European Journal of Medicinal Chemistry 84 (2014) 146–154

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Zwitterionic pyrimidinium adducts as antioxidants with therapeutic potential as nitric oxide scavenger

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ARTICLE INFO

Article history:
Received 19 May 2014
Received in revised form
4 July 2014
Accepted 8 July 2014
Available online 9 July 2014

Keywords:
Reactive oxygen species (ROS)
Nitric oxide (NO)
Zwitterion
Barbituric acids
Cancer
Diabetes
Chronic inflammatory disease and heart failure

ABSTRACT

A variety of zwitterionic adducts were synthesized by using means green chemistry method. The products contain the biologically active barbituric acid moiety embedded in zwitterion products. Both features are pharmaceutically relevant. The chemical structures were deduced by ^{1}H-, ^{13}C-, NMR and HRMS spectral analysis, and X-ray diffraction techniques. *In vitro* evaluation for the antioxidant activities were carried out towards the inhibition of nitric oxide (NO) radical, known to regulate a mechanism of signals for various cellular functions. NO also play an important role as a mediator of various pathological conditions responsible for cellular damages such as strokes, cancers, diabetes, chronic heart failure and inflammatory disease and various neurodegenerative disorders. All tested compounds were found to be more potent nitric oxide scavengers as compared to standard drug ascorbic acid (IC_{50} = 618 ± 2.0 μM). Compounds **4c** and **e** exhibiting several hundred fold more activity against nitric oxide radical with IC_{50} values of 69 ± 1.66 and 70.1 ± 0.89 μM respectively, as compared to standard drug ascorbic acid (IC_{50} = 618 ± 2.0 μM).

1. Introduction

The recent literature supports that the excess of reactive nitrogen (RNS) and oxygen species (ROS) plays an important role in the degenerative or pathological processes leading various diseases, such as liver and lung damages, aging, Alzheimer's and coronary heart disease, neurodegenerative disorders, atherosclerosis, as well as breast cancers through damage to DNA and other vital biomolecules [1–7]

Antioxidants are therefore considered as significant factors for the remediation and prevention of degenerative diseases including cancer [8]. It is assumed that compounds functionalized with functional groups having potential antioxidant properties could be useful in chemoprevention and chemotherapy. Several anti-necrotic, anti-inflammatory, neuroprotective, digestive, and hepatoprotective drugs have recently been shown to act through anti-radical and/or antioxidant scavenging mechanisms [9,10].

Previous studies have suggested that chemopreventive and antioxidant properties of numerous compounds such as ascorbic acid, α-tocopherol, carotenoids, proteins, peptides, amino acids, flavonoids and polyphenols, are due to the presence of phenolic hydroxyl group(s) [11–14], and heterocyclic systems [15].

The six membered pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione scaffold is an interesting heterocyclic ring system present in a large number of natural or synthetic compounds and have wide applications as pharmaceuticals. Moreover, literature revealed that the 5-alkylated barbituric acids have special significance in the design and synthesis of novel chemotherapeutic agents with remarkable activities such as anticancer, HIV-1 and HIV-2 protease inhibitors, anticonvulsant, sedative-hypnotic [16–19], etc.

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http://dx.doi.org/10.1016/j.ejmech.2014.07.026
0223-5234/

Similarly, the related pyrimidine-trione derivatives are the key components of hypnotic and anti-inflammatory drugs, such as seconal, veronal, sodium pentothal phenobarbital, and bucolome (Fig. 1) [20–23].

In the light of the aforementioned facts, and in continuation for our interest in the synthesis of biologically active heterocyclic compounds, we describe here the synthesis of a series of previously reported [24,25] and new zwitterionic adducts. These compounds were subjected to biological evaluation to determine antioxidant activity and inhibition of NO production.

2. Results and discussion

2.1. Chemistry

Green chemistry is the most robust and economical approach for the generation of diverse classes of compounds. Recently several, research groups are involved in employing green chemistry for the generation of valuable scaffolds with interesting biological activities. The chemistry which involves aqueous diethylamine medium catalyzed formation of zwitterionic adduct **4**, by 3 components reactants is a powerful tool for the generation of privileged therapeutic scaffolds, due to its efficiency, selectivity, and mild and environmentally benign conditions.

The synthesis of compound **4** has been achieved earlier through a very mild and one-pot synthesis [24–28] (Scheme 1). Products were obtained by reacting barbituric acid **1**, dimedone **2**, with aldehyde **3**, for 1–5 h, in presence of aqueous diethylamine medium. This one-pot three-component reaction completed spontaneously at room temperature. "Trimolecular adduct salts" **4a**–**4w** were obtained in quantitative yields by simple filtration.

Products **4f**, **i**, **n**, **r**, **v** are new products and obtained as described for previous zwitterion derivatives [24].

The structures of the compounds were deduced by ^{1}H NMR, ^{13}C NMR, MS and IR, spectroscopy and elemental analysis, and X-ray crystallography.

2.2. X-ray diffraction

The structures of compounds **4a**, **b**, **o**, **c**, **e** and **i** were unambiguously deduced by single-crystal X-ray diffraction (Fig. 2) technique. Crystallographic data for the structures were deposited to the Cambridge Crystallographic Data Center as supplementary publication No. CCDC-957026, CCDC-957025, CCDC-933624, CCDC-1001798, CCDC-1001799 and CCDC-1001745 respectively [24].

The compounds of **4c**, **e**, **i** were obtained as crystals by slow diffusion of diethyl ether solution of pure compounds **4c**, **e**, **i** in dichloromethane at room temperature followed by allowed to stand for 2 days. The structures were resolved by direct methods by using the SHELXS97 program [29] in the SHELXTL-plus package, and refined by a full-matrix least-squares procedure on *F2* using SHELXS97. Diffraction data were collected on a Bruker SMART APEXII CCD diffractometer. The crystal structure and refinement data of compounds **4c**, **e**, **i** are listed in Table 1. The final atomic coordinates for all atoms and a complete listing of bond distance and angles are presented in Supplementary Tables 1–6. ORTEP drawings of final X-ray model of compounds **4c**, **e**, **i** with the atomic numbering scheme is presented in Fig. 3, while crystal packing presentation of compounds **4c**, **e**, and **i** is shown in Fig. 4.

2.3. Biological activity

Three different series of zwitterionic pyrimidinium adducts were evaluated for their nitric oxide scavenging potential and results are presented in (Table 2). Series 1 (**4a**–**4i**) comprises of compounds having *bis*(6-hydroxy-*N*,*N*-1,3-dimethyl pyrimidinium-2,4,6-trione) rings linked by phenyl substituted methane bridge. Whereas members of series 2 (**4j**–**4m**) lack the *N*,*N*-dimethyl groups. In compounds of series 3 (**4o**–**4r**) one of the (6-hydroxy-*N*,*N*-1,3-dimethyl pyrimidinium-2,4,6-trione) ring is substituted by dimethyl 6-oxocyclohexene ring. All of these compounds showed several hundred times more nitric oxide scavenging activity (IC_{50} = 75 ± 2.0–466 ± 4.0 μM), than the standard ascorbic acid (IC_{50} = 681 ± 2.0 μM).

The electron withdrawing *p*–NO_2 substituted phenyl ring containing compound **4c** (IC_{50} = 69 ± 1.66 μM) was found to be the most active member of series 1. The NO scavenging potential found to be several hundred fold more than the standard ascorbic acid (IC_{50} = 681 ± 2.0 μM). A slight decrease in activity was observed when *p*-NO_2 phenyl ring is replaced with *p*-chloro (**4a**, IC_{50} = 75 ± 4.33 μM), *m*-bromo (**4e**, IC_{50} = 70.1 ± 0.89 μM) phenyl and naphthalene (**4h**, IC_{50} = 79.1 ± 2.92 μM) rings.

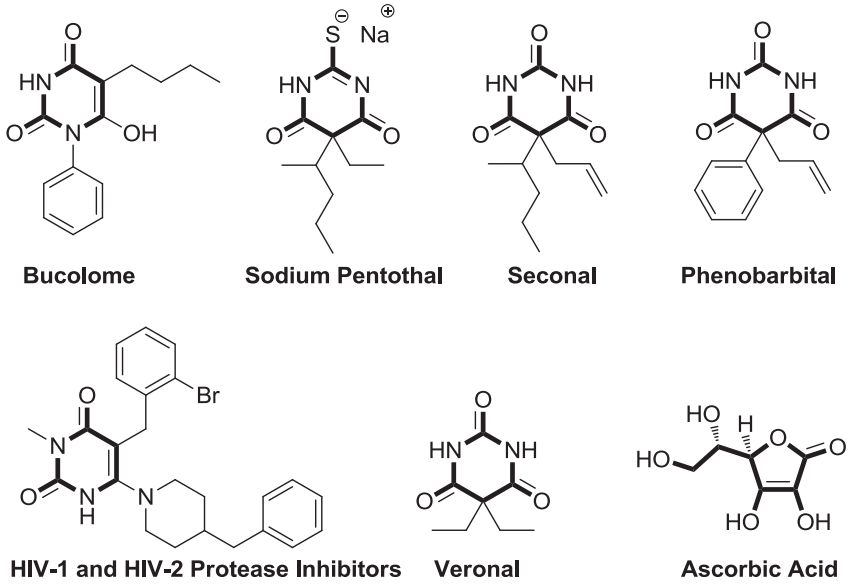
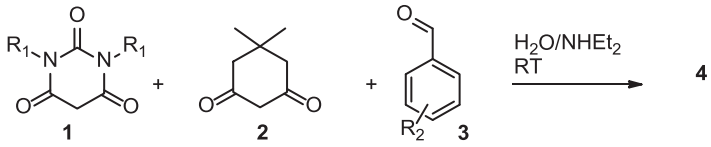
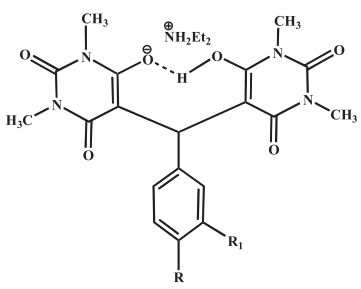
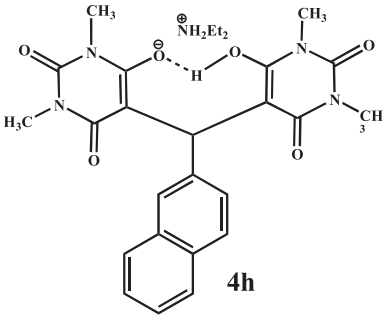


Fig. 1. Biologically active barbituric acids derivatives and ascorbic acid.

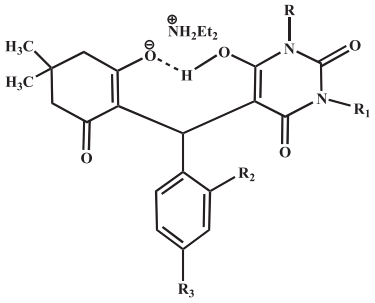




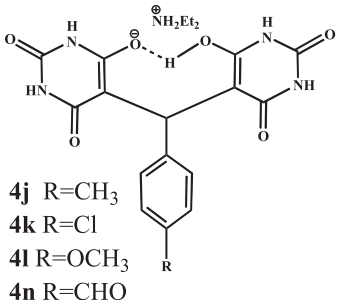
4a R=Cl, R_1=H
4b R=CH_3, R_1=H
4c R=NO_2, R_1=H
4d R=OCH_3, R_1=H
4e R=H, R_1=Br
4f R=OH, R_1=H
4g R=H, R_1=CH_3
4i R=CHO, R_1=H



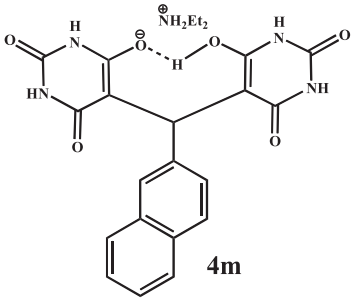
4h



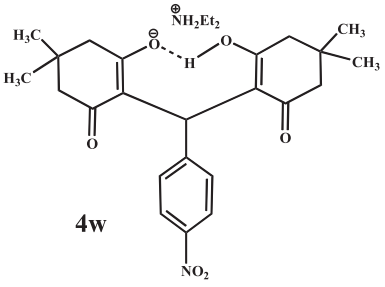
4o R=R_1=CH_3, R_2=R_3=H
4p R=R_1=CH_3, R_2=R_3=Cl
4q R=R_1=CH_3, R_2=H, R_3=NO_2
4r R=R_1=CH_3, R_2=H, R_3=CHO
4s R=R_1=R_2=R_3=H
4t R=R_1=R_2=H, R_3=CH_3
4v R=R_1=R_2=H, R_3=CHO



4j R=CH_3
4k R=Cl
4l R=OCH_3
4n R=CHO



4m



4w

Scheme 1. Synthesis of compounds **4**.

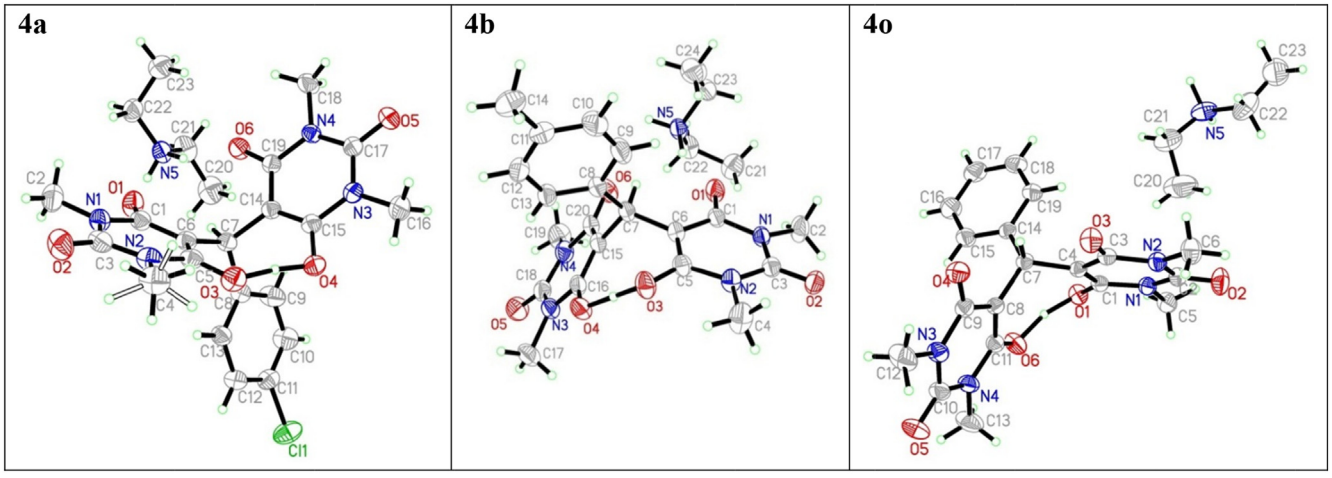


Fig. 2. ORTEP representation of the structure of **4a**, **b**, **o**.

Table 1
The crystal and experimental data of compounds **c** and **e**, **i**.

	Compound **c**	Compound **e**	Compound **i**
Empirical formula	$C_{24}H_{29}N_5O_8$	$C_{23}H_{29}BrN_5O_6$	$C_{24}H_{30}N_5O_7$
Formula weight	515.52	551.42	500.53
Temperature	273 (2)	273 (2)	273° K
Wavelength	0.71073 Å	0.71073 Å	0.71073 Å
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	P2 1/*n*	P2 (1)/*n*	P2 (1)/*n*
a	11.6202 (10) Å	9.9073 (11) Å	11.4427 (15) Å
b	14.1044 (13) Å	14.5554 (16) Å	14.229 (2) Å
c	15.5263 (14)Å	17.8380 (18) Å	15.643 (2) Å
α	90°	90°	90°
β	92.511 (2)°	98.696 (3)°	91.800 (4)°
γ	90°	90°	90°
Volume	2542.3 (4) A^3	2542.8 (5) A^3	2545.7 (6) A^3
Z	4	4	4
Calculated density	1.347 mg/m^3	1.440 mg/m^3	1.306 mg/m^3
Absorption coefficient	0.103 mm^{-1}	1.664 mm^{-1}	0.097 mm^{-1}
F (000)	1088	1140	1060
Crystal size	0.52 × 0.38 × 0.35 mm	0.26 × 0.14 × 0.11 mm	0.54 × 0.34 × 0.28 mm
θ range	1.95–25.49°	1.81–25.50°	1.94–25.50°
Reflections collected	14,821	14,853	14,914
Reflections unique	4629	4621	4610
(R_{int})	0.0182	0.0639	0.0452
R_1 with $I > 2\sigma(I)$	0.0624	0.0515	0.1051
R_2 with $I > 2\sigma(I)$	0.1790	0.1096	0.3109
R_1 for all data	0.0774	0.1117	0.1748
R_2 for all data	0.1962	0.1316	0.3786
Goodness of fit	1.041	1.014	1.165
max/min ρ e$A^{\circ-3}$	0.427 and −0.316	0.332 and −0.368	1.442 and −0.453
CCDC number	1001798	1001799	1001945

p-Methoxy phenyl ring containing compound **4l** (IC_{50} = 206.5 ± 1.0 μM) was the most active member of series 2 (**4j**–**4m**) with several hundred times more scavenging potential than the standard, ascorbic acid (IC_{50} = 681 ± 2.0 μM). The antioxidant potential significantly decreased when methoxy group is replaced with chloro (**4k**, IC_{50} = 304 ± 4.5 μM), napthyl (**4m**, IC_{50} = 308 ± 2.0 μM) and methyl (**4j**, IC_{50} = 377 ± .21 μM) substituents.

Third series of compounds **4o**–**4r**, having cyclohexene and *N*,*N*-dimethyl pyrimidone rings, also showed a potent nitric oxide scavenging ability and found to be more active than ascorbic acid. The most active *o*,*p*-dicholoro (IC_{50} = 174.9 ± 3.65 μM) phenyl ring containing compound **4p** showed a five hundred times more antioxidant potential than ascorbic acid.

However, when we compared the activities of *p*-nitro substituted phenyl ring containing compounds **4q** (IC_{50} = 466 ± 1.40 μM) and **4c** (IC_{50} = 69 ± 1.66 μM), several fold more potent activity of **4c** indicated the significant role of 6-hydroxy *N*,*N*-dimethyl pyrimidone ring to enhance the nitric oxide scavenging potential. The observation further supported by the decrease activity of **4r** (IC_{50} = 380 ± 4.08 μM), having dimethyl cyclohexene and *N*,*N*-dimethyl pyrimidone rings, as compared to *bis*-6-hydroxy *N*,*N*-dimethyl pyrimidone ring containing compound **4i** (IC_{50} = 113 ± 0.53 μM).

3. Conclusion

In conclusion, we have developed an efficient methodology for the construction of zwitterionic adducts through tandem Aldol/Michael addition reactions between barbituric acid and dimedone with aldehydes catalyzed by aqueous diethylamine. The final products were obtained in high yields. This provides a rapid means of synthesizing a variety of trimolecular adducts. Generality and applicability of a wide range of substrates is the key advantage of our method for the synthesis of zwitterionic adduct derivatives, potentially as important bioactive compounds. All compounds were found to be potent antioxidant against NO free radicals and more active than the standard ascorbic acid. The NO radicals play a key role in pathogenesis of various types of cancers, diabetes, strokes, cardiovascular complications associated to chronic inflammatory disease and heart failure, various neurodegenerative disorders and atherosclerosis and its inhibition has therapeutic significance. The promising results indicate that synthesized zwitterionic compounds can be potential candidates to use as antioxidant in future to overcome associated health disorders.

4. Experimental

General: All the chemicals were purchased from Aldrich, Sigma–Aldrich, Fluka, etc, and used without further purification, unless otherwise stated. All melting points were measured on a Gallenkamp 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 Jeol-400 NMR spectrometer. ^1H NMR (400 MHz), and ^{13}C NMR (100 MHz) were recorded in either deuterated dimethylsulphoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$) on Bruker 400 MHz. Chemical shifts (δ) are referred in terms of ppm and *J*-coupling constants are given in Hz. Mass spectra were recorded on a Jeol of JMS-600H. Elemental analysis was carried out on Elmer 2400 Elemental Analyzer; CHN mode.

4.1. General procedure for Aldol condensation Michael addition for the synthesis of **4** (GP1)

A mixture of aldehyde **3** (1.5 mmol), **1** and **2** (3 mmol) as well as Et_2NH (1.5 mmol, 155 μL) in 3 mL of degassed H_2O (bubbling nitrogen through the water) was stirred at room temperature for 1–5 h until TLC showed complete disappearance of the reactants.

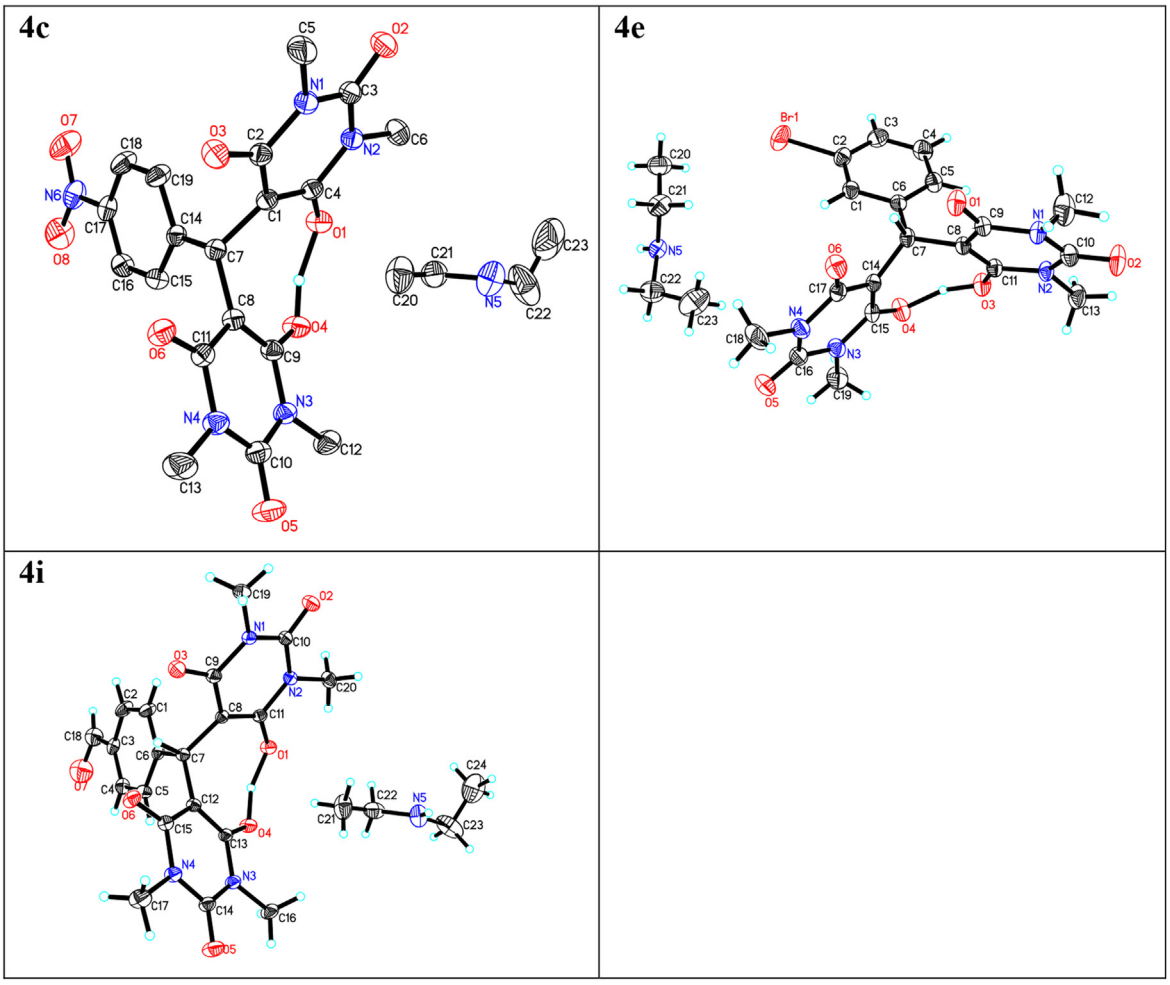


Fig. 3. ORTEP representation of the structure of **4c**, **e**, **i**.

The precipitate was removed by filtration and washed with ether (3 × 20 mL). The solid was dried to afford pure products **4a**–**w**.

*4.1.1. 5,5′-((4-Chlorophenyl)methylene)bis(1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione) diethylaminium salt (**4a**)*

4a was prepared from 1,3-dimethylbarbituric acid **1a**, and *p*-chlorobenzaldehyde according to the general procedure (**GP1**) yielding colorless crystals (1.44 g, 2.85 mmol, 95%). m.p.: 103 °C; IR (KBr, cm^{-1}): 3450, 3198, 2988, 1698, 1603, 1479, 1385; ^1H NMR (400 MHz, $CDCl_3$): δ 17.66 (s, 1H, OH), 7.18 (d, 2H, J = 8.8 Hz, Ph), 7.05 (d, 2H, J = 8.8 Hz, Ph), 5.80 (s, 1H, benzyl-H), 3.34 (s, 12H, $4CH_3$), 3.06 (q, 4H, J = 7.3 Hz, CH_2CH_3), 1.30 (t, 6H, J = 7.3 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.3, 164.3, 151.7, 138.6, 134.8, 128.2, 126.3, 91.7, 42.1, 34.2, 28.9, 28.7, 11.5; LC/MS (ESI): 507 $[M]^+$; Anal. for $C_{23}H_{30}ClN_5O_6$; Calcd: C, 54.38; H, 5.95; Cl, 6.98; N, 13.79; Found: C, 54.38; H, 5.94; Cl, 7.01; N, 13.81.

The structure of **4a** was unambiguously deduced by single-crystal X-ray diffraction structure analysis (Bruker AXS GmbH). CCDC-957026 contains the supplementary crystallographic data for this compound. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. A colorless crystal suitable for X-ray analysis was obtained from recrystallization the compound from DCM/Et_2O at room temperature after 2 days.

*4.1.2. 5,5′-(p-Tolylmethylene)bis(1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione) diethylaminium salt (**4b**)*

4b was prepared from 1,3-dimethylbarbituric acid **1a**, and *p*-tolualdehyde according to the general procedure (**GP1**) yielding colorless needle materials (1.41 g, 2.91 mmol, 97%). m.p.: 152 °C; IR (KBr, cm^{-1}): 3455, 3210, 2984, 2820, 1560, 1449, 1359; ^1H 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, $4CH_3$), 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}C NMR (100 MHz, $CDCl_3$): δ = 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; LC/MS (ESI): 487$[M]^+$; Anal. for $C_{24}H_{35}N_5O_6$; Calcd: C, 59.12; H, 6.82; N, 14.36; Found: C, 59.13; H, 6.81; N, 14.35.

The structure of **4b** was confirmed by X-ray crystal structure analysis (Bruker AXS GmbH). CCDC-957025 contains the supplementary crystallographic data for this compound. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. A colorless crystal suitable for X-ray analysis was obtained from recrystallization the compound from DCM/Et_2O at room temperature after 2 days.

*4.1.3. 5,5′-((4-Nitrophenyl)methylene)bis(1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione) diethylaminium salt (**4c**)*

4c was prepared from 1,3-dimethylbarbutric acid **1a**, and *p*-nitrobenzaldehyde according to the general procedure (**GP1**) yielding a yellow powder (1.35 g, 2.61 mmol, 87%); m.p.: 195 °C; IR (KBr, cm^{-1}): 3453, 3205, 2987, 2904, 1675, 1608, 1576, 1511, 1438,

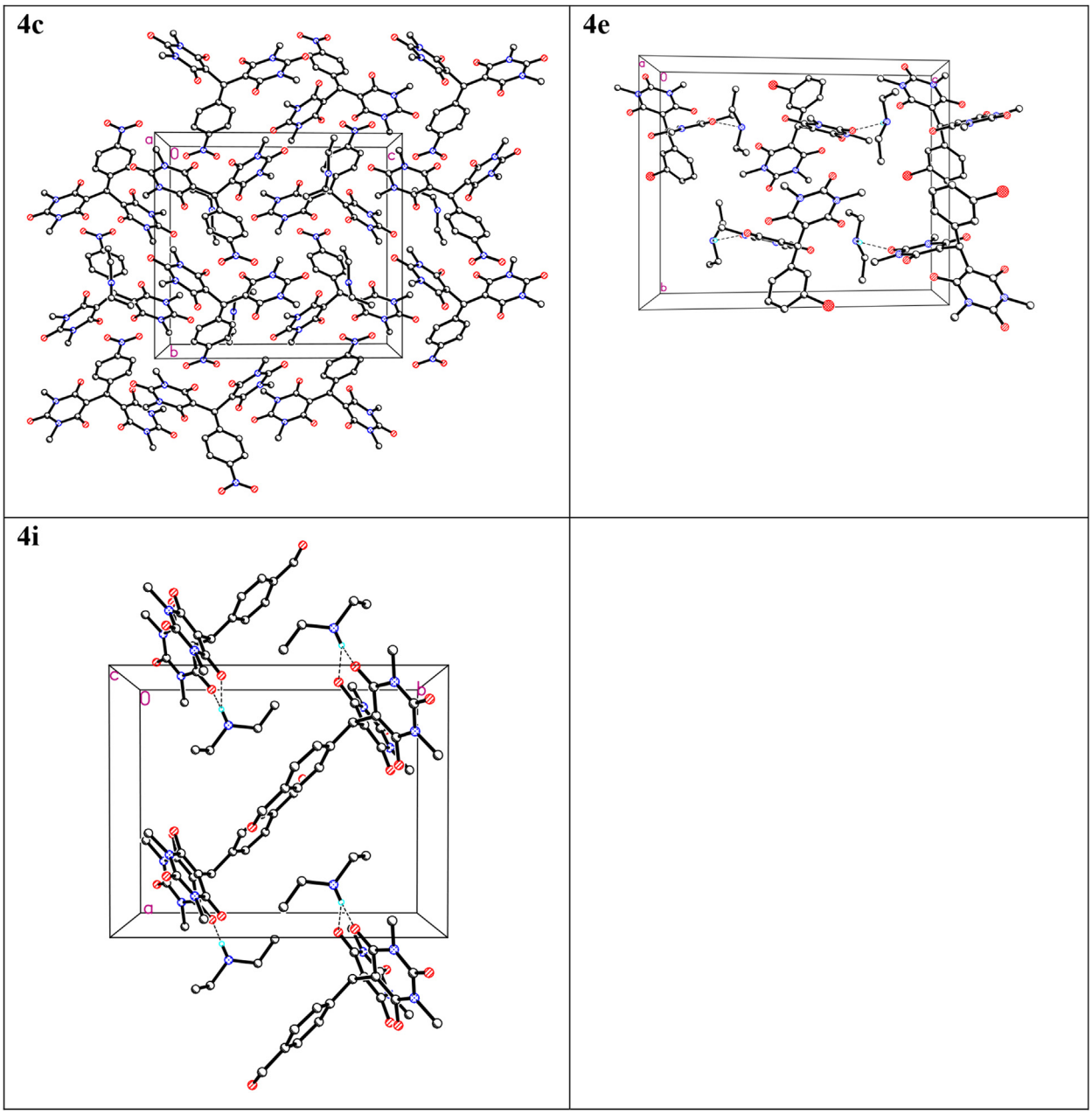


Fig. 4. Crystal packing of **4c**, **e**, **i**.

1343, 1254; ^{1}H NMR (400 MHz, $CDCl_3$): δ 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, $4CH_3$), 3.07 (q, 4H, J = 7.3 Hz, CH_2CH_3), 1.29 (t, 6H, J = 7.3 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 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; LC/MS (ESI): 518[M]$^{+}$; Anal. for $C_{23}H_{30}N_6O_8$; Calcd: C, 53.28; H, 5.83; N, 16.21; Found: C, 53.29; H, 5.85; N, 16.23.

The structure of **4c** was confirmed by X-ray crystal structure analysis (Bruker SMART APEXII CCD diffractometer). CCDC-1001798 contains the supplementary crystallographic data for this compound. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. A colorless crystal suitable for X-ray analysis was obtained from recrystallization the compound from DCM/Et_2O at room temperature after 2 days.

*4.1.4. 5,5′-((4-Methoxyphenyl)methylene)bis(1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione) diethylaminium salt (**4d**)*

4d was prepared from 1,3-dimethylbarbutric acid **1a**, and *p*-methoxybenzaldehyde according to the general procedure (**GP1**) yielding rose-colored crystalline materials (1.35 g, 2.7 mmol, 90%). m.p.: 160 °C; IR (KBr, cm^{-1}): 3445, 3195, 2977, 2836, 1689, 1664, 1613, 1504, 1447, 1378, 1242; ^{1}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, $4CH_3$), 2.99 (q, 4H, J = 7.3 Hz, CH_2CH_3), 1.26 (t, 6H, J = 7.3 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 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; LC/MS (ESI): 503[M]$^{+}$; Anal. for $C_{24}H_{33}N_5O_7$; Calcd: C, 57.25; H, 6.61; N, 13.91; Found: C, 57.26; H, 6.61; N, 13.90.

Table 2
Result of nitric oxide scavenging assay.

S. no.	Compounds	Nitric oxide scavenging assay IC_{50} ± SEM [μM]
1	**4a**	75 ± 4.33
2	**4b**	230 ± 4.95
3	**4c**	69 ± 1.66
4	**4d**	234 ± 2.30
5	**4e**	70.1 ± 0.89
6	**4f**	310 ± 3.67
7	**4g**	174.5 ± 4.80
8	**4h**	79.1 ± 2.92
9	**4i**	113 ± 0.53
10	**4j**	377 ± 2.21
11	**4k**	304 ± 4.5
12	**4l**	206.5 ± 1.0
13	**4m**	308 ± 2.0
14	**4n**	330 ± 2.02
15	**4o**	290 ± 4.0
16	**4p**	174.9 ± 3.65
17	**4q**	466 ± 4.20
18	**4r**	380 ± 4.08
19	**4s**	361 ± 4.89
20	**4t**	432 ± 3.17
21	**V**	439.2 ± 3.7
22	**4w**	281 ± 2.48
STD	**Ascorbic acid**	618 ± 2.00

*4.1.5. 5,5′-((3-Bromophenyl)methylene)bis(1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione) diethylaminium salt (**4e**)*

4e was prepared from 1,3-dimethylbarbutric acid **1a**, and *m*-bromobenzaldehyde according to the general procedure (**GP1**) yielding colorless crystalline materials (1.5 g, 2.76 mmol, 92%). m.p.: 169 °C; IR (KBr, cm^{-1}): 3450, 3120, 2982, 1694, 1667, 1615, 1577, 1445, 1250; ^{1}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, $2CH_3$), 3.32 (s, 6H, $2CH_3$), 3.02 (q, 4H, *J* = 7.3 Hz, CH_2CH_3), 1.27 (t, 6H, *J* = 7.3 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 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; LC/MS (ESI): 552[M]$^+$; Anal. for $C_{23}H_{30}BrN_5O_6$; 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.

The structure of **4e** was confirmed by X-ray crystal structure analysis (Bruker SMART APEXII CCD diffractometer). CCDC-1001799 contains the supplementary crystallographic data for this compound. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. A colorless crystal suitable for X-ray analysis was obtained from recrystallization the compound from DCM/Et_2O at room temperature after 2 days.

*4.1.6. 5,5′-((4-hydroxyphenyl)methylene)bis(6-hydroxy-1,3-dimethylpyrimidine-2,4(1H,3H)-dione) diethylaminium salt (**4f**)*

4f was prepared from 1,3-dimethylbarbutric acid **1a**, and *p*-hydroxybenzaldehyde according to the general procedure (**GP1**) yielding a yellow powder (1.3 g, 2.64 mmol, 88%); m.p.: 180 °C; IR (KBr, cm^{-1}): 3458, 3200, 2980, 2904, 1677, 1620, 1572, 1511, 1438, 1343, 1254; ^{1}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, $4CH_3$), 3.03 (q, 4H, *J* = 7.3 Hz, CH_2CH_3), 1.27 (t, 6H, *J* = 7.3 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 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; LC/MS (ESI): 489.52 [M]$^+$; Anal. for $C_{23}H_{31}N_5O_7$; Calcd: C, 56.43; H, 6.38; N, 14.31; Found: C, 56.44; H, 6.36; N, 14.30.

*4.1.7. 5,5′-(3-Tolylmethylene)bis(1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione) diethylaminium salt (**4g**)*

4g was prepared from 1,3-dimethylbarbituric acid **1a**, and *m*-tolualdehyde according to the general procedure (**GP1**) yielding rose-colored crystalline materials (1.41 g, 2.91 mmol, 97%). m.p.: 135 °C; IR (KBr, cm^{-1}): 3455, 3201, 2988, 1693, 1667, 1611, 1573, 1443; ^{1}H NMR (400 MHz, $CDCl_3$): δ 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, $4CH_3$), 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}C NMR (100 MHz, $CDCl_3$): δ = 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; LC/MS (ESI): 487 [M]$^+$; Anal. for $C_{24}H_{35}N_5O_6$; Calcd: C, 59.12; H, 6.82; N, 14.36; Found: C, 59.13; H, 6.81; N, 14.35.

*4.1.8. 5,5′-(Naphthalen-2-ylmethylene)bis(1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione) diethylaminium salt (**4h**)*

4h was prepared from 1,3-dimethylbarbutric acid **1a**, and 2-naphthaldehyde **2i** according to the general procedure (**GP1**) yielding beige powder (1.47 g, 2.82 mmol, 94%). m.p.: 146 °C; IR (KBr, cm^{-1}): 3454, 3200, 2967, 1668, 1585, 1438, 1250; ^{1}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, $4CH_3$), 3.01 (q, 4H, *J* = 7.3 Hz, CH_2CH_3), 1.30 (t, 6H, *J* = 7.3 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 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; LC/MS (ESI): 523 [M]$^+$; Anal. for $C_{27}H_{33}N_5O_6$; Calcd: C, 61.94; H, 6.35; N, 13.38; Found: C, 61.95; H, 6.34; N, 13.40.

*4.1.9. 4-(bis(6-Hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl)benzaldehyde diethylaminium salt (**4i**)*

Pure product **4i** was obtained according to **GP1** as colorless crystal (1.5 g, 2.76 mmol, 92%). IR (cm^{-1}): 3450, 3000, 2872, 1670, 1582, 1510, 1466, 1384, 1339; ^{1}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, $4CH_3$), 3.06 (q, 4H, *J* = 7.3 Hz, CH_2CH_3), 1.27 (t, 6H, *J* = 7.3 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 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; LC/MS (ESI): 501.53 [M]$^+$; Anal. for $C_{24}H_{31}N_5O_7$; Calcd: C, 57.48; H, 6.23; N, 13.96; Found: C, 57.50; H, 6.25; N, 14.00.

*4.1.10. 5,5′-(p-Tolylmethylene)bis(6-hydroxypyrimidine-2,4(1H,3H)-dione) diethylaminium salt (**4j**)*

4j was prepared from barbituric acid **1b**, and *p*-tolualdehyde according to the general procedure (**GP1**) yielding white powder (1.22 g, 2.85 mmol, 95%); m.p.: 205 °C; IR (KBr, cm^{-1}): 3459, 3120, 2978, 2811, 1689, 1612, 1325, 1252; ^{1}H NMR (400 MHz, 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}C NMR (100 MHz, DMSO-d_6): δ = 164.8, 164.1, 151.3, 142.1, 133.5, 128.5, 127.1, 91.6, 42.6, 30.6, 21.1, 13.0; LC/MS (ESI): 431[M]$^+$; Anal. for $C_{20}H_{25}N_5O_6$; Calcd: C, 55.68; H, 5.84; N, 16.23; Found: C, 55.67; H, 5.83; N, 16.22.

*4.1.11. 5,5′-((4-Chlorophenyl)methylene)bis(6-hydroxypyrimidine-2,4(1H,3H)-dione) diethylaminium salt (**4k**)*

4k was prepared from barbituric acid **1b**, and *p*-chlorobenzaldehyde according to the general procedure (**GP1**) yielding a white powder (1.28 g, 2.85 mmol, 95%); m.p.: 221 °C; IR (KBr, cm^{-1}): 3435, 3185, 2978, 2830, 1677, 1548, 1448, 1345, 1250; ^{1}H

NMR (400 MHz, 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}C NMR (100 MHz, DMSO-d_6): δ = 164.7, 164.0, 151.2, 144.6, 133.5, 129.9, 129.1, 127.8, 91.3, 42.1, 30.7, 11.8; LC/MS (ESI): 451[M]$^+$; Anal. for $C_{19}H_{22}ClN_5O_6$; 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.

4.1.12. 5,5′-((4-Methoxyphenyl)methylene)bis(6-hydroxypyrimidine-2,4(1H,3H)-dione) diethylaminium salt (**4l**)

4l was prepared from barbituric acid **1b**, and *p*-methoxybenzaldehyde according to the general procedure (**GP1**) yielding a beige powder (1.22 g, 2.73 mmol, 91%); m.p.: 195 °C; IR (KBr, cm^{-1}): 3449, 3190, 2991, 2835, 1688, 1592, 1505, 1383, 1247; ^{1}H NMR (400 MHz, 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}C NMR (100 MHz, DMSO-d_6): δ = 164.6, 164.0, 157.0, 151.2, 137.2, 132.4, 115.1, 91.7, 55.4, 42.1, 30.7, 11.6; LC/MS (ESI): 447[M]$^+$; Anal. for $C_{20}H_{25}N_5O_7$; Calcd C, 53.69; H, 5.63; N, 15.65; Found: C, 53.69; H, 5.63; N, 15.66.

4.1.13. 5,5′-(Naphthalen-2-ylmethylene)bis(6-hydroxypyrimidine-2,4(1H,3H)-dione) diethylaminium salt (**4m**)

4m was prepared from barbituric acid **1b**, and 2-naphthaldehyde according to the general procedure (**GP1**) yielding a beige powder (1.3 g, 2.79 mmol, 93%); m.p.: 192 °C; IR (KBr, cm^{-1}): 3459, 3208, 2994, 1677, 1579, 1448, 1386, 1354; ^{1}H NMR (400 MHz, 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}C NMR (100 MHz, DMSO-d_6): δ = 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; LC/MS (ESI): 467[M]$^+$; Anal. for $C_{23}H_{25}N_5O_6$; Calcd C, 59.09; H, 5.39; N, 14.98; Found: C, 59.12; H, 5.40; N, 15.01.

4.1.14. 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 diethylaminium salt (**4n**)

Pure product **4n** was obtained according to **GP1** as white solid (1.20 g, 88%). IR (cm^{-1}): 3455, 3305, 3000, 2910, 1677, 1582, 1510, 1466, 1384, 1339; ^{1}H NMR ($CDCl_3$, 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, $4CH_3$), 3.03 (q, 4H, J = 7.3 Hz, CH_2CH_3), 1.25 (t, 6H, J = 7.3 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 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; LC/MS (ESI): 473.48 [M]$^+$; Anal. for $C_{22}H_{27}N_5O_7$; Calcd: C, 55.81; H, 5.75; N, 14.79; Found: C, 55.83; H, 5.76; N, 14.81.

4.1.15. 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 (**4o**)

4o was prepared from 1,3-dimethylbarbituric acid **1a**, dimedone **2** and benzaldehyde according to the general procedure (**GP1**) yielding colorless crystalline material (671 mg, 1.47 mmol, 98%). m.p: 159 °C; IR (KBr, cm^{-1}): 3150, 2959, 1667, 1617, 1585, 1422, 1256, 1227; ^{1}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, $4CH_3$), 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}C NMR (100 MHz, $CDCl_3$): δ = 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; LC/MS (ESI): 457 [M]$^+$; Anal. for $C_{25}H_{35}N_3O_5$; calcd: C, 65.62; H, 7.71; N, 9.18; Found: C, 65.61; H, 7.73; N, 9.20.

The structure of **4o** was confirmed by X-ray crystal structure analysis. CCDC-933624 contains the supplementary crystallographic data for this compound. This data can be obtained free of charge from the Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif. A colorless crystal suitable for X-ray analysis was obtained from recrystallization of the compound from $CHCl_3$/Et_2O at room temperature after 2 days.

4.1.16. 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 (**4p**)

4p was prepared from 1,3-dimethylbarbituric acid **1a**, dimedone **2** and 2,4-dichlorobenzaldehyde according to the general procedure (**GP1**) yielding a beige solid material (710 mg, 1.35 mmol, 90%). m.p: 164 °C; IR (KBr, cm^{-1}): 3059, 2995, 2867, 2114, 1741, 1658, 1591, 1463, 1429, 1370, 1341, 1256, 1201^{1}H NMR (400 MHz, $CDCl_3$): 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, $4CH_3$), 3.07(q, 4H, J = 7.3 Hz, CH_2CH_3), 2.37 (s, 2H, CH_2), 2.27 (d, 2H, J = 5.1 Hz, CH_2), 1.34(t, 6H, J = 7.3 Hz, CH_2CH_3), 1.04(s, 3H, CH_3), 1.01(s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 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; LC/MS (ESI): 526 [M]$^+$; Anal. for $C_{25}H_{33}Cl_2N_3O_5$; 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.

4.1.17. 5-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(4-nitrophenyl)methyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate (**4q**)

4q was prepared from 1,3-dimethylbarbituric acid **1a**, dimedone **2** and *p*-nitrobenzaldehyde according to the general procedure (**GP1**) yielding a beige material (700 mg, 1.39 mmol, 93%). m.p: 148 °C; IR (KBr, cm^{-1}): 3050, 2950, 2865, 2500, 1669, 1580, 1510, 1427, 1373, 1255, 1214;^{1}H NMR (400 MHz, $CDCl_3$): δ 15.26 (s, 1H, OH), 6.99 (d, 2H, J = 8.0 Hz, Ph), 6.72 (d, 2H, J = 8.8 Hz, Ph), 5.69 (s, 1H, benzyl-H), 3.71 (s, 12H, $4CH_3$), 2.85(q, 4H, J = 7.3 Hz, CH_2CH_3), 2.31(d,4H, J = 14.7 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}C NMR (100 MHz, $CDCl_3$): δ = 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; LC/MS (ESI): 502[M]$^+$; Anal. for $C_{25}H_{34}N_4O_7$; calcd: C, 59.75; H, 6.82; N, 11.15; Found: C, 59.73; H, 6.81; N, 11.17.

4.1.18. 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 (**4r**)

Pure product **4r** was obtained according to **GP1** as solid (1.26 g, 90%). IR (cm^{-1}): 3156, 2950, 2872, 1678, 1590, 1508, 1375, 1256, 1232, 1167; ^{1}H 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, CH_3), 2.92 (q, 4H, J = 7.3 Hz, CH_2CH_3), 2.31 (m, 4H, $2CH_2$), 1.26 (t, 6H, J = 7.3 Hz, CH_2CH_3), 1.05 (s, 3H, CH_3), 1.00 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 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; LC/MS (ESI): 485.57 [M]$^+$; Anal. for $C_{26}H_{35}N_3O_6$; Calcd: C, 64.31; H, 7.27; N, 8.65; Found: C, 64.30; H, 7.26; N, 8.63.

4.1.19. 5-((2-Hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(phenyl)methyl)-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-olate (**4s**)

4s was prepared from barbituric acid **1b**, dimedone **2** and benzaldehyde according to the general procedure (**GP1**) yielding a white solid material (598 mg, 1.39 mmol, 93%). m.p: 215 °C; IR (KBr, cm^{-1}): 3027, 2948, 2867, 2156, 1683, 1593, 1451, 1374, 1291, 1257,

1141; ^{1}H NMR (400 MHz, $CDCl_3$): 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, CH_2CH_3), 2.45 (d, 4H, J = 5.1 Hz, CH_2), 1.24(t, 6H, J = 7.3 Hz, CH_2CH_3), 1.09(s, 3H, CH_3), 1.03(s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 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; LC/MS (ESI): 429[M]$^+$; Anal. for $C_{23}H_{31}N_3O_5$; calcd: C, 64.32; H, 7.27; N, 9.78; Found: C, 64.29; H, 7.29; N, 9.80.

4.1.20. 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 (**4t**)

4t was prepared from barbituric acid **1a**, dimedone **2** and tolualdehyde according to the general procedure (**GP1**) yielding a white solid material (604 mg, 1.36 mmol, 91%). m.p: 213 °C; IR (KBr, cm^{-1}): 3150, 2955, 2867, 1690, 1592, 1508, 1375, 1256, 1232, 1167; ^{1}H NMR (400 MHz, $CDCl_3$): 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, CH_2CH_3), 2.31 (d, 4H, J = 5.1 Hz, CH_2), 2.23 (s, 3H, CH_3), 1.19(t, 6H, J = 7.3 Hz, CH_2CH_3), 1.04(s, 3H, CH_3), 1.02(s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 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; LC/MS (ESI): 443 [M]$^+$; Anal. for $C_{24}H_{33}N_3O_5$; calcd: C, 64.99; H, 7.50; N, 9.47; Found: C, 64.95; H, 7.49; N, 9.50.

4.1.21. 4-((6-Hydroxy-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 (**4v**)

Pure product **4v** was obtained according to **GP1** as solid (1.35 g, 95%). IR (cm^{-1}): 3150, 2955, 2867, 1690, 1592, 1508, 1375, 1256, 1232, 1167; ^{1}H NMR ($CDCl_3$, 400 MHz) 15.16 (s, 1H, OH), 9.93 (s, 1H, CHO), 9.66 (brs, 2H, NH), 7.71 (d, 2H, J = 7.3 Hz, Ph), 7.23 (d, 2H, J = 7.3 Hz, Ph), 5.93 (s, 1H, benzyl-H), 2.92 (q, 4H, J = 7.3 Hz, CH_2CH_3), 2.2 (m, 4H, $2CH_2$), 1.15 (t, 6H, J = 7.3 Hz, CH_2CH_3), 1.02 (s, 3H, CH_3), 1.00 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 193.0, 166.0, 154.6, 151.8, 134.5, 129.5, 128.1, 126.3, 115.9, 89.6, 51.1, 42.7, 31.9, 31.6, 29.8, 27.9, 11.7; LC/MS (ESI): 457.52 [M]$^+$; Anal. for $C_{24}H_{31}N_3O_6$; Calcd: C, 63.00; H, 6.83; N, 9.18; Found: C, 63.03; H, 6.86; N, 9.21.

4.1.22. Diethylammonium 2-((2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)(4-nitrophenyl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate (**4w**)

Pure product **4w** was obtained according to **GP1** as solid (1.26 g, 90%). IR (cm^{-1}): 2872 (s), 1582 (s), 1510 (s), 1466 (s), 1384 (s), 1339 (s), 757 (s), 487 (s); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.91–1.06 (m, 12H, C**H**$_3$), 1.21 (t, J = 7.32 Hz, 6H, $NH_2CH_2CH_3$),2.29 (s, 8H, CH_2^+ $COCH_2$), 2.94 (q, J = 7.32 Hz, 4H, $NHCH_2CH_3$), 5.92 (s, 1H, PhC**H**), 7.21 (d, J = 8.80 Hz, 2H, Ar**H**), 8.01 (m, J = 8.80 Hz, 2H.Ar**H**),8.32 (bs,2H. N**H**$_2$), 15.12 (s, O**H**); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 11.4 (**C**H_3CH_2NH), 31.6{**C**$(CH_3)_2$}, 32.2 (CH_3)$_2$, 34.1 (Ph-**C**), 42.5 (CH_3**C**H_2NH), 45.2, 50.3, 114.8, 123.2 (Ph**C3**), 127.7 (Ph**C2**), 145.5 (Ph**C4**), 151.9 (Ph**C1**), 186.8 (**C**–OH), 194.9 (**C**=O); Anal. Calcd. for $C_{27}H_{38}N_2O_6$: 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]$^+$.

4.2. Procedure for nitric oxide scavenging assay

Nitric oxide scavenging activities of compounds were determined by using the following method.

In the present investigation Griess–Ilosavay methodology was modified by using naphthylethylenediamine dihydrochloride (0.1% w/v). The reaction mixture (250 μL), containing 12 μL of 1.0 mM of test sample (in DMSO), 38 μL of potassium phosphate buffer (10 mM, pH 7.4) and 100 μL of sodium nitropruside (10 mM), was incubated at 25 °C for 150 min for the formation of nitrite ions. After incubation, 50 μL of sulphanalic acid reagent (0.33% in 20% glacial acetic acid) was added and allowed to stand for 5 min for completion of diazotisation. Then 50 μL of N-(1-naphthyl)ethylenediamine dihydrochloride (0.1% w/v) was added and stirred, and allowed to stand for 1–2 min. A pink-colored chromophore was formed in diffused light. The absorbance of the solution was measured at 546 nm against the corresponding blank solution. Ascorbic acid and DMSO was used as the positive control and blank respectively. The IC_{50} value was calculated by using the kinetic program Ezi-Fit Enzyme Kinetic Program (Perrella Scientific Inc., Amherst, U.S.A.).

The % radical scavenging activity (RSA) was calculated according to the following formula:

% RSA = 1 − [A of test compound/A of control) − 100].

Where; RSA is radical scavenging activity and A is absorbance.

Acknowledgments

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project Number RGP-VPP-257.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.07.026.

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