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ORIGINAL ARTICLE

# Phthalazine-triones: Calix[4]arene-assisted synthesis using green solvents and their anticancer activities against human cancer cells



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*p*-Sulfonic acid calix[4]arene

**Abstract** Fourteen phthalazine-triones bearing different substituents at C-4 position were synthesized through multicomponent reactions (MCR) by using phthalhydrazide, dimedone and different aldehydes as starting materials, *p*-sulfonic acid calix[4]arene as catalyst and ethyl lactate as solvent under microwave irradiation. Compounds **7–16** were obtained in excellent to moderate yields (94–51%) in only 10 min of reaction using this methodology. The antiproliferative activity against cancer cells was disclosed, for the first time, for synthesized compounds. The capacity of all compounds to inhibit cancer cells growth was dependent on the histological origin of cells. Compound **20** was active against more than one strain.

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## 1. Introduction

Phthalazine derivatives (Fig. 1) are nitrogen heterocycle compounds constituting a bridgehead hydrazine (Khurana and Magoo, 2009). This class of compounds has been shown to possess a range of biological and pharmacological properties such as anticonvulsant (Grasso et al., 2000), cardiotoxic (Nomoto et al., 1990), vasorelaxant (Watanabe et al., 1998) and antiproliferative (Scott et al., 2007), as well as their unique luminescence properties (Wu et al., 2009).

The phthalazine **2** (Fig. 1) was described by Grasso and coworkers (Grasso et al., 2000) as a potent anticonvulsant agent, while compound 1-chloro-4-(3-chloro-4-methoxybenzylamino)-6-phthalazine carbonitrile, **3**, (Fig. 1) has vasorelaxant activity *via* inhibition of cyclic nucleotide phosphodiesterase 5 (PDE5). This compound was able to inhibit the activity of PDE5 at level of 50% when used at only 3.5 nM (Watanabe et al., 1998). Antiproliferative activities were also described for phthalazines, being the most notable example is vatalanib (**4**), an inhibitor of vascular growth factor, which is in clinical phase III for metastatic colorectal cancer (Fig. 1) (Scott et al., 2007). Despite the diverse biological profile exhibited by phthalazine derivatives, phthalazine-triones have their biological activities poorly explored. In the only reports, Berber et al., demonstrated that urea- and  $\beta$ -lactam-phthalazine-trione derivatives inhibit the human carbon anhydrase (Berber et al., 2013, 2015).

These compounds can be obtained using multicomponent reactions (MCR). This approach is based on three-component condensations with aldehydes, 5,5-dimethylcyclohexane-1,3-dione (dimedone) and 2,3-dihydro-1,4-phthalazinedione (phthalhydrazide) and employs different catalysts such as *p*-toluenesulfonic acid (PTSA) (Sayyafi et al., 2008),  $\text{Ce}(\text{SO}_4)_2 \cdot 4\text{H}_2\text{O}$  (Mosaddegh and Hassankhani, 2011), dodecylphosphonic acid (DPA) (Kidwai et al., 2012), camphorsulfonic acid (CSA) (Shukla et al., 2011), silica supported polyphosphoric acid (Shaterian et al., 2009) and *N*-halosulfonamides (Ghorbani-Vaghei et al., 2011). However, so far there are no reports of the use of calix[*n*]arenes as catalysts in the synthesis of phthalazine derivatives.

Calix[*n*]arenes are macrocyclic cavity-shaped molecules obtained from the *ortho*-condensation of *para*-substituted phenols and formaldehyde in a basic medium (de Fátima et al., 2009; Simoes et al., 2012). Over the last few decades, these supramolecules have

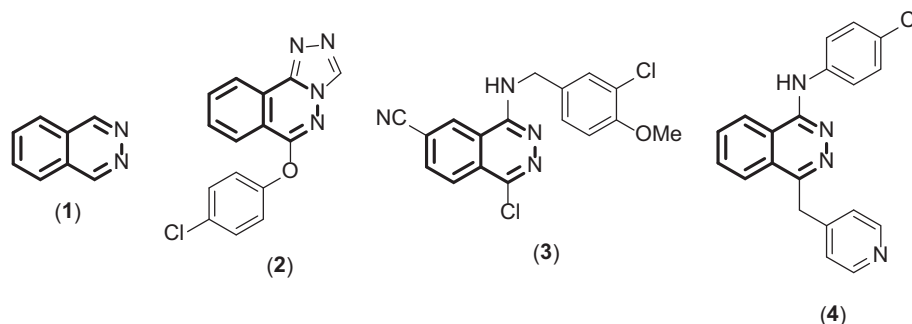
received increasing attention, particularly because of their applications as molecular hosts and organocatalysts (Gutsche, 2008; Varejão et al., 2013). Among all of the calix[*n*]arenes known so far, *p*-sulfonic acid calix[4]arene (**5**) and *p*-sulfonic acid calix[6]arene (**6**) (Fig. 2) have been shown to be the most efficient catalysts for different multicomponent reactions (da Silva et al., 2011, 2015; Simões et al., 2013, 2014).

Solvents are responsible for a large share of the environmental impact of the production processes of the chemical industry and are directly related to factors such as cost, safety and performance (Capello et al., 2007). In this context, the search for “green solvents” aims to reduce the environmental impact resulting from the use of solvents in chemical production processes (Capello et al., 2007). These solvents have been characterized for their low toxicity, easy biodegradability under environmental conditions, high boiling point and easy recyclability after use (Capello et al., 2007). Ethyl lactate is a “green solvent” derived from processing raw biomass material. This compound is the ester of lactic acid, and some lactate esters are solvents commonly used in the paints and coatings industry and have numerous attractive advantages, including being 100% biodegradable, easy to recycle, non-corrosive, non-carcinogenic and non-ozone depleting (Pereira et al., 2011). Dimethyl and diethyl carbonate also have properties that can lead to them being considered “green solvents”, such as their very low solubility in water, high boiling point and low vapor pressure.

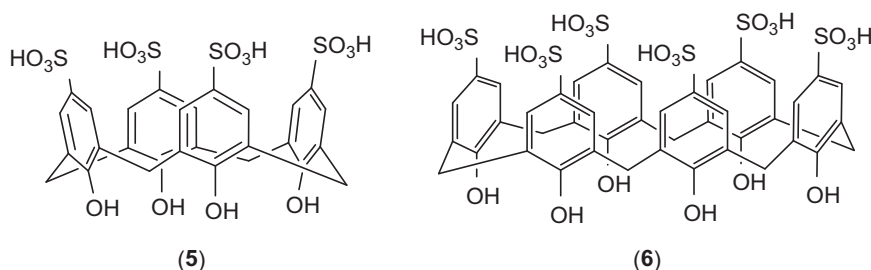
In this paper, we report a facile synthesis of 2*H*-indazolo[2,1-*b*]phthalazine-trione derivatives by the microwave-assisted one-pot three-component condensation of aromatic or aliphatic aldehydes, 5,5-dimethylcyclohexane-1,3-dione and phthalhydrazide in the presence of a catalytic amount of *p*-sulfonic acid calix[4]arene using ethanol, ethyl lactate, dimethyl carbonate and diethyl carbonate as “green solvents”. This procedure is very interesting because it combines a MCR synthetic protocol using microwave irradiation in the presence of green solvents. We also report the biological evaluation of fourteen phthalazine-triones as inhibitors of cancer cells proliferation.

## 2. Materials and methods

All of the chemicals were obtained from commercially available sources and used without further purification. Melting



**Figure 1** Phthalazine moiety (**1**) and chemical structures of bioactive phthalazine derivatives (**2–4**).



**Figure 2** Chemical structures of *p*-sulfonic acid calix[4]arene (**5**) and calix[6]arene (**6**).

points (uncorrected) were determined on a Mettler FP 80 HT apparatus. Infrared spectra were recorded on a Perkin Elmer Spectrum One spectrometer (KBr).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker AVANCE DPX-200 spectrometer in  $\text{CDCl}_3$  or  $\text{DMSO}-d_6$ . High resolution mass spectra were acquired on a Shimadzu LCMS-IT-TOF instrument. Single crystal X-ray diffraction measurements were carried out on an Oxford-Diffraction GEMINI-Ultra diffractometer (LabCri-UFMG) using graphite-Enhance Source Mo  $\text{K}\alpha$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ) at 150(2) K. The data collection, cell refinements, and data reduction were performed using the CrysalisPro software package (Oxford Diffraction, 2010). An absorption correction based on a multi-scan method was applied (Oxford Diffraction, 2010). The crystal structure was solved by direct methods using SHELXS-97 (Sheldrick, 2008). A full-matrix least-squares refinement procedure on  $F^2$  with anisotropic thermal parameters was carried on using SHELXL (Sheldrick, 2008). Positional and anisotropic atomic displacement parameters were refined for all non-hydrogen atoms. Hydrogen atoms were placed geometrically, and their positional parameters were refined using a riding model. PLATON was used to prepare the molecular graphics (Spek, 2009).

### 2.1. General procedure for the synthesis of phthalazine-triones 7–20

2,3-Dihydrophthalazine-1,4-dione (1.0 mmol), 5,5-dimethylcyclohexane-1,3-dione (1.2 mmol), aldehyde (1.5 mmol) and *p*-sulfonic acid calix[4]arene (1.5 mol%) were dissolved in 1 mL of ethyl lactate. The mixture was irradiated in a DiscoverCem® reactor for 10 min at 130 °C. The reaction was cooled to room temperature and water was added. The mixture was placed in a freezer at  $-20^\circ\text{C}$  to form the precipitate. The solid was isolated by filtration and purified by chromatography on a silica gel column using hexane/ethyl acetate as the eluent to obtain the phthalazine-trione of interest.

#### 2.1.1. 3,3-Dimethyl-13-phenyl-2,3,4,13-tetrahydro-1H-indazolo[1,2-*b*]phthalazine-1,6,11-trione (7) (Sayyafi et al., 2008)

Yellow solid. mp: 189–190 °C. IR (KBr) ( $\nu_{\text{max}}/\text{cm}^{-1}$ ): 3086, 3026, 2960, 2926, 1666, 1628, 1600, 1468, 1420, 1358, 1314, 1268, 1168, 1142, 1100, 1082, 798, 702, 560.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.11 (bs, 6H, 2x  $\text{CH}_3$ ), 2.23 (bs, 2H,  $\text{CH}_2$ ), 3.14 (d, 1H,  $J = 18.0 \text{ Hz}$ ,  $\text{CH}_2$ ), 3.33 (d, 1H,  $J = 18.0 \text{ Hz}$ ,  $\text{CH}_2$ ), 6.34 (s, 1H, CH), 7.09–7.46 (m, 5H, ArH), 7.66–7.85 (m, 2H, ArH), 8.08–8.36 (m, 2H, ArH);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.3 ( $\text{CH}_3$ ), 28.5 ( $\text{CH}_3$ ), 34.5 (C), 37.8 ( $\text{CH}_2$ ), 50.7 ( $\text{CH}_2$ ), 64.8 (CH), 118.4 (C), 127.0 (CH), 127.5 (CH), 127.8 (CH), 128.5 (CH), 128.7 (C), 128.8 (C), 133.4 (CH), 134.4 (CH), 136.2 (C), 150.7 (C), 154.1 (C), 155.8 (C), 192.0 (C). HRMS (ESI, IT-TOF) calculated for  $\text{C}_{23}\text{H}_{21}\text{N}_2\text{O}_3^+$ : 373.1547; found: 373.1596.

#### 2.1.2. 13-(Benzo[d][1,3]dioxol-5-yl)-3,3-dimethyl-2,3,4,13-tetrahydro-1H-indazolo[1,2-*b*]phthalazine-1,6,11-trione (8) (Veeranarayana Reddy et al., 2012)

Yellow solid. mp: 256–257 °C. IR (KBr) ( $\nu_{\text{max}}/\text{cm}^{-1}$ ): 2962, 2898, 1662, 1624, 1490, 1364, 1314, 1268, 1244, 1100, 1034, 924, 788, 700.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.21 (s, 3H,  $\text{CH}_3$ ), 1.22 (s, 3H,  $\text{CH}_3$ ), 2.34 (bs, 2H,  $\text{CH}_2$ ), 3.22 (d, 1H,

$J = 18.0 \text{ Hz}$ ,  $\text{CH}_2$ ), 3.41 (d, 1H,  $J = 18.0 \text{ Hz}$ ,  $\text{CH}_2$ ), 5.90 (s, 2H,  $\text{CH}_2$ ), 6.36 (s, 1H, CH), 6.75 (d, 1H,  $J = 8.0 \text{ Hz}$ , ArH), 6.84 (s, 1H, ArH), 6.94 (d, 1H,  $J = 8.0 \text{ Hz}$ , ArH), 7.76–7.95 (m, 2H, ArH), 8.19–8.42 (m, 2H, ArH);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.5 ( $\text{CH}_3$ ), 28.6 ( $\text{CH}_3$ ), 34.6 (C), 38.0 ( $\text{CH}_2$ ), 50.9 ( $\text{CH}_2$ ), 64.7 (CH), 101.2 ( $\text{CH}_2$ ), 107.4 (CH), 108.4 (CH), 118.4 (C), 121.2 (CH), 127.6 (CH), 127.9 (CH), 128.9 (C), 129.1 (C), 130.1 (C), 133.5 (CH), 134.5 (CH), 147.9 (C), 150.8 (C), 154.3 (C), 156.0 (C), 192.1 (C). HRMS (ESI, IT-TOF) calculated for  $\text{C}_{24}\text{H}_{21}\text{N}_2\text{O}_5^+$ : 417.1445; found: 417.1442.

#### 2.1.3. 13-(4-Chlorophenyl)-3,3-dimethyl-2,3,4,13-tetrahydro-1H-indazolo[1,2-*b*]phthalazine-1,6,11-trione (9) (Sayyafi et al., 2008)

Yellow solid. mp: 227–229 °C. IR (KBr) ( $\nu_{\text{max}}/\text{cm}^{-1}$ ): 3166, 3032, 2958, 2932, 2894, 1654, 1624, 1492, 1468, 1362, 1312, 1268, 1148, 1106, 1090, 1014, 840, 794, 698, 530.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.20 (bs, 6H, 2x  $\text{CH}_3$ ), 2.33 (bs, 2H,  $\text{CH}_2$ ), 3.23 (d, 1H,  $J = 19.0 \text{ Hz}$ ,  $\text{CH}_2$ ), 3.41 (d, 1H,  $J = 19.0 \text{ Hz}$ ,  $\text{CH}_2$ ), 6.41 (s, 1H, CH), 7.29 (d, 2H,  $J = 8.5 \text{ Hz}$ , ArH), 7.37 (d, 2H,  $J = 8.5 \text{ Hz}$ , ArH), 7.78–7.90 (m, 2H, ArH), 8.18–8.41 (m, 2H, ArH);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.3 ( $\text{CH}_3$ ), 28.6 ( $\text{CH}_3$ ), 34.6 (C), 37.9 ( $\text{CH}_2$ ), 50.7 ( $\text{CH}_2$ ), 64.2 (CH), 117.9 (C), 127.6 (CH), 127.9 (CH), 128.4 (CH), 128.7 (C), 128.8 (C, CH), 133.6 (CH), 134.4 (C), 134.5 (CH), 134.8 (C), 150.1 (C), 154.3 (C), 155.9 (C), 192.0 (C). HRMS (ESI, IT-TOF) calculated for  $\text{C}_{23}\text{H}_{20}\text{ClN}_2\text{O}_3^+$ : 407.1157; found: 407.1144.

#### 2.1.4. 13-(4-Fluorophenyl)-3,3-dimethyl-2,3,4,13-tetrahydro-1H-indazolo[1,2-*b*]phthalazine-1,6,11-trione (10) (Sayyafi et al., 2008)

Yellow solid. mp: 196–198 °C. IR (KBr) ( $\nu_{\text{max}}/\text{cm}^{-1}$ ): 3070, 2960, 2926, 2878, 1666, 1628, 1602, 1508, 1468, 1420, 1360, 1314, 1268, 1220, 1144, 1098, 1080, 1028, 850, 798, 702, 528.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.21 (bs, 6H, 2x  $\text{CH}_3$ ), 2.34 (bs, 2H,  $\text{CH}_2$ ), 3.23 (d, 1H,  $J = 19.2 \text{ Hz}$ ,  $\text{CH}_2$ ), 3.42 (d, 1H,  $J = 19.2 \text{ Hz}$ ,  $\text{CH}_2$ ), 6.43 (s, 1H, CH), 6.92–7.11 (m, 2H, ArH), 7.32–7.49 (m, 2H, ArH), 7.78–7.93 (m, 2H, ArH), 8.20–8.42 (m, 2H, ArH);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.4 ( $\text{CH}_3$ ), 28.6 ( $\text{CH}_3$ ), 34.6 (C), 38.0 ( $\text{CH}_2$ ), 50.9 ( $\text{CH}_2$ ), 64.2 (CH), 115.7 (d,  $J_{\text{C-F}} = 21.8 \text{ Hz}$ , CH), 118.2 (C), 127.7 (CH), 128.0 (CH), 128.9 (C), 129.0 (d,  $J_{\text{C-F}} = 8.6 \text{ Hz}$ , CH), 132.2 (C), 133.6 (CH), 134.6 (CH), 151.0 (C), 154.3 (C), 156.0 (C), 192.2 (C). HRMS (ESI, IT-TOF) calculated for  $\text{C}_{23}\text{H}_{20}\text{FN}_2\text{O}_3^+$ : 391.1452; found: 391.1466.

Crystals suitable for single-crystal X-ray diffraction were obtained from slow evaporation of a saturated methanolic solution of the product.

#### 2.1.5. 13-(4-Nitrophenyl)-3,3-dimethyl-2,3,4,13-tetrahydro-1H-indazolo[1,2-*b*]phthalazine-1,6,11-trione (11) (Sayyafi et al., 2008)

Yellow solid. mp: 205–206 °C. IR (KBr) ( $\nu_{\text{max}}/\text{cm}^{-1}$ ): 3076, 2970, 2956, 1694, 1660, 1618, 1522, 1470, 1366, 1312, 1276, 1256, 1146, 1018, 856, 792, 696, 578.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.20 (s, 3H,  $\text{CH}_3$ ), 1.22 (s, 3H,  $\text{CH}_3$ ), 2.34 (bs, 2H,  $\text{CH}_2$ ), 3.26 (d, 1H,  $J = 18.8 \text{ Hz}$ ,  $\text{CH}_2$ ), 3.43 (d, 1H,  $J = 18.8 \text{ Hz}$ ,  $\text{CH}_2$ ), 6.51 (s, 1H, CH), 7.61 (d, 2H,  $J = 8.0 \text{ Hz}$ , ArH), 7.82–8.00 (m, 2H, ArH), 8.12–8.50

(m, 4H, ArH);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.3 ( $\text{CH}_3$ ), 28.6 ( $\text{CH}_3$ ), 34.7 (C), 38.0 ( $\text{CH}_2$ ), 50.7 ( $\text{CH}_2$ ), 64.1 (CH), 117.2 (C), 124.0 (CH), 127.7 (CH), 128.0 (CH), 128.2 (CH), 128.5 (C), 128.9 (C), 133.9 (CH), 134.8 (CH), 143.4 (C), 147.8 (C), 151.7 (C), 154.5 (C), 155.9 (C), 192.1 (C). HRMS (ESI, IT-TOF) calculated for  $\text{C}_{23}\text{H}_{20}\text{N}_3\text{O}_5^+$ : 418.1397; found: 418.1388.

**2.1.6. 13-(3-Hydroxyphenyl)-3,3-dimethyl-2,3,4,13-tetrahydro-1H-indazolo[1,2-b]phthalazine-1,6,11-trione (12)** (Ghorbani-Vaghei et al., 2011)

White solid. mp: 260–261 °C. IR (KBr) ( $\nu_{\text{max}}/\text{cm}^{-1}$ ): 3356, 3116, 2952, 2872, 1662, 1630, 1592, 1470, 1432, 1360, 1314, 1288, 1266, 1234, 1144, 1102, 1084, 784, 700, 686, 532.  $^1\text{H}$  NMR (200 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  1.11 (s, 3H,  $\text{CH}_3$ ), 1.12 (s, 3H,  $\text{CH}_3$ ), 2.27 (bs, 2H,  $\text{CH}_2$ ), 3.16 (dd, 1H,  $J = 18.8$  Hz,  $J = 2.1$  Hz,  $\text{CH}_2$ ), 3.27–3.37 (m, 1H,  $\text{CH}_2$ ), 6.18 (s, 1H, CH), 6.62–6.68 (m, 1H, ArH), 6.79–6.85 (m, 2H, ArH), 7.08 (t, 1H,  $J = 8.1$  Hz, ArH), 7.93–8.01 (m, 2H, ArH), 8.08–8.15 (m, 1H, ArH), 8.23–8.30 (m, 1H, ArH), 9.39 (s, 1H, OH);  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  27.8 ( $\text{CH}_3$ ), 28.0 ( $\text{CH}_3$ ), 34.3 (C), 37.2 ( $\text{CH}_2$ ), 50.3 ( $\text{CH}_2$ ), 64.2 (CH), 114.4 (CH), 114.9 (CH), 117.6 (C), 117.8 (CH), 126.8 (CH), 127.6 (CH), 128.7 (C), 128.9 (C), 129.1 (CH), 133.7 (CH), 134.6 (CH), 138.8 (C), 151.0 (C), 153.7 (C), 155.3 (C), 157.2 (C), 191.8 (C). HRMS (ESI, IT-TOF) calculated for  $\text{C}_{23}\text{H}_{21}\text{N}_2\text{O}_4^+$ : 389.1496; found: 389.1472.

**2.1.7. 13-(4-Cyanophenyl)-3,3-dimethyl-2,3,4,13-tetrahydro-1H-indazolo[1,2-b]phthalazine-1,6,11-trione (13)** (Hasaninejed et al., 2012)

Yellow solid. mp: 213–215 °C. IR (KBr) ( $\nu_{\text{max}}/\text{cm}^{-1}$ ): 3130, 3040, 2958, 2932, 2230, 1654, 1620, 1502, 1468, 1390, 1362, 1312, 1270, 1150, 1106, 1026, 844, 792, 700, 558, 498.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.19 (s, 3H,  $\text{CH}_3$ ), 1.22 (s, 3H,  $\text{CH}_3$ ), 2.34 (bs, 2H,  $\text{CH}_2$ ), 3.25 (d, 1H,  $J = 19.2$  Hz,  $\text{CH}_2$ ), 3.41 (d, 1H,  $J = 19.2$  Hz,  $\text{CH}_2$ ), 6.45 (s, 1H, CH), 7.55 (d, 2H,  $J = 8.1$  Hz, ArH), 7.63 (d, 2H,  $J = 8.1$  Hz, ArH), 7.81–7.96 (m, 2H, ArH), 8.19–8.43 (m, 2H, ArH);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.3 ( $\text{CH}_3$ ), 28.6 ( $\text{CH}_3$ ), 34.6 (C), 37.9 ( $\text{CH}_2$ ), 50.7 ( $\text{CH}_2$ ), 64.3 (CH), 112.3 (C), 117.2 (C), 118.3 (C), 127.6 (CH), 127.8 (CH), 128.1 (CH), 128.5 (C), 128.8 (C), 132.5 (CH), 133.8 (CH), 134.7 (CH), 141.5 (C), 151.5 (C), 154.4 (C), 155.8 (C), 192.0 (C). HRMS (ESI, IT-TOF) calculated for  $\text{C}_{24}\text{H}_{20}\text{N}_3\text{O}_3^+$ : 398.1499; found: 398.1471.

**2.1.8. 13-(3-Methoxyphenyl)-3,3-dimethyl-2,3,4,13-tetrahydro-1H-indazolo[1,2-b]phthalazine-1,6,11-trione (14)** (Hasaninejed et al., 2012)

Yellow solid. mp: 221–222 °C. IR (KBr) ( $\nu_{\text{max}}/\text{cm}^{-1}$ ): 3030, 3022, 2971, 2937, 1654, 1600, 1502, 1372, 1221, 1180, 1031, 744, 535, 496.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.21 (bs, 6H, 2x  $\text{CH}_3$ ), 2.33 (bs, 2H,  $\text{CH}_2$ ), 3.22 (d, 1H,  $J = 19.3$  Hz,  $\text{CH}_2$ ), 3.41 (d, 1H,  $J = 19.3$  Hz,  $\text{CH}_2$ ), 3.78 (s, 3H,  $\text{OCH}_3$ ), 6.41 (s, 1H, CH), 6.82 (d, 1H,  $J = 8.0$  Hz, ArH), 6.95 (s, 1H, ArH), 7.00 (d, 1H,  $J = 8.0$  Hz, ArH), 7.25 (t, 1H,  $J = 8.0$  Hz, ArH), 7.77–7.94 (m, 2H, ArH), 8.20–8.43 (m, 2H, ArH);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.4 ( $\text{CH}_3$ ), 28.6 ( $\text{CH}_3$ ), 34.6 (C), 38.0 ( $\text{CH}_2$ ), 50.9 ( $\text{CH}_2$ ), 55.2 ( $\text{CH}_3$ ), 64.7 (CH), 113.1 (CH), 113.8 (CH), 118.5 (C), 119.4 (CH), 127.7 (CH), 127.9 (CH), 128.9 (C), 129.0 (C), 129.7 (CH), 133.5

(CH), 134.5 (CH), 137.9 (C), 150.8 (C), 154.2 (C), 156.0 (C), 159.7 (C), 192.1 (C). HRMS (ESI, IT-TOF) calculated for  $\text{C}_{24}\text{H}_{23}\text{N}_2\text{O}_4^+$ : 403.1652; found: 403.1626.

**2.1.9. 13-(4-Hydroxy-3,5-dimethoxyphenyl)-3,3-dimethyl-2,3,4,13-tetrahydro-1H-indazolo[1,2-b]phthalazine-1,6,11-trione (15)**

Yellow solid. mp: 210–212 °C. IR (KBr) ( $\nu_{\text{max}}/\text{cm}^{-1}$ ): 3510, 3168, 3018, 2962, 1656, 1624, 1518, 1492, 1466, 1432, 1362, 1314, 1266, 1218, 1146, 1112, 1082, 826, 792, 700, 628.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.24 (bs, 6H, 2x  $\text{CH}_3$ ), 2.36 (bs, 2H,  $\text{CH}_2$ ), 3.21 (d, 1H,  $J = 19.1$  Hz,  $\text{CH}_2$ ), 3.47 (d, 1H,  $J = 19.1$  Hz,  $\text{CH}_2$ ), 3.85 (bs, 6H, 2x  $\text{OCH}_3$ ), 5.68 (bs, 1H, OH), 6.38 (s, 1H, CH), 6.65 (s, 2H, ArH), 7.79–7.95 (m, 2H, ArH), 8.21–8.43 (m, 2H, ArH);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.0 ( $\text{CH}_3$ ), 28.9 ( $\text{CH}_3$ ), 34.5 (C), 38.0 ( $\text{CH}_2$ ), 50.8 ( $\text{CH}_2$ ), 56.3 (2x  $\text{CH}_3$ ), 65.0 (CH), 104.4 (CH), 118.3 (C), 127.2 (C), 127.6 (CH), 127.9 (CH), 128.8 (C), 129.0 (C), 133.5 (CH), 134.5 (CH), 136.2 (C), 147.0 (C), 150.7 (C), 154.4 (C), 156.1 (C), 192.2 (C). HRMS (ESI, IT-TOF) calculated for  $\text{C}_{25}\text{H}_{25}\text{N}_2\text{O}_6^+$ : 449.1707; found: 449.1691.

**2.1.10. 3,3-Dimethyl-13-(4-(trifluoromethyl)phenyl)-2,3,4,13-tetrahydro-1H-indazolo[1,2-b]phthalazine-1,6,11-trione (16)** (Wang et al., 2010)

Yellow solid. mp: 248–250 °C. IR (KBr) ( $\nu_{\text{max}}/\text{cm}^{-1}$ ): 2960, 2934, 2874, 1658, 1624, 1468, 1424, 1360, 1310, 1266, 1162, 1130, 1066, 854, 702.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.20 (s, 3H,  $\text{CH}_3$ ), 1.22 (s, 3H,  $\text{CH}_3$ ), 2.34 (bs, 2H,  $\text{CH}_2$ ), 3.25 (dd, 1H,  $J = 19.2$  Hz,  $J = 2.2$  Hz,  $\text{CH}_2$ ), 3.41 (dd, 1H,  $J = 19.2$  Hz,  $J = 1.0$  Hz,  $\text{CH}_2$ ), 6.49 (s, 1H, CH), 7.54 (d, 2H,  $J = 8.4$  Hz, ArH), 7.61 (d, 2H,  $J = 8.4$  Hz, ArH), 7.84–7.93 (m, 2H, ArH), 8.22–8.42 (m, 2H, ArH);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.4 ( $\text{CH}_3$ ), 28.7 ( $\text{CH}_3$ ), 34.7 (C), 38.0 ( $\text{CH}_2$ ), 50.8 ( $\text{CH}_2$ ), 64.4 (CH), 117.8 (C), 125.8 (q,  $J = 3.9$  Hz, CH), 127.4 (CH), 127.8 (CH), 128.1 (CH), 128.8 (C), 128.9 (C), 130.7 (q,  $J = 32.5$  Hz, C), 133.8 (CH), 134.7 (CH), 140.3 (C), 151.3 (C), 154.4 (C), 156.0 (C), 192.1 (C). HRMS (ESI, IT-TOF) calculated for  $\text{C}_{24}\text{H}_{20}\text{F}_3\text{N}_2\text{O}_3^+$ : 441.1421; found: 441.1441.

**2.1.11. 3,3-Dimethyl-13-(4-(methylthio)phenyl)-2,3,4,13-tetrahydro-1H-indazolo[1,2-b]phthalazine-1,6,11-trione (17)** (Ghorbani-Vaghei et al., 2011)

Yellow solid. mp: 216–218 °C. IR (KBr) ( $\nu_{\text{max}}/\text{cm}^{-1}$ ): 3024, 2960, 2946, 2924, 1656, 1630, 1466, 1360, 1312, 1268, 1148, 1104, 1092, 704.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.20 (bs, 6H, 2x  $\text{CH}_3$ ), 2.33 (bs, 2H,  $\text{CH}_2$ ), 2.42 (s, 3H,  $\text{CH}_3$ ), 3.22 (dd, 1H,  $J = 19.1$  Hz,  $J = 1.8$  Hz,  $\text{CH}_2$ ), 3.42 (d, 1H,  $J = 19.1$  Hz,  $\text{CH}_2$ ), 6.40 (s, 1H, CH), 7.19 (d, 2H,  $J = 8.3$  Hz, ArH), 7.33 (d, 2H,  $J = 8.3$  Hz, ArH), 7.77–7.89 (m, 2H, ArH), 8.19–8.40 (m, 2H, ArH);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  15.6 ( $\text{CH}_3$ ), 28.4 ( $\text{CH}_3$ ), 28.6 (CH), 34.5 (C), 38.1 ( $\text{CH}_2$ ), 51.0 ( $\text{CH}_2$ ), 64.5 (CH), 118.4 (C), 126.1 (CH), 127.5 (CH), 127.6 (CH), 127.9 (CH), 129.0 (C), 129.1 (C), 133.2 (C), 133.4 (CH), 134.4 (CH), 139.2 (C), 150.8 (C), 154.3 (C), 156.0 (C), 191.9 (C). HRMS (ESI, IT-TOF) calculated for  $\text{C}_{24}\text{H}_{23}\text{N}_2\text{O}_3\text{S}^+$ : 419.1424; found: 419.1424.



**2.1.12. 3,3-Dimethyl-13-propyl-2,3,4,13-tetrahydro-1H-indazolo[1,2-b]phthalazine-1,6,11-trione (18)** (Ghorbani-Vaghei et al., 2011)

Yellow solid. mp: 186–187 °C. IR (KBr) ( $\nu_{\max}/\text{cm}^{-1}$ ): 3158, 3016, 1646, 1622, 1515, 1490, 1456, 1422, 1382, 1310, 1260, 1141, 1110, 1081, 824, 790, 666.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.87 (t, 3H,  $J = 7.2$  Hz,  $\text{CH}_3$ ), 1.06–1.30 (m, 8H,  $\text{CH}_2$ , 2x  $\text{CH}_3$ ), 1.90–2.53 (m, 4H, 2x  $\text{CH}_2$ ), 3.13 (d, 1H,  $J = 19.3$  Hz,  $\text{CH}_2$ ), 3.34 (d, 1H,  $J = 19.3$  Hz,  $\text{CH}_2$ ), 5.70 (s, 1H, CH), 7.79–7.97 (m, 2H, ArH), 8.28–8.42 (m, 2H, ArH);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  13.7 ( $\text{CH}_3$ ), 16.7 ( $\text{CH}_2$ ), 28.5 ( $\text{CH}_3$ ), 28.7 ( $\text{CH}_3$ ), 31.5 ( $\text{CH}_2$ ), 34.5 (C), 38.0 ( $\text{CH}_2$ ), 51.0 ( $\text{CH}_2$ ), 62.8 (CH), 117.3 (C), 127.5 (CH), 127.9 (CH), 128.9 (C), 129.0 (C), 133.4 (CH), 134.5 (CH), 151.6 (C), 154.7 (C), 156.1 (C), 193.1 (C). HRMS (ESI, IT-TOF) calculated for  $\text{C}_{20}\text{H}_{23}\text{N}_2\text{O}_3^+$ : 339.1703; found: 339.1691.

**2.1.13. 13-Butyl-3,3-dimethyl-2,3,4,13-tetrahydro-1H-indazolo[1,2-b]phthalazine-1,6,11-trione (19)**

Yellow solid. mp: 146–148 °C. IR (KBr) ( $\nu_{\max}/\text{cm}^{-1}$ ): 3078, 2962, 2928, 2856, 1654, 1626, 1468, 1434, 1366, 1294, 1274, 1152, 1086, 788, 696.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.81 (t, 3H,  $J = 7.1$  Hz,  $\text{CH}_3$ ), 1.01–1.37 (m, 10H, 2x  $\text{CH}_2$ , 2x  $\text{CH}_3$ ), 2.01–2.55 (m, 4H, 2x  $\text{CH}_2$ ), 3.13 (dd, 1H,  $J = 19.2$  Hz,  $J = 2.3$  Hz,  $\text{CH}_2$ ), 3.35 (d, 1H,  $J = 19.2$  Hz,  $\text{CH}_2$ ), 5.70 (s, 1H, CH), 7.76–7.96 (m, 2H, ArH), 8.23–8.43 (m, 2H, ArH);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  13.8 ( $\text{CH}_3$ ), 22.3 ( $\text{CH}_2$ ), 25.5 ( $\text{CH}_2$ ), 28.4 ( $\text{CH}_3$ ), 28.7 ( $\text{CH}_3$ ), 29.3 ( $\text{CH}_2$ ), 34.4 (C), 38.1 ( $\text{CH}_2$ ), 51.1 ( $\text{CH}_2$ ), 62.9 (CH), 117.4 (C), 127.5 (CH), 127.8 (CH), 129.0 (C), 129.1 (C), 133.3 (CH), 134.3 (CH), 151.5 (C),

154.7 (C), 156.1 (C), 192.7 (C). HRMS (ESI, IT-TOF) calculated for  $\text{C}_{21}\text{H}_{25}\text{N}_2\text{O}_3^+$ : 353.1860; found: 353.1797.

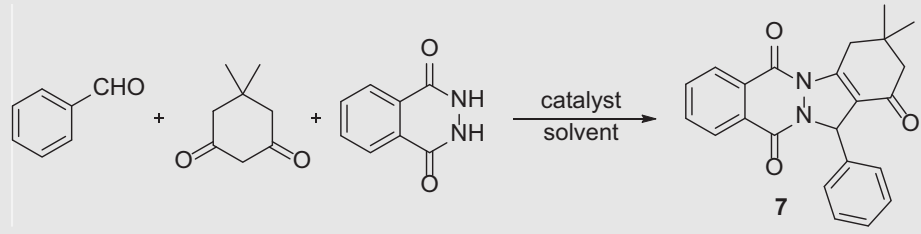
**2.1.14. 3,3-Dimethyl-2,3,4,13-tetrahydro-1H-indazolo[1,2-b]phthalazine-1,6,11-trione (20)**

Yellow solid. mp: 150–152 °C. IR (KBr) ( $\nu_{\max}/\text{cm}^{-1}$ ): 3168, 3022, 2958, 2892, 1656, 1494, 1360, 1270, 1082, 848, 792, 700.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.18 (br, 6H, 2x  $\text{CH}_3$ ), 2.37 (bs, 2H,  $\text{CH}_2$ ), 3.20 (br, 2H,  $\text{CH}_2$ ), 4.93 (bs, 2H,  $\text{CH}_2$ ), 7.73–7.96 (m, 2H, ArH), 8.22–8.39 (m, 2H, ArH);  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.5 (2x  $\text{CH}_3$ ), 34.6 (C), 38.0 ( $\text{CH}_2$ ), 49.5 ( $\text{CH}_2$ ), 50.7 ( $\text{CH}_2$ ), 114.9 (C), 127.4 (CH), 127.9 (CH), 128.6 (C), 129.0 (C), 133.4 (CH), 134.3 (CH), 152.3 (C), 154.5 (C), 155.9 (C), 192.7 (C). HRMS (ESI, IT-TOF) calculated for  $\text{C}_{17}\text{H}_{17}\text{N}_2\text{O}_3^+$ : 297.1234; found: 297.1506.

**2.2. Antiproliferative assay**

Human tumor cell lines U251 (glioma), MCF7 (breast) NCI-ADR/RES (multiple drug-resistant ovarian), 786-0 (renal), NCI-H460 (lung, non-small cells), PC-3 (prostate), OVCAR-03 (ovarian) HT-29 (colon) and K562 (leukemia) were kindly provided by the Frederick Cancer Research & Development Center, National Cancer Institute (Frederick, MA, USA). Stock cultures were grown in RPMI 1640 (GIBCO BRL, Life Technologies) supplemented with 5% of fetal bovine serum and penicillin (final concentration of 1 mg/mL) and streptomycin (final concentration of 200 U/mL). Cells in 96-well plates (100  $\mu\text{L}$  cells/well) were exposed to phthalazine-triones (0.25–250  $\mu\text{g}/\text{mL}$ ) for 48 h at 37 °C and 5% of  $\text{CO}_2$ . Afterward

**Table 1** Optimization of the reaction conditions for the synthesis of phthalazine-trione **7**<sup>a</sup>.

					
Entry	Catalyst (mol%)	Solvent	Temperature (°C)	Time (min)	Yield (%) <sup>b</sup>
1	–	Ethanol	Reflux	10	0
2	<b>5</b> (2.5)	Ethanol	Reflux	10	41
3	<b>5</b> (2.0)	Ethanol	Reflux	10	22
4	<b>5</b> (1.5)	Ethanol	Reflux	10	75
5	<b>5</b> (1.0)	Ethanol	Reflux	10	45
6	<b>5</b> (0.5)	Ethanol	Reflux	10	39
7	PHSA <sup>c</sup> (6.0)	Ethanol	Reflux	10	12
8	PHSA (9.0)	Ethanol	Reflux	10	60
9	<b>5</b> (1.5)	Ethanol	Reflux	5	35
10	<b>5</b> (1.5)	Dimethyl carbonate	Reflux	10	5
11	<b>5</b> (1.5)	Diethyl carbonate	Reflux	10	6
12	<b>5</b> (1.5)	Ethyl lactate	130	10	81

<sup>a</sup> Reagents and conditions: benzaldehyde/5,5-dimethylcyclohexane-1,3-dione/phthalhydrazide (molar ratio = 1.5:1.2:1.0), under microwave irradiation.

<sup>b</sup> Isolated yields.

<sup>c</sup> PHSA = *p*-hydroxybenzenesulfonic acid.

cells were fixed with 50% trichloroacetic acid, submitted to sulforhodamine B assay for cell proliferation quantification at 540 nm (Monks et al., 1991). The concentration of compound that inhibits cell growth by 50% ( $GI_{50}$ ) was determined through non-linear regression analysis using software ORIGIN 7.5 (OriginLab Corporation). Doxorubicin was used as a reference drug.

### 3. Results and discussion

#### 3.1. Synthesis of phthalazine-triones

Sulfonic acid calix[*n*]arenes have proven to be efficient catalysts in different types of organic reactions (Fernandes et al., 2012; Natalino et al., 2014; Liu et al., 2008; Shimizu et al., 2006). Recently, we demonstrated that these macrocycles are effective in promoting synthesis of xanthenones by multicomponent reactions (da Silva et al., 2015). Thus, in the present study, we evaluated the efficiency of *p*-sulfonic acid calix[4]arene **5** in the synthesis of phthalazine-triones.

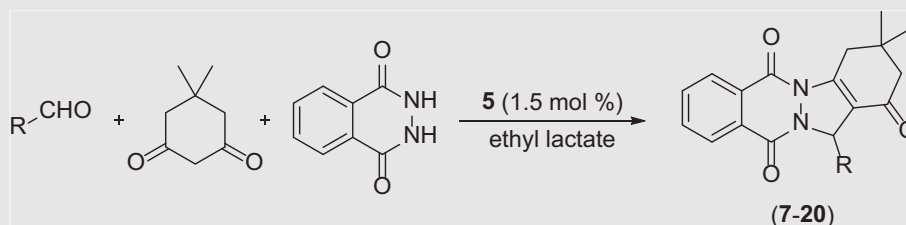
A preliminary investigation aiming to optimize the reaction conditions was performed. For a model reaction, benzaldehyde, 5,5-dimethylcyclohexane-1,3-dione and phthalhydrazide were employed (Table 1). The reactions were performed in a DiscoverCem® reactor for a period of 10 min using ethanol as the solvent. Initially, we evaluated the catalytic activity of *p*-sulfonic acid calix[4]arene (**5**) in promoting the reaction. This catalyst was tested in ratios ranging from 2.5 to 0.5 mol% (Table 1, entries 2–6). The best yield was obtained when **5**

was used at a concentration of 1.5 mol% (Table 1, entry 4). Interestingly, the yields decreased with an increase in the molar ratio of the catalyst (Table 1, entries 2 and 3). The use of *p*-sulfonic acid calix[6]arene (**6**) as the catalyst furnished the desired products in low yields (<40%) (data not shown). To explore the role of the spatial arrangement imposed by the calix[4]arene **5** on the efficiency of the catalysis process, we used *p*-hydroxybenzenesulfonic acid (PHSA) as a catalyst. Because each molecule of *p*-sulfonic acid calix[4]arene corresponds to four units of PHSA, this catalyst was used at a concentration of 6.0 mol% (Table 1, entry 7). At this concentration, PHSA led to the formation of phthalazine-trione **7** in only a 12% yield. Even at a concentration of 9.0 mol%, PHSA was still less effective than *p*-sulfonic acid calix[4]arene (Table 1, entry 8). These results suggest that the spatial organization of the macrocycle is important for the catalytic activity. No product formation was observed in the reaction carried out without any catalyst under the tested experimental conditions (Table 1, entry 1).

Once the optimal concentration of *p*-sulfonic acid calix[4]arene was obtained, we evaluated the effect of reducing the reaction time. Thus, a new test reaction was carried out for a period of 5 min. However, the reduction in the reaction time led to a significant decrease in the yield (Table 1, entry 9).

The promising results obtained with calix[4]arene **5** prompted us to further investigate the effect of solvents in the synthesis of phthalazine-trione **7** catalyzed by this macrocyclic compound. Dimethyl carbonate, diethyl carbonate, and ethyl lactate were evaluated. Dimethyl and diethyl carbonate furnished **7** in low yields (5% and 6%, respectively)

**Table 2** Use of different aldehydes in the synthesis of phthalazine-trione derivatives<sup>a</sup>.



Entry	R	Compound	Yield (%) <sup>b</sup>
1	Phenyl	<b>7</b>	81
2	3,4-OCH <sub>2</sub> O-phenyl	<b>8</b>	51
3	4-Cl-phenyl	<b>9</b>	85
4	4-F-phenyl	<b>10</b>	83
5	4-NO <sub>2</sub> -phenyl	<b>11</b>	83
6	3-OH-phenyl	<b>12</b>	67
7	4-CN-phenyl	<b>13</b>	87
8	3-OCH <sub>3</sub> -phenyl	<b>14</b>	75
9	3,5-OCH <sub>3</sub> , 4-OH-phenyl	<b>15</b>	61
10	4-CF <sub>3</sub> -phenyl	<b>16</b>	94
11 <sup>b</sup>	4-SCH <sub>3</sub> -phenyl	<b>17</b>	31
12 <sup>b</sup>	<i>n</i> -propyl	<b>18</b>	30
13 <sup>b</sup>	<i>n</i> -butyl	<b>19</b>	35
14 <sup>b</sup>	H	<b>20</b>	11

<sup>a</sup> Reagents and conditions: aldehyde/5,5-dimethylcyclohexane-1,3-dione/phthalhydrazide (molar ratio = 1.5:1.2:1.0) using 1 mL ethyl lactate at 130 °C under microwave irradiation.

<sup>b</sup> These reactions were carried out at 85 °C under microwave irradiation.

(Table 1, entries 10 and 11). The best yield, 81%, was achieved using ethyl lactate as the solvent (Table 1, entry 12). Reactions carried out without solvents furnished phthalazine-trione **7** in yields lower than 40% (data not shown).

After establishing the best solvent, the scope of this protocol was further investigated. Aliphatic and aromatic aldehydes bearing electron-donating or electron-withdrawing groups were employed in the synthesis of a series of phthalazine-trione derivatives, and the expected products were obtained, mainly, in good to moderate yields (Table 2).

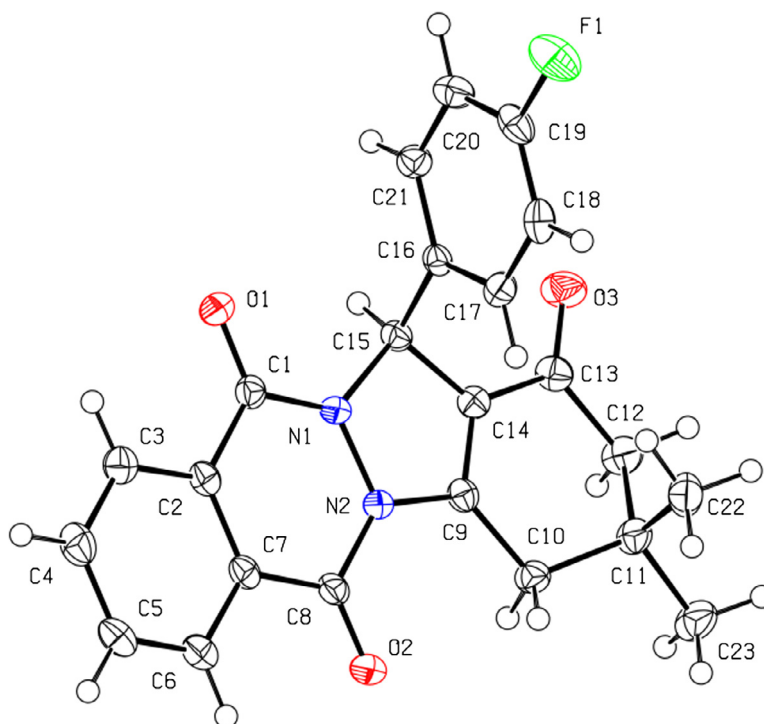
In general, aromatic aldehydes led to good yields, except for piperonal and 4-(methylthio)benzaldehyde (Table 2, entries 2 and 11, respectively). Aldehydes with electron-withdrawing groups led to higher yields (83–94%) than do those with electron-donating groups. However, non-aromatic aldehydes were less reactive than aromatic aldehydes and afforded only low yields (Table 2, entries 12–14).

Once synthesized, the structural features of the compounds were determined from the corresponding IR, NMR, and ESI-HRMS data. Crystals of **10** were formed, and its crystal structure was determined using single-crystal X-ray diffractometry. The atom arrangements and atom numbering scheme for **10** are shown in Fig. 3 while the crystal data and refinement results are listed in Table 3.

Phthalazine-trione **10** crystallized in the monoclinic space group  $P2_1/n$  with one independent molecule in the asymmetric unit. Although the molecule shown in Fig. 3 has a chiral center located on atom C15, the compound crystallized as a racemate because it crystallizes in a centrosymmetric space group. The

**Table 3** Crystal data and structural refinement results for **10**.

CCDC number	1,021,277
Empirical formula	C <sub>23</sub> H <sub>19</sub> FN <sub>2</sub> O <sub>3</sub>
Molecular weight (g mol <sup>-1</sup> )	390.40
Crystal system	Monoclinic
Space group	$P2_1/n$
Unit cell dimensions	$a$ (Å) 8.5083(2)
	$b$ (Å) 20.3729(4)
	$c$ (Å) 11.3768(3)
	$\alpha = \gamma$ (°) 90
	$\beta$ (°) 111.220(3)
$V$ (Å <sup>3</sup> )	1838.33(7)
$Z$	4
Density calculated (g cm <sup>-3</sup> )	1.411
Absorption coefficient (mm <sup>-1</sup> )	0.101
$F(000)$	816
Crystal size (mm <sup>3</sup> )	0.64 × 0.59 × 0.25
$\theta$ range for data collection (°)	2.00–26.37
Index ranges	$-10 \leq h \leq 10$
	$-25 \leq k \leq 25$
	$-14 \leq l \leq 14$
Reflections collected	36,936
Independent reflections	3762 [ $R(\text{int}) = 0.0351$ ]
Completeness to $\theta = 26.37^\circ$ (%)	100.0
Max. and min. transmission	1.00000 and 0.83326
Data/restraints/parameters	3762/0/264
Goodness-of-fit on $F^2$	1.059
Final $R$ indices [ $I > 2\sigma(I)$ ]	$R_1 = 0.0362$ , $wR_2 = 0.0902$
$R$ indices (all data)	$R_1 = 0.0409$ , $wR_2 = 0.0938$
Largest diff. peak and hole (eÅ <sup>-3</sup> )	0.235 and -0.314



**Figure 3** Molecular plot of **10** showing the labeling scheme of the non-H atoms and their displacement ellipsoids at a 50% probability level. The hydrogen atoms are shown as circles of arbitrary radii.

**Table 4** Concentration of phthalazine-triones (GI<sub>50</sub> in µg/mL) that elicit cancer cells<sup>a</sup> growth inhibition by 50%.

Compound	Cell Line									
	U251	MCF7	NCI-ADR/RES	786-0	NCI-H460	PC-3	OVCAR-3	HT-29	K-562	HaCaT
<b>7</b>	115.1	> 250	> 250	131.0	> 250	17.5	136.6	> 250	> 250	16.8
<b>8</b>	> 250	> 250	> 250	140.9	> 250	> 250	> 250	> 250	> 250	> 250
<b>9</b>	> 250	> 250	> 250	> 250	> 250	204.7	> 250	> 250	> 250	> 250
<b>10</b>	90.3	243.5	41.5	74.0	83.7	29.2	26.8	> 250	175.1	201.4
<b>11</b>	> 250	> 250	> 250	> 250	> 250	> 250	> 250	> 250	> 250	> 250
<b>12</b>	27.0	21.3	9.1	39.6	40.5	23.8	15.6	70.7	54.1	25.4
<b>13</b>	> 250	225.4	129.8	247.1	> 250	> 250	51.0	> 250	> 250	152.1
<b>14</b>	139.8	101.4	> 250	79.4	> 250	52.2	95.2	> 250	> 250	> 250
<b>15</b>	147.5	> 250	210.0	128.8	> 250	203.7	72.4	> 250	> 250	> 250
<b>16</b>	> 250	> 250	> 250	> 250	> 250	> 250	93.5	> 250	7.4	> 250
<b>17</b>	127.8	> 250	> 250	58.9	> 250	> 250	119.9	> 250	28.6	> 250
<b>18</b>	48.8	132.9	10.5	42.1	122.2	26.2	23.5	60.5	41.4	43.2
<b>19</b>	> 250	> 250	51.7	> 250	> 250	138.8	> 250	> 250	2.6	> 250
<b>20</b>	171.4	124.0	> 250	> 250	> 250	5.3	186.2	> 250	9.4	> 250
Dox <sup>b</sup>	0.03	0.06	0.05	0.03	0.01	0.03	0.24	0.08	0.03	0.2

<sup>a</sup> U251, glioma cells; MCF7, breast cancer cells; NCI-ADR/RES, multiple drug-resistant ovarian cancer cells; 786-0, renal cancer cells; NCI-H460, non-small lung cancer cells; PC-3, prostate cancer cells; OVCAR-03, ovarian cancer cells; HT-29, colon cancer cells; K562, leukemia.

<sup>b</sup> Dox, reference drug doxorubicin.

geometrical parameters for the indazolophthalazine-1,6, 11-trione skeleton of **10** are very similar to those reported for 13-(4-bromophenyl)-3,3-dimethyl-2,3,4,13-tetrahydro-1*H*-indazolo[1,2-*b*]phthalazine-1,6,11-trione (Sayyafi et al., 2008).

### 3.2. Antiproliferative activities

The effect of synthesized phthalazine-triones (0.25–250 µg/mL) on glioma (U251), breast (MCF-7), adriamycin-resistant ovarian cancer (NCI-ADR/RES), kidney cancer (786-0), lung non-small cancer (NCI-H460), prostate cancer (PC-3), ovarian cancer (OVCAR-03), colon cancer (HT-29) and leukemia (K562) cells was also studied. Cell proliferation was determined by the sulforhodamine B method using doxorubicin (Dox) as a positive control. The concentration of phthalazine-triones that elicited the inhibition of cell growth by 50% (GI<sub>50</sub>) is summarized in Table 4. The selectivity index (SI), herein is defined as the ratio of the GI<sub>50</sub> of pure compound in HaCaT cell line to the GI<sub>50</sub> of the same pure compound in a cancer cell line, for the most promising compounds and doxorubicin.

Compounds **12** and **18** were the most active compounds against NCI-ADR/RES cell line (Table 4) with GI<sub>50</sub> values lower than 15 µg/mL. Compound **20** had the most promising activity against PC-3 cancer cells, with GI<sub>50</sub> value of 5.3 µg/mL (Table 4) and SI of over 47, 15-fold more than required to define a highly selective compound (Prayong et al., 2008). For K562 cells, compounds **16**, **19** and **20** were active with GI<sub>50</sub> values of 7.4, 2.6 and 9.4 µg/mL (Table 4) and SI greater than 33.8, 96.2 and 26.6, respectively. It is noteworthy that doxorubicin's SI for PC-3 and K562 cell lineages was equal to 0.2, demonstrating no differentiation of normal cells and cancerous cells.

Among the cell lines tested K562 was the most sensitive, and three phthalazine-triones were active against these cells. For U251, MCF7, 786-0, NCI-H460, OVCAR-3 and HT29 cell lines, none of the compounds tested proved to be active. Compound **20** was the only active against more than one strain. Overall, the potency of phthalazine-triones adducts was dependent on the histological origin of cancer cells.

### 4. Conclusion

A new and efficient green methodology for obtaining 2*H*-indazolo[2,1-*b*]phthalazine-trione derivatives was developed using *p*-sulfonic acid calix[4]arene as a catalyst and ethyl lactate as the solvent. Fourteen compounds were obtained in moderate to good yields in only 10 min of reaction time. This procedure is of great interest because it allows the synthesis of compounds of biological and technological interest through an environmentally friendly methodology.

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### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.arabjc.2016.04.007>.

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