Res Chem Intermed (2016) 42:4041–4053
DOI 10.1007/s11164-015-2257-1

# Molecular structure investigation and biological evaluation of Michael adducts derived from dimedone

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Received: 19 March 2015 / Accepted: 29 August 2015 / Published online: 11 September 2015


**Abstract** Trimolecular salt Michael adducts **2a–c** were synthesized in excellent yields up to 92 % via one-pot multicomponent reactions in an aqueous medium. The chemical structures of compounds **2a–c** were characterized by X-ray single-crystal diffraction techniques. Calculations of the density functional theory for the synthesized compound were performed. The stability of the products was deduced by TGA analysis. Compounds **2a–c** were screened in vitro for different bio-assays such as thymidine phosphorylase inhibition assay, urease inhibition assay, $\beta$-glucuronidase inhibition assays and cytotoxicity against PC-3 and HeLa cell lines.

**Keywords** Michael adduct · Dimedone · Thymidine phosphorylase assay · Urease inhibition assay · $\beta$-Glucuronidase inhibition assay

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**Electronic supplementary material** The online version of this article (doi:10.1007/s11164-015-2257-1) contains supplementary material, which is available to authorized users.

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## Introduction

5,5-Dimethylcyclohexane-1,3-dione, commonly known as dimedone, belongs to the family of cyclic 1,3-diketones –class of organic compounds. A wide range of applications of dimedones includes its use as reagent for various analytical techniques [1, 2], as well as a versatile synthon for synthesis of several *spiro* and heterocyclic compounds [3], such as xanthene derivatives, which have emerged as an important class of compound because of their industrial importance [4] and other synthetic applications [5].

The versatile chemistry [6–10] and ready availability of cyclohexane-1,3-diones [11–13] and its derivatives make them suitable precursors for the preparation of divergent organic compounds, for example chromene derivatives, which possess anticancer, antioxidant, spasmolytic, anti-anaphylactic, anti-HIV and anti-bacterial activities [14, 15], oxozolidinones with antibacterial activity [16–19], substituted xanthene derivatives with several uses in dyes [20, 21], laser technology [22], fluorescent compounds [23], and more importantly, have been reported to show a variety of biological activity [24–26]. Additionally, acridine and its derivatives display antimalarial, anticancer, antibacterial, and mutagenic properties, while phenylbutazones exhibit unique pharmacological uses for pain treatment associated with Tietze's syndrome and rheumatoid arthritis [27].

On the other hand, with the increasing environmental concerns, green chemistry has attracted major scientific and commercial interest in recent years. Multicomponent one-pot reactions are an efficient and economical procedure, with wide uses in the preparation of heterocycles molecules [28–30]. On the other hand, organic transformation takes place in aqueous media gives a clean, environmentally safe, and cost-effective approach. Recently, Barakat and coworkers reported examples for MCR (one-pot fission) for example reaction of substituted cyclohexanedione with alkanal mediated by diethylamine in water [31–36].

In the current study, the structure of salt Michael adducts **2a–c** were elucidated by X-ray single-crystal diffraction and TGA study. In addition, density functional theory (DFT) using B3LYP/6-311G(d,p) method were used to study different structural aspects of the compounds. Also, the synthesized compounds **2a–c** were evaluated for a set of in vitro biochemical assays.

## Experimental

### General

Chemical reagents were purchased from Fluka, Sigma-Aldrich, Aldrich, etc., and were used without further purification, unless otherwise stated. The crystal data were collected on a Bruker APEX-II CCD area diffractometer, crystallographic data for the compounds, **2a**, **2b** and **2c** are deposited with the CCDC-993141, 993142, and 993140 respectively.

*Diethylammonium 2-((4-bromophenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate (**2a**)*

Compound **2a** was synthesized from dimedone and *p*-bromobenzaldehyde **1a** as a white crystal. The structure of **2a** was unambiguously deduced by X-ray diffraction analysis. A suitable colorless cubic crystal of **2a** was grown in $CHCl_3/Et_2O$ at rt after 48 h.

*Diethylammonium 2-((3-bromophenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate (**2b**)*

Compound **2b** was synthesized from dimedone and *m*-bromobenzaldehyde **1b** as a crystalline compound. Colorless cubic crystals of compound **2**, found suitable for X-ray analysis, were grown in $CH_2Cl_2$/pet. ether at rt after standing for 24 h.

*Diethylammonium 2-((2,4-dichlorophenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-5,5-dimethyl-3-oxocyclohex-1-enolate (**2c**)*

Compound **2c** was synthesized from dimedone and 2,4-dichlorobenzaldehyde (**1c**) as a colorless cubical crystals in $CH_2Cl_2$/pet. ether at rt. The crystals suitable for X-ray analysis were obtained after 3 days.

## DFT calculations

In the present work, DFT calculations using the B3LYP method, together with local or non-local functionals, were performed that unites the Becke's three-parameter exchange functional (B3) with the Lee, Yang, and Parr correlation functional (LYP). The density functional theory (DFT) calculations were carried out utilizing GAMESS program. The input geometry of the synthesized compounds were optimized using at B3LYP/6-311G(d,p) basis set for C, O, N, and H atoms without imposing any external constraint on the potential energy surfaces.

## Biological assay

The *p*-nitrophenyl-*β*-D-glucuronide (N-1627) and *β*-glucuronidase (E.C. 3.2.1.31, from bovine liver, G-0251) were purchased from Sigma Chemical Co. (U.S.A.). $Na_2CO_3$ anhydrous and all other reagents of standard grade were getting from E. Merck. The anhydrous EtOH and $CHCl_3$were used in experiment and these were dried by using the standard methods. All other solvents and reagents were of standard grade like the benzoyl chloride.

## *β*-D-glucuronidase assay

*β*-D-glucuronidase inhibition assay was performed as described by Khan et al. [37].

### Urease assay and inhibition

The assay was performed as described by Khan et al. [38] and Weatherburn et al. [39].

### Thymidine phosphorylase assay protocol

The assay was performed as described by Krenitsky et al. [40].

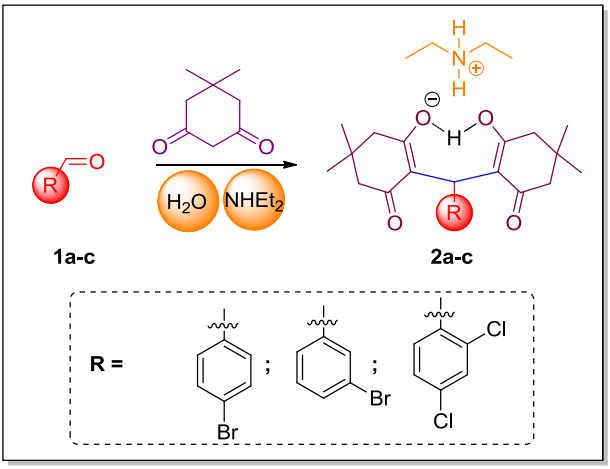
### Cytotoxic activity

The assay was performed as described by Mosmann et al. [41].

## Results and discussion

### Synthesis of the salt of Michael adduct

Recently, there has been a lot of interest in the development of highly efficient transformations for the synthesis of biologically active organic molecules, with potential utilities in agrochemical or pharmaceutical industries. There is also interest in discovering new, strategically important protocols, which are efficient and environmentally benign, and lead to the complex structural variations with simple work-up procedures in high yields [32–35]. During the current study, diethylamine in aqueous medium mediated the one-pot two-component Aldol-Michael addition of 5,5-dimethylcyclohexane-1,3-dione (dimedone), with different arylaldehyde **2a–c** in 2:1 molar ratio to provide trimolecular Michael adducts **2a–c** in excellent yields up to 92 % (Scheme 1) [31].




**Scheme 1** Synthetic protocol used in the present work

## X-ray crystal structures

The chemical structures of the final adduct **2a–c** were unambiguously deduced by single-crystal X-ray diffraction technique (Fig. 1a–c). Tables 1, 2, 3 and 4 display the crystal data and main geometrical parameters of the compounds.

Slow evaporation of compounds **2a** ($CHCl_3/Et_2O$, 3 days), **2b** (DCM/pet. ether, 24 h), and **2c** (DCM/pet. ether, 2 days), at room temperature yielded pure crystals of **2a–c**. Crystals of dimensions 0.38 × 0.37 × 0.24 (**2a**), 0.47 × 0.42 × 0.14 (**2b**) and 0.31 × 0.21 × 0.20 (**2c**) mm were selected for X-ray diffraction analysis on a

**Table 1** The crystal data of **2a–c**

| | **2a** | **2b** | **2c** |
|---|---|---|---|
| Empirical formula | $C_{27}H_{37}BrNO_4$ | $C_{27}H_{37}BrNO_4$ | $C_{27}H_{36}Cl_2NO_4$ |
| Formula weight | 519.49 | 519.41 | 509.47 |
| Temperature | 273 (2) | 273 (2) | 273 K |
| Wavelength | 0.71073 Å | 0.71073 Å | 0.71073 Å |
| Crystal system | Monoclinic | Monoclinic | Monoclinic |
| Space group | $P2_1/n$ | $P2_1/c$ | $P2_1/n$ |
| A | 10.2861 (11) Å | 12.6829 (13) Å | 10.3400 (10) Å |
| B | 18.1701 (19) Å | 12.2729 (13) A | 18.2984 (18) Å |
| C | 15.6171 (16) Å | 18.5831 (19) Å | 15.0374 (15) Å |
| $\alpha$ | 90° | 90° | 90° |
| $\beta$ | 106.5 (2)° | 108.283 (3)° | 98.257 (2)° |
| $\gamma$ | 90° | 90° | 90° |
| Volume | 2798.5 (5) $A^3$ | 2746.5 (5) $A^3$ | 2815.7 (5) $A^3$ |
| $Z$ | 4 | 4 | 4 |
| Calculated density | 1.233 $mgm^{-3}$ | 1.256 $mgm^{-3}$ | 1.202 $mgm^{-3}$ |
| Absorption coefficient | 1.498 $mm^{-1}$ | 1.527 $mm^{-1}$ | 0.261 $mm^{-1}$ |
| F(000) | 1092 | 1092 | 1084 |
| Crystal size | 0.38 × 0.37 × 0.24 mm | 0.47 × 0.42 × 0.14 mm | 0.31 × 0.21 × 0.20 mm |
| $\theta$ range | 1.76–25.50° | 1.69–25.50° | 1.76–28.37° |
| Reflections collected | 16,291 | 15,992 | 20,357 |
| Reflections unique | 5202 | 5050 | 7014 |
| ($R_{int}$) | 0.0384 | 0.0597 | 0.0442 |
| $R_1$ with $I > 2\sigma(I)$ | 0.0553 | 0.0518 | 0.0605 |
| $R_2$ with $I > 2\sigma(I)$ | 0.1273 | 0.1142 | 0.1358 |
| $R_1$ for all data | 0.1068 | 0.1192 | 0.1092 |
| $R_2$ for all data | 0.1528 | 0.1438 | 0.1609 |
| Goodness of fit | 1.081 | 0.972 | 1.017 |
| Max/min $\rho$ $e^{-3}$Å | 0.567 and −0.730 | 0.326 and −0.258 | 0.474 and −0.225 |
| CCDC | 993,141 | 993,142 | 993,140 |

**Table 2** Geometric parameters (Å, °) of **2a** (selected)

| Bond | Experimental | Calculated |
|---|---|---|
| Br1–C3 | 1.902 (4) | 1.8905 |
| O2–C13 | 1.286 (4) | 1.2595 |
| O4–C19 | 1.334 (4) | 1.3831 |
| N1–C26 | 1.481 (6) | 1.4785 |
| N1–C25 | 1.492 (6) | 1.4786 |
| C8–C13 | 1.388 (4) | 1.3831 |
| C14–C19 | 1.353 (4) | 1.3558 |
| **Bond angle** | **Experimental** | **Calculated** |
| C26—N1—C25 | 114.5 (4) | 117.6644 |
| O2—C13—C8 | 122.2 (3) | 122.5294 |
| O2—C13—C12 | 115.4 (3) | 111.9032 |
| O4—C19—C14 | 124.3 (3) | 127.8520 |
| CO4—C19—C18 | 110.9 (3) | 113.9985 |
| N1—C26—C27 | 110.3 (4) | 109.9599 |
| C24—C25—N1 | 111.7 (5) | 111.5620 |

**Table 3** Geometric parameters (Å, °) of **2b** (selected)

| Bond | Experimental | Calculated |
|---|---|---|
| Br1–C2 | 1.897 (4) | 1.8930 |
| O1–C9 | 1.329 (4) | 1.2650 |
| O3–C17 | 1.290 (3) | 1.2301 |
| N1–C25 | 1.467 (4) | 1.4768 s |
| N1–C26 | 1.501 (5) | 1.4770 |
| C8–C9 | 1.358 (4) | 1.3560 |
| C16–C17 | 1.386 (4) | 1.3646 |
| **Bond angle** | **Experimental** | **Calculated** |
| C25—N1—C26 | 112.2 (3) | 117.6644 |
| O1—C9—C8 | 124.6 (3) | 121.5499 |
| O1—C9—C10 | 111.4 (3) | 119.5021 |
| O3—C17—C16 | 122.6 (3) | 121.3063 |
| O3— C17— C18 | 116.2 (3) | 111.9032 |
| C24— C25— N1 | 112.8 (3) | 111.5620 |
| C27—C26—N1 | 111.9 (4) | 109.9599 |

Bruker Smart Apex II diffractometer, equipped with CCD detector and graphite monochromatic MoK$\alpha$ radiations ($\lambda = 0.71073$ Å) at 293 (2) K. Cell refinement and data reduction were carried out by Bruker SAINT [14]. The chemical structure

**Table 4** Geometric parameters (Å, °) of **2c** (selected)

| Bond | Experimental | Calculated |
|---|---|---|
| Cl1–C9 | 1.741(2) | 1.7354 |
| C12–C11 | 1.736(3) | 1.7207 |
| O2–C5 | 1.299(2) | 1.2307 |
| O4–C19 | 1.329(3) | 1.2639 |
| N1–C26 | 1.472(4) | 1.4774 |
| N1–C25 | 1.482(4) | 1.4775 |
| C4–C5 | 1.508(3) | 1.5113 |
| C14–C19 | 1.360(3) | 1.3646 |
| **Bond angle** | **Experimental** | **Calculated** |
| O2–C5–C6 | 121.97(19) | 121.9985 |
| O2–C5–C4 | 115.08(18) | 118.2618 |
| O4–C19–C14 | 125.1(2) | 120.7989 |
| O4–C19–C18 | 110.92(19) | 119.0782 |
| C8–C9–Cl1 | 120.03(19) | 119.9182 |
| C11–C10–C9 | 118.8(2) | 119.8890 |
| C12–C11–C10 | 120.0(2) | 120.1922 |
| C12–C11–Cl2 | 120.1(2) | 119.9325 |
| N1–C26–C27 | 110.9(3) | 110.2876 |
| C24–C25–N1 | 112.1(3) | 110.1151 |

was solved by using SHELXS-97 [15, 16]. The listed crystallographic parameters in Table 1 indicate that compounds **2a**, **2b**, and **2c** were crystallized in monoclinic crystal system with space group $P2_1/n$ for **2a** and **2c** and $P2_1/c$ for **2b**. The crystal structures **2a**, **2b**, and **2c** (Fig. 1a) were finally refined with R factor of 5.5, 5.1, and 6.0 %, respectively. The molecule of diethyl amine solvate played an important role to stabilize the structures in crystal lattice through intermolecular hydrogen bondings (Fig. S7–S9; Table S1–S3; see supplementary material).

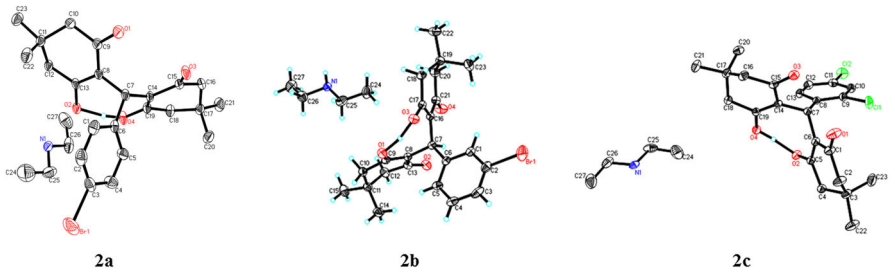



**Fig. 1** The structure of **2a–c** (ORTEP diagrams)

## DFT calculations

### *Optimized molecular geometry*

From our XRD data, it is clear that compounds **2a, 2b**, and **2c** possess monoclinic crystal structures. The cell dimensions and other data are presented in Table 1. Selected values of calculated DFT and experimental geometric parameters for the synthesized molecules were found to be in good agreement, and are listed in Tables 2, 3 and 4. Figures 2 and 3 depict the optimized structures and HOMO–LUMO for compounds **2a, 2b**, and **2c**.

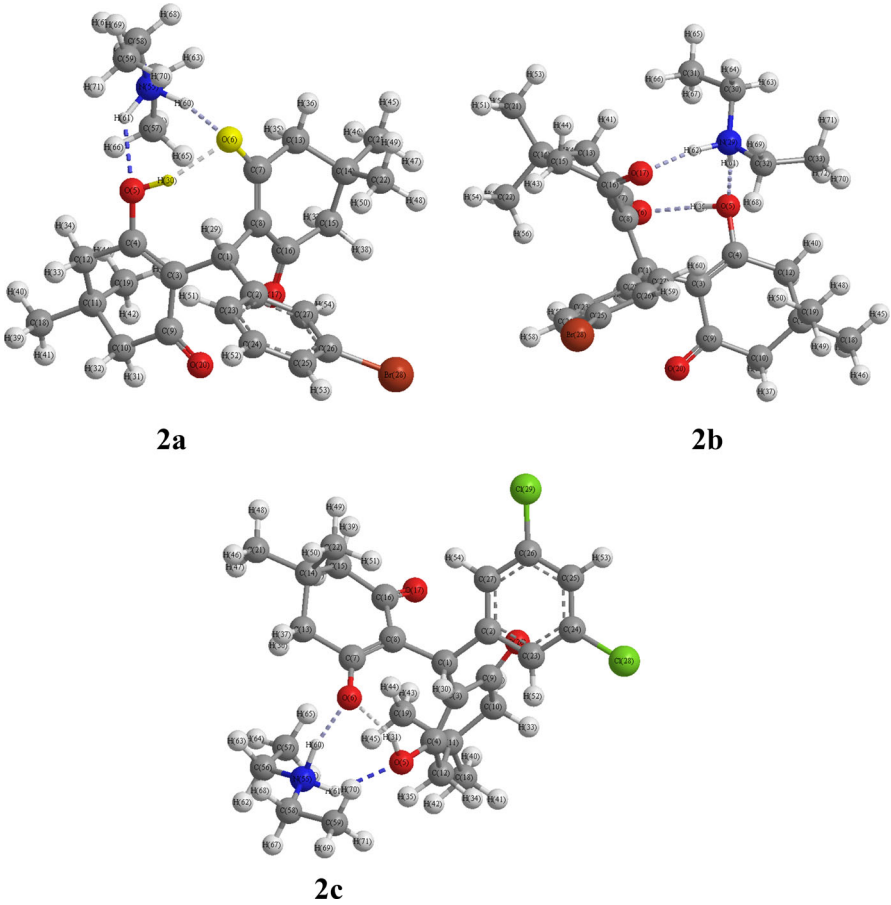



**Fig. 2** Optimized molecular structures of compounds **2a, 2b**, and **2c**

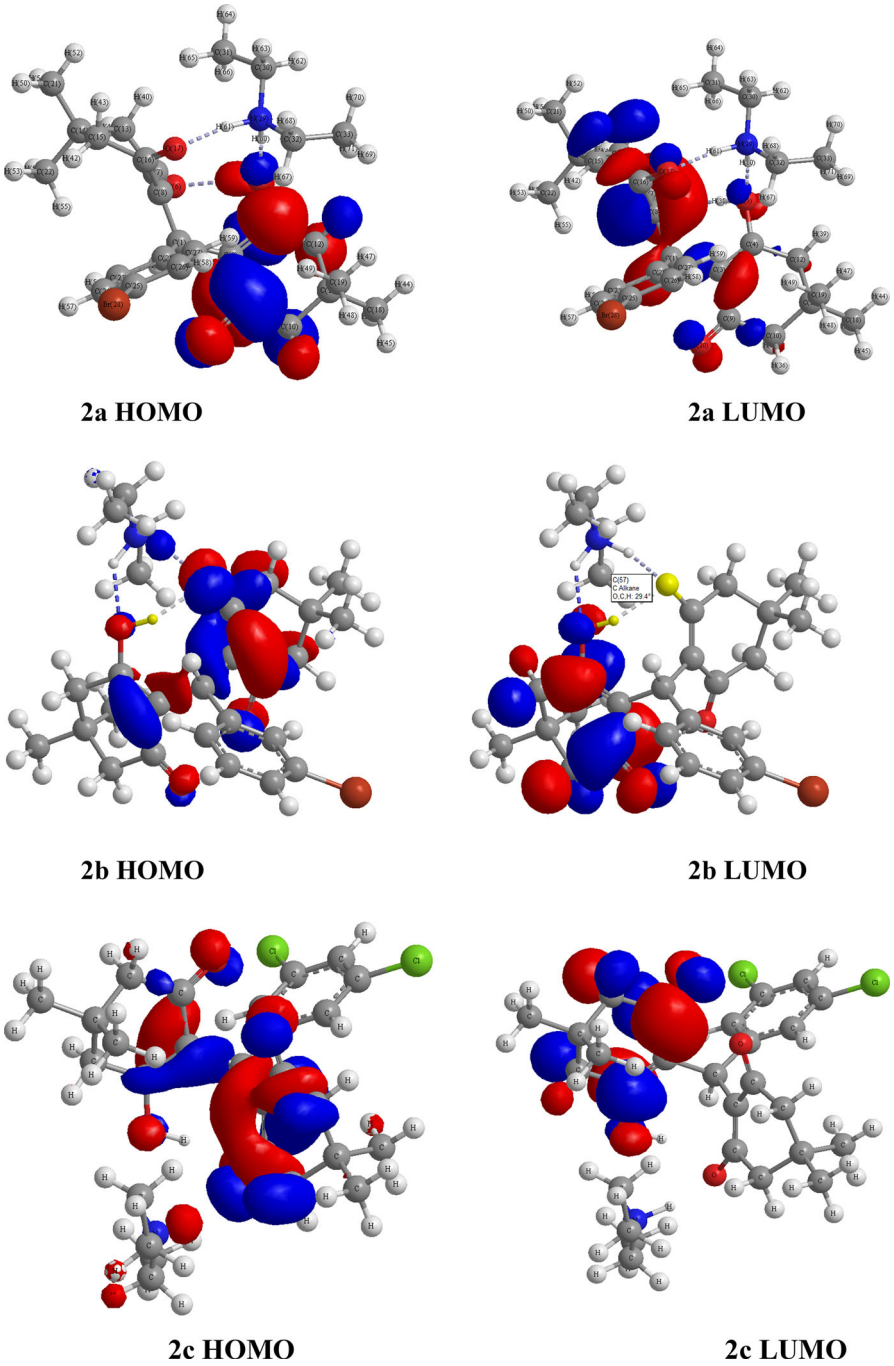



**Fig. 3** HOMO and LUMO for compounds **2a, 2b**, and **2c**

*Thermal gravimetric analysis (TGA)*

Thermal stability of the synthesized compounds was investigated by thermogravimetric analysis (TGA). All the samples were made under nitrogen atmosphere in the temperature ranges between 0 and 800 °C with a heating ramp rate of 10 °C per min (Table 5). It has shown that all the synthesized compounds demonstrated thermal stability up to 200 °C, with a percentage weight loss of 31.45, 22.42, and 16.12 for the compounds **2a**, **2b**, and **2c**, respectively. When the temperature was further raised to 300 °C, dramatic percentage weight loss of up to 98.9, 97.3, and 97.7 for the compounds **2a**, **2b**, and **2c** respectively. Hence, it can be said that the synthetic compounds are thermally stable up to 200 °C, and from there on a rapid degradation occur (Fig. 4).

*Biological activity evaluation*

Compounds **2a–c** were screened in vitro for different bio-assays such as thymidine phosphorylase, urease, and $\beta$-glucuronidase inhibition assays. These compounds

**Table 5** Weight loss of synthesized molecules with temperature

| Temperature | Weight loss (%) | | |
|---|---|---|---|
| | **2a** | **2b** | **2c** |
| 100 | 0.07 | 0.24 | 0 |
| 200 | 31.45 | 22.42 | 16.12 |
| 300 | 98.907 | 97.301 | 97.794 |
| 400 | 99.5205 | 98.977 | 98.17 |
| 500 | 99.8727 | 99.318 | 98.212 |
| 600 | 99.6842 | 99.621 | 98.045 |
| 700 | 99.6564 | 99.4393 | 97.682 |
| 800 | 99.8236 | 99.088 | 97.215 |

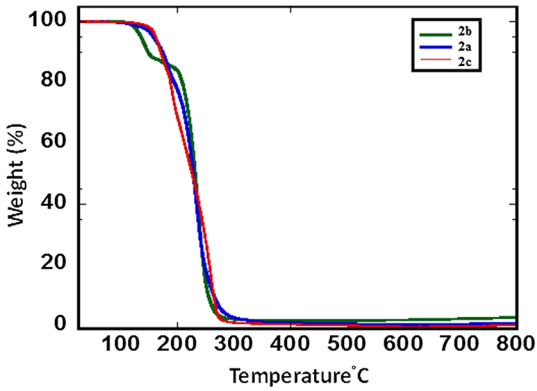



**Fig. 4** The TGA curve of the studied compounds **2a**, **2b** and **2c**

**Table 6** Results of biological activity of the synthesized compounds **2a–c**

| Compounds | Cytotoxicity (PC-3 cell line) | Cytotoxicity (HeLa cell line) | Thymidine phosphorylase inhibition $IC_{50} \pm SEM$ (μM) | Urease inhibition $IC_{50} \pm SEM$ (μM) | β-glucuronidase inhibition $IC_{50} \pm SEM$ (μM) |
|---|---|---|---|---|---|
| **2a** | NA | NA | NA | 154.8 ± 1.33 | 351.6 ± 4.12 |
| **2b** | >30 | >30 | 283.3 ± 0.7 | 75.4 ± 1.46 | NA |
| **2c** | >30 | >30 | 243.3 ± 1.5 | 108.2 ± 1.3 | NA |
| Std. | Doxorubicin 0.912 ± 0.12 | Doxorubicin 0.506 ± 0.15 | 7-Deazaxanthine 41 ± 1.64 | Thiourea 21.2 ± 1.3 | D-Saccharic acid 1-4 lactone 45.75 ± 2.16 |

were also evaluated for their cytotoxic effect against PC-3 and HeLa cell lines. Results are summarized in Table 6.

Compounds **2b, c** showed a varying degree of thymidine phosphorylase inhibition with $IC_{50}$ values 283.3 ± 0.7 and 243.3 ± 1.5 μM, respectively, against the tested standard drug 7-deazaxanthine ($IC_{50} = 41 \pm 1.64$ μM). Compound **2a** found to be inactive (Table 6).

The tested compound **2a–c** ($IC_{50} = 154.8 \pm 1.33$, 75.4 ± 1.46, and 108.2 ± 1.3 μM) showed weak urease inhibition activity against the standard compound thiourea ($IC_{50} = 21.2 \pm 1.3$ μM). Michael adducts **2a–c** were also evaluated for their in vitro $\beta$ -glucuronidase inhibitory potential and compound **2a** ($IC_{50} = 351.6 \pm 4.12$ μM) showed weak inhibition of $\beta$-glucuronidase enzyme in comparison to the standard drug D-saccharic acid 1-4 lactone ($IC_{50} = 45.75 \pm 2.16$ μM). Michael adducts **2b** and **2c** found to be inactive.

Michael adducts **2b–c** were found to be non cytotoxic against of PC-3 normal and HeLa cancer cell lines, and showed >30 % inhibition while compound **2a** was found to be inactive against PC-3 normal and HeLa cancer cell lines (Table 6).

## Conclusions

In the present study, three Michael adducts were synthesized by using a simple, high-yielding, and economical synthetic scheme. The structures were established with the help of physico-chemical properties and single-crystal X-ray diffraction technique. Single-crystal X-ray analysis and DFT calculations revealed that H-bonding plays a crucial role in stability of the molecules. It was observed that all the synthesized compounds demonstrated a thermal stability up to 200 °C. Compounds **2a–c** were also evaluated for their biological activities in various in vitro biological assays.

**Acknowledgments** The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at king Saud University for its funding this Research group NO (RG -257-1435-1436).

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