃), 1.03 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 198.5, 180.1, 152.8, 140.5, 131.4, 130.7, 128.7, 128.6, 118.5, 115.6, 91.0, 50.9, 42.8, 31.6, 31.5, 29.2, 28.3, 27.8, 11.3; Anal. for C₂₃H₃₀BrN₃O₅; calcd: C, 54.34; H, 5.95; Br, 15.72; N, 8.27; Found: C, 54.35; H, 5.96; Br, 15.69; N, 8.30; LC/MS (ESI): *m/z* = 508 [M]⁺.

5-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(p-tolyl)methyl)-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylammonium salt 5p

5p; a white solid material; m.p: 213 °C; (91 %, 604 mg, 1.36 mmol). IR (KBr, cm⁻¹): 3150, 2955, 2867, 1690, 1592, 1508, 1375, 1256, 1232, 1167; ¹H NMR (400 MHz, CDCl₃): δ 13.31 (s, 1H, OH), 8.83 (brs, 2H, NH), 7.27 (d, 2H, *J* = 8.0 Hz, Ph), 7.00 (d, 2H, *J* = 8.0 Hz, Ph), 5.88 (s, 1H, benzyl-H), 2.83 (q, 4H, *J* = 7.3 Hz, CH₂CH₃), 2.31 (d, 4H, *J* = 5.1 Hz, CH₂), 2.23 (s, 3H, CH₃), 1.19 (t, 6H, *J* = 7.3 Hz, CH₂CH₃), 1.04 (s, 3H, CH₃), 1.02 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 196.5, 180.1, 152.8, 140.5, 131.4, 130.7, 128.7, 128.6, 118.5, 115.6, 91.0, 50.9, 42.8, 31.6, 31.5, 29.2, 28.3, 27.8, 20.9, 11.3; Anal. for C₂₄H₃₃N₃O₅; calcd: C, 64.99; H, 7.50; N, 9.47; Found: C, 64.95; H, 7.49; N, 9.50; LC/MS (ESI): *m/z* = 443 [M]⁺.

2-((4-Formylphenyl)(6-hydroxy-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate diethylammonium salt 5q

5q; a white solid material; m.p: 205 °C; (87 %, 594 mg, 1.3 mmol). IR (KBr, cm^{-1}): 3145, 2950, 2870, 1677, 1550, 1510, 1375, 1256, 1232, 1167; ^1H NMR (400 MHz, CDCl_3): δ 13.35 (s, 1H, OH), 9.92 (s, 1H, CHO), 8.80 (brs, 2H, NH), 7.30(d, 2H, $J = 8.0$ Hz, Ph), 7.05(d, 2H, $J = 8.0$ Hz, Ph), 5.85 (s, 1H, benzyl-H), 2.89(q, 4H, $J = 7.3$ Hz, CH_2CH_3), 2.30 (d, 4H, $J = 5.1$ Hz, CH_2), 2.26(s, 3H, CH_3), 1.22(t, 6H, $J = 7.3$ Hz, CH_2CH_3), 1.08(s, 3H, CH_3), 1.05(s, 3H, CH_3); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 198, 181.3, 152.8, 140.5, 131.4, 130.7, 128.7, 128.6, 118.5, 115.6, 91.0, 50.9, 42.8, 31.6, 31.5, 29.2, 28.3, 27.8, 20.9, 11.3$; Anal. for $\text{C}_{24}\text{H}_{31}\text{N}_3\text{O}_6$; calcd: C, 63.00; H, 6.83; N, 9.18; Found: C, 63.01; H, 6.84; N, 9.18; LC/MS (ESI): $m/z = 457$ [M] $^+$.

5-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(naphthalen-2-yl)methyl)-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate diethylammonium salt 5r

5r; an oily product (90 %, 646 mg, 1.35 mmol). IR (KBr, cm^{-1}): 3049, 2948, 2863, 2725, 1685, 1594, 1508, 1371, 1252, 1216; ^1H NMR (400 MHz, CDCl_3): δ 14.25 (s, 1H, OH), 7.46-7.22(m, 7H, naphthyl), 6.21 (s, 1H, benzyl-H), 3.27 (s, 6H, 2 CH_3), 3.25 (s, 6H, 2 CH_3), 3.14(q, 4H, $J = 7.3$ Hz, CH_2CH_3), 2.41 (q, 4H, $J = 5.1$ Hz, CH_2), 2.23 (s, 2H, CH_2), 1.37(t, 6H, $J = 7.3$ Hz, CH_2CH_3), 1.07(s, 3H, CH_3), 1.01(s, 3H, CH_3); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 199.1, 180.5, 165.5, 164.2, 152.5, 149.7, 136.8, 131.5, 129.9, 126.5, 124.2, 115.5, 114.7, 89.9, 50.9, 45.5, 41.7, 31.3, 30.7, 28.2, 11.3$; Anal. for $\text{C}_{27}\text{H}_{33}\text{N}_3\text{O}_5$; calcd: C, 67.62; H, 6.94; N, 8.76; Found: C, 67.65; H, 6.96; N, 8.80; LC/MS (ESI): $m/z = 479$ [M] $^+$.

2-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(naphthalen-2-yl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate diethylammonium salt 5s

5s; as solid (93 %, 1.33 g). IR (cm^{-1}): 3053 (s), 2943 (s), 2866 (s), 1688 (s), 1566 (s), 1511 (s), 1383 (s), 1241 (s), 1035 (s), 774 (s), 482 (s), 554 (s); ^1H -NMR (CDCl_3 , 400 MHz) δ 1.01 (bs, 12H, CH_3), 1.19 (t, $J = 7.3$ Hz, 6H, $\text{NH}_2\text{CH}_2\text{CH}_3$), 2.29 (bs, 8H, $\text{CH}_2 + \text{COCH}_2$), 2.88 (q, $J = 7.3$ Hz, 4H, NHCH_2CH_3), 6.32 (s, 1H, PhCH), 7.55-7.64 (m, 2H, ArH), 7.69 (t, $J = 7.4$ Hz, 1H, ArH), 7.91 (d, $J = 8.8$ Hz, 1H, ArH), 7.99 (d, $J = 6.6$ Hz, 1H, ArH), 8.10 (d, $J = 8.1$ Hz, 1H, ArH), 9.25 (d, $J = 8.0$ Hz, 1H, ArH), 1039 (s, 2H, NH_2), 14.25 (s, OH); ^{13}C -NMR (CDCl_3 , 100 MHz): δ 193.6, 182.8, 136.8, 135.4, 133.8, 131.5, 124.7, 116.8, 50.5, 130.6, 128.6, 129.1, 127.0, 45.3, 42.2, 33.9, 31.4, 29.8, 11.7; Anal. Calcd. for $\text{C}_{30}\text{H}_{39}\text{NO}_4$: C, 75.83; H, 8.05; N, 3.03; O, 13.29; Found: C, 75.71;

H, 8.23; N, 2.91; O, 13.40; LC/MS (ESI): $m/z = 477.29$ [M] $^+$.

Procedure for In vitro Urease Inhibition Assay

Reaction mixture comprising of 25 μL of enzyme (jack bean urease) (1 unit/well) solution and 55 μL of phosphate buffers (4 mM) containing 100 mM urea were incubated with 5 μL of test compounds dissolved in methanol (0.5 mM concentration) at 30 °C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weather burn [30]. Briefly, 45 μL each 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. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (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). The entire assays were performed at pH 6.8. Percentage inhibitions were calculated from the formula $100 - (\text{OD}_{\text{testwell}}/\text{OD}_{\text{control}}) \times 100$. Thiourea was used as the standard inhibitor of urease [31, 32].

Materials and methods for MD simulation and molecular docking studies

Receptor and ligand preparation

The crystal structure of helicobacter pylori (HP) urease in complex with acetohydroxamic acid, (PDB entry code 1E9Y) was retrieved from the protein data bank [33]. All the water molecules were removed from the PDB crystal structure and hydrogen atoms were added. This structure was followed by energy minimization with amber99 force field (<http://www.chempcomp.com>) in the molecular operating environment (MOE) Software packages [34]. The three dimensional structure of the compounds were constructed via Builder module implemented in MOE. Subsequently all the compounds structures were minimized by using MMFF94 force field [35] in MOE preceding to molecular docking studies.

Protocol selection

Initially docking was performed for both the isomers i.e. keto and enol form. For docking purpose, default docking parameters of MOE is used such as Triangle Matcher Algorithm with two different rescoring functions. London dG and GBVI/WSA dG were used to generate 30 poses of each ligand and were saved in MOE database. Finally, docking results were analyzed by visualizing several interactions of compounds within binding pocket of proteins.

Molecular dynamic simulation

The keto and enol complexes were energy-minimized to eliminate possible steric strain up to 0.1 gradients by using AMBER99 force field. The relaxed complexes were then subjected to MD simulations using MOE 2013.0801 software. Each complex was gradually simulated at 300 K for 100 ps, in order to simulate the physiological conditions, system is allowed to maintain at physiological temperature of 300 K. The temperature is attained gradually, to avoid protein destruction, over a period of 100 ps. Initially, protein is heated from 0 to 50 K, followed by its ramping to 100, 200 and finally 300 K and then equilibrated at 300 K for even distribution of water molecules keeping protein molecule constrained. After equilibration step MD simulation was performed for 5 ns by using the Nose-Poincare-Anderson (NPA) method [36]. To make ensemble trajectories NVT ensemble was used.

The trajectory output files were saved after every 1 ps for future analysis. Equilibration was monitored by convergence in terms of the temperature, energy, density and the RMSD (root-mean-squared deviations) of the backbone atoms as compared to the crystal structure of both complexes.

Results and discussion

Chemistry

In our continued interest [30, 37–47] in the development of highly expedient methods for the synthesis of diverse heterocyclic compounds of biological importance via one-pot multi-component reactions (MCRs) and avoiding organic solvents during the reactions in organic synthesis leads to efficient, environmentally benign reagents, clean, and economical technology (Green Chemistry Concepts). In the present investigation, reaction of equimolar amounts of barbituric acid **1a,b** dimedone **2** with aldehyde **3** in presence of aqueous diethylamine medium at RT afforded zwitterionic adducts **4a–z** and **5a–s** in quantitative yields by simple filtration (Scheme 1).

Biological activity

Thirty-two new derivatives of barbituric acid as zwitterionic adducts of diethyl ammonium salts having *bis*(6-hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl) (**4a–h**), *bis*-(6-hydroxypyrimidine-2,4(1*H*,3*H*)-dione) (**4i–4l**), (2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate (**4m–4z**), 4-((6-Hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(6-hydroxy-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl) benzaldehyde (**5l**), (2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl) methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate (**5m–5r**) and twelve derivatives of dimedone as

zwitterionic adducts of diethyl ammonium salts having *bis*-(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en) (**5a–k** and **5s**) as basic nucleus were screened in vitro for their urease enzyme inhibition potential against thiourea ($IC_{50} = 21.2 \pm 1.3 \mu M$), as a standard tested compounds (Table 1).

Among barbituric acid zwitterionic adducts (**4a–h**) having *bis*(6-Hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl) ring as basic nucleus, all compounds **4a**, **4b**, **4d**, **4e**, **4g** and **4f** showed IC_{50} values 39.3 ± 0.36 , 34.4 ± 1.57 , 31.6 ± 0.79 , 27.5 ± 0.12 , 28.5 ± 0.41 , and $40.3 \pm 0.32 \mu M$ respectively, and were found to be the potent urease inhibitors except compounds **4c** ($IC_{50} = 54.2 \pm 0.47 \mu M$) and **4f** ($IC_{50} = 54.2 \pm 0.83 \mu M$), while compared with the standard compound thiourea ($IC_{50} = 21.2 \pm 1.3 \mu M$).

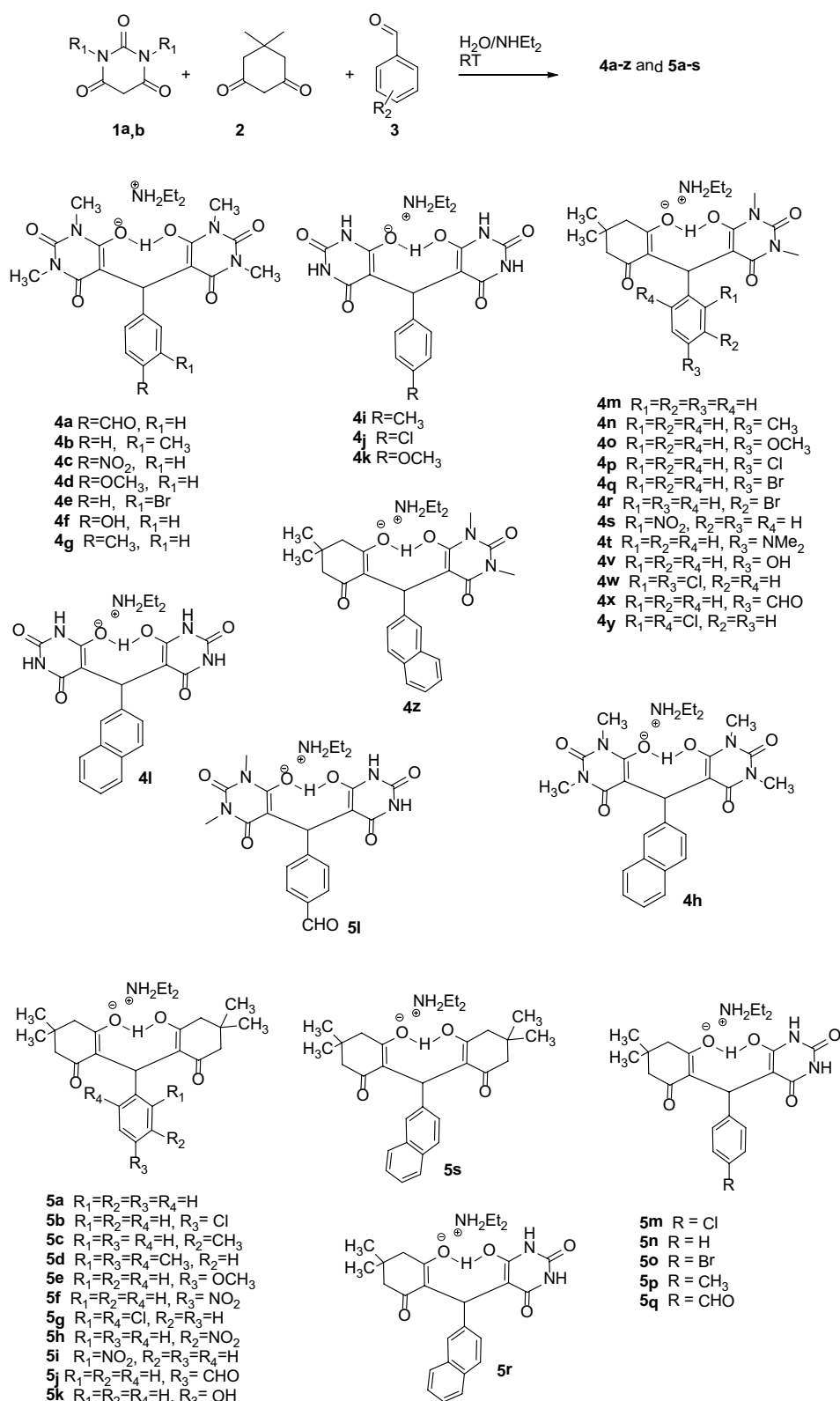
Among the barbituric acid derived derivatives (**4i–4l**), having *bis*(6-hydroxypyrimidine-2,4(1*H*,3*H*)-dione) as backbone, all tested compounds *i.e.* **4i** ($IC_{50} = 17.6 \pm 0.23 \mu M$), **4j** ($IC_{50} = 22.3 \pm 0.73 \mu M$), **4k** ($IC_{50} = 25.8 \pm 0.23 \mu M$) and **4l** ($IC_{50} = 22.7 \pm 0.20 \mu M$) were found to be potent inhibitors of urease enzyme. Methyl substituted phenyl ring containing compound **4i** ($IC_{50} = 17.6 \pm 0.23 \mu M$) was the most active candidate of the series.

Third series of the derivatives of barbituric acids having (2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate ring as basic nucleus (**4m–4z**) were also evaluated for their urease enzyme inhibition. Compounds **4m** ($IC_{50} = 39.3 \pm 0.79 \mu M$), **4n** ($IC_{50} = 41.2 \pm 0.58 \mu M$), **4p** ($IC_{50} = 39.7 \pm 0.70 \mu M$), **4q** ($IC_{50} = 24.6 \pm 0.42 \mu M$), **4r** ($IC_{50} = 27.5 \pm 0.19 \mu M$), **4x** ($IC_{50} = 38.5 \pm 0.28 \mu M$), and **4z** ($IC_{50} = 39.8 \pm 1.38 \mu M$) was found to be potent urease inhibitors against the standard thiourea.

Among fourth series of the derivatives of barbituric acid having (2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate) ring as basic nucleus (**5m–5r**), compound **5n** ($IC_{50} = 23.7 \pm 0.57 \mu M$), **5o** ($IC_{50} = 34.6 \pm 0.79 \mu M$), **5p** ($IC_{50} = 27.4 \pm 0.54 \mu M$), and **5q** ($IC_{50} = 41.6 \pm 0.41 \mu M$), showed potent urease inhibition. All other compounds found to be weak urease inhibitors.

Similarly dimedone derivatives, *bis*-(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en) ring containing compounds **5a–s** were also evaluated for their in vitro urease enzyme inhibition potential. Compounds **5b** ($IC_{50} = 29.7 \pm 0.67 \mu M$), **5e** ($IC_{50} = 39.8 \pm 0.75 \mu M$), and **5k** ($IC_{50} = 43.8 \pm 0.33 \mu M$), showed good enzyme inhibition. All other compounds found to be significant to weak urease inhibitors ($IC_{50} = 49.0 \pm 0.55$ – $210.1 \pm 0.29 \mu M$).

On the basis of the evaluated urease inhibition abilities of the above five different series of barbituric acid and



Scheme 1 Synthesis of compounds 4a–z and 5a–s

Table 1 In vitro urease inhibition activity of compounds 4a–z and 5a–s

Compound	Urease inhibition IC ₅₀ ± SEM [μM]	Compound	Urease inhibition IC ₅₀ ± SEM [μM]
4a	39.3 ± 0.36	4x	38.5 ± 0.28
4b	34.4 ± 1.57	4y	83.4 ± 1.00
4c	54.2 ± 0.47	4z	39.8 ± 1.38
4d	31.6 ± 0.79	5a	74.5 ± 0.88
4e	27.5 ± 0.12	5b	29.7 ± 0.67
4f	54.2 ± 0.83	5c	61.4 ± 1.12
4g	28.5 ± 0.41	5d	51.3 ± 0.45
4h	40.3 ± 0.32	5e	39.8 ± 0.75
4i	17.6 ± 0.23	5f	106.4 ± 1.49
4j	22.3 ± 0.73	5g	170.7 ± 1.55
4k	25.8 ± 0.23	5h	49.0 ± 0.55
4l	22.7 ± 0.20	5i	210.1 ± 0.29
4m	39.3 ± 0.79	5j	72.6 ± 0.59
4n	41.2 ± 0.58	5k	43.8 ± 0.33
4o	83.0 ± 0.66	5l	17.2 ± 0.44
4p	39.7 ± 0.70	5m	65.9 ± 0.61
4q	24.6 ± 0.42	5n	23.7 ± 0.57
4r	27.5 ± 0.19	5o	34.6 ± 0.79
4s	109.7 ± 1.10	5p	27.4 ± 0.54
4t	142.1 ± 0.64	5q	41.6 ± 0.41
4v	52.2 ± 1.26	5r	82.8 ± 0.72
4w	59.4 ± 0.98	5s	123.2 ± 0.37
STD. Thiourea	21.2 ± 1.3		

dimedone derivatives as zwitter ion adduct compounds **4i** (IC₅₀ = 17.6 ± 0.23 μM) and **5l** (IC₅₀ = 17.2 ± 0.44 μM) found to be the most active compounds and showed more urease inhibition potential than the standard compound thiourea.

Molecular modeling and docking studies

In order to obtain deep insight into the binding mechanism of barbituric acid derivatives within the active site of urease enzyme and to obtain further validations of experimental results, MD simulation studies were performed.

Forty-four barbituric acid derivatives (1–44) were docked into the binding pocket of urease. All the compounds were observed to accept analogous conformations with similar binding mode around the binding site of urease and these compounds were found to interact with nickel metal ions and the hotspot binding pocket residues (His137, His138, Ala169, KCX219, Asp362, Ala366 etc.) Visual inspection for predicted binding conformations of most potent compounds **5l** and **4i** (IC₅₀ = 17.2 ± 0.44 M and IC₅₀ = 17.6 ± 0.23 M) revealed that both compounds can adopt conformation for a better fit into the binding groove of urease.

Further analysis of the top ranked poses of these compounds revealed that these compounds involved in multiple hydrogen bonding interactions with His138, Ala169, KCX219, Gly279, Asp362 and Arg338 residues. Compound **5l** was found to be the least potent among the active ligands with IC₅₀ value of 210.1 ± 0.29 μM. Additionally; compounds **4a–4h**, **4j–4r**, and **4v–5e**, **5m–5q**, **5h** and **5k** showed a good urease inhibitory activity. In case of most active compound **5l** the amine moiety adjacent to the carbonyl group formed hydrogen bond with the side chain oxygen of modified lysine KCX 219 at a distance of 2.61 Å. Another hydrophilic interaction found between carbonyl moiety of aldehyde group at para position of benzene ring and NH of His323 at a distance of 2.82 Å. The compound is further stabilized by the numerous hydrophilic interactions provided by catalytic residues Ala169 (2.75 Å), Gly279 (1.97 Å) and Asp362 (3.14 Å) NH moiety with carbonyl oxygen of compound **5l**. Another important residue Arg338 formed two hydrogen bonds with carbonyl oxygen of pyrimidine ring at a distance of 2.32 and 3.03 Å, respectively. The best docked conformation of compound **5l** predicted by MOE showed that the compound is deeply inserted within the urease binding site which is further stabilize by multiple hydrophilic and hydrophobic interactions, contributing to the higher activity of this compound. The binding mode of **5l** is represented in Fig. 1a.

The binding mechanism of compound **4i** reveals that multiple hydrogen bonding interactions found between ligand and hotspot residues. The carbonyl of pyrimidine moiety engaged in hydrogen bonding interaction with the NH of KCX219 and Arg338 at a distance of 2.71 and 2.35 Å, respectively. The NH of pyrimidine ring form hydrogen bond with the oxygen of Ala169, Gly279 and Asp362 at a distance of 2.88, 1.91 and 3.38 Å, respectively. Moreover these interactions are further stabilized by polar interactions with the His138. Due to the absence of aldehyde group at *meta* position of compound **4i**, it is unable to make hydrophilic interaction with His323. The binding pattern of **4i** in the ligand binding site of urease is shown in Fig. 1b.

Compounds **4j** and **5n** adopted a similar binding mechanism to **5l** with some minor changes. The carbonyl oxygen of these compounds are hydrogen bonded to NH of Arg338 (2.19 and 2.13 Å) and KCX219 (2.7 and 2.91 Å), while NH of pyrimidine moiety of these compounds are engaged in hydrogen bond interactions with Ala169 (2.86 and 2.88 Å), Gly279 (1.87 and 1.96 Å) and Asp 362 (3.01 and 3.33 Å) as observed in **5l** (Fig. 1). The non substituted benzene ring of the molecule is exposed to the surface and not found to be involved in such interactions. The docked orientation of **4j** and **5n** is shown in Fig. 1c, d.

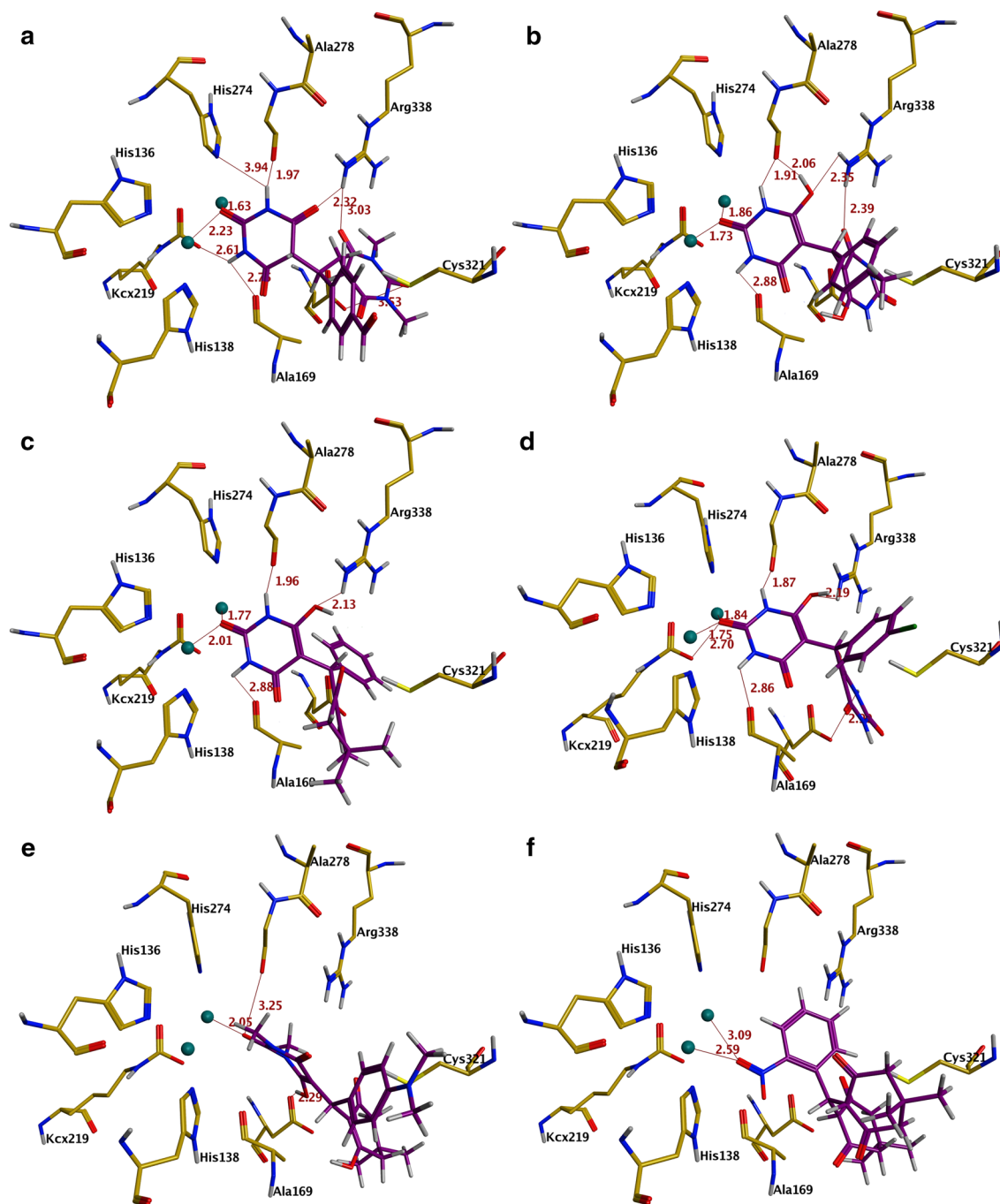


Fig. 1 The docked poses of urease inhibitors: most active **5i** (a), **4i** (b), active **5n** (c), **4j** (d) and least active **4t** (e), **5g** (f). The interacting residues are presented in yellow stick while the ligands are shown in purple sticks

To explain the inactivity of compounds **4s–4t**, **5f–5g**, **5i** and **5s** all the inactive compounds were also docked in the urease binding cavity by using MOE. By docking pose analysis, it is evident that all the inactive compounds were poorly occupied in the binding site. The carbonyl

oxygen of **4t**, **5g** and **5i** is replaced by an alkyl group as a result, hydrogen bonding interactions with catalytic residues are lost. The docked poses are shown in Fig. 1e, f. Similarly, the compounds **4s**, **5i** containing nitro group at R1 position instead of electron donating group may

be one of the reasons for the inactivity of these compounds. These compounds interact with nickel ions but interactions with the catalytic residues are not as effective as observed in case of active compounds and shown in Fig. 1e, f). Consequently, absence of hydrogen bond interactions of these inactive compounds with crucial residues Ala169, Arg338 and Asp362 might be the reason for the inactivity of these compounds in the in vitro assay.

Molecular dynamic simulations

In order to understand the binding mechanism of barbituric acid derivatives molecular dynamic (MD) simulation was performed. The enol form of barbituric acid derivatives is found to be more stable during MD simulation as compared to its keto form. The keto form established only a weak interaction with nickel as compared to the enol. Interaction with nickel ion is crucial for inhibitory mechanism of urease inhibitors. The two forms disagree after 500 ps simulation as the distance between Ni and keto form increases gradually. To obtain further interaction pattern for two different form of barbituric acid derivatives, docking was also performed with both the possible forms. In the docking experiment, similar interactions with catalytic residues were observed except interaction with nickel metal. The obtained conformation explained the three dimensional structure of protein, which can be changed without fluctuating covalent bonds. The RMSD plot of protein conformation verses time for both the complexes is given in Fig. 2, which support the stability of enol form as nickel complex.

Conclusion

This study conclude that a simple one step chemistry can generate extra-ordinary array bioactive compounds. During this study, we synthesized barbituric acid derivatives by simple filtration and evaluated for their urease inhibitory activity. Compounds (4a–4z

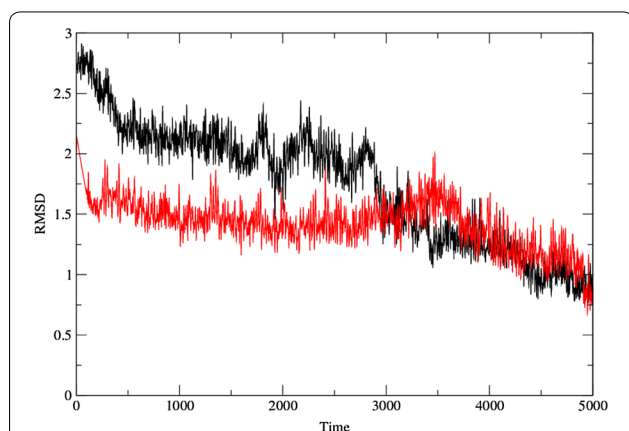


Fig. 2 The RMSD plot of barbituric acid derivatives enol and keto complexes. *Red color* represents the enol form and *black color* represents the keto form RMSD

and 5a–s) were evaluated for their urease inhibition potential in vitro against the standard compound thiourea ($IC_{50} = 128.8 \pm 2.1 \mu\text{M}$). Compounds 4i ($IC_{50} = 17.6 \pm 0.23 \mu\text{M}$) and 5l ($IC_{50} = 17.2 \pm 0.44 \mu\text{M}$) were found to be the most active members of the series with several fold more urease inhibition activity than the standard compound thiourea. The promising result of the current study indicates that barbituric acid derivatives can be investigated for the treatment of urease associated complications, such as peptic ulcer.

Additional file

Additional file 1. Supplementary information containing the spectra of the synthesized compounds.

Authors' contributions

AB proposed and designed research subject; GL performed research; FA carried out the assay of urease inhibition; SJ carried out the computation studies; AB, SY, and ZUH wrote the paper. AMA and MIC helped in the result and discussion and edit the final manuscript; All authors read and approved the final manuscript.

Author details

¹ Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia. ² Department of Chemistry, Faculty of Science, Alexandria University, P.O. Box 426, Ibrahimia, Alexandria 21321, Egypt. ³ Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt. ⁴ H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan. ⁵ Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan.

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Competing interests

The authors declare that they have no competing interests.

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