Bioorganic & Medicinal Chemistry 20 (2012) 2645–2650

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Free radical scavenging and antiproliferative properties of Biginelli adducts

Daniel L. da Silva [a], Fabiano S. Reis [a,b], Dandara R. Muniz [b], Ana Lúcia T.G. Ruiz [c], João E. de Carvalho [c], Adão A. Sabino [a], Luzia V. Modolo [b,*], Ângelo de Fátima [a,*]

[a] Grupo de Estudos em Química Orgânica e Biológica (GEQOB), Departamento de Química, ICEx, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[b] Grupo de Estudos em Bioquímica de Plantas (GEBioPlan), Departamento de Botânica, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[c] Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA), Universidade Estadual de Campinas, Paulínia, SP, Brazil

ARTICLE INFO

Article history:
Received 4 December 2011
Revised 5 February 2012
Accepted 13 February 2012
Available online 22 February 2012

Keywords:
Biginelli adducts
Free radical scavenging
Antiproliferative activity
Cancer cells

ABSTRACT

A series of Biginelli adducts bearing different substituents at C-4 position were synthesized by using *p*-sulfonic acid calix[4]arene as a catalyst. The in vitro potential to scavenge reactive nitrogen/oxygen species (RNS and ROS) and the ability to inhibit cancer cells growth were then investigated. Four adducts were found to be potent scavengers of 2,2-diphenyl-1-picrylhydrazyl (RNS) and/or superoxide anion (ROS) radicals. The antiproliferative activity against cancer cells was disclosed for the first time for 16 monastrol analogs. The capacity of all compounds to inhibit cancer cells growth was dependent on the histological origin of cells, except for **BA24**, which was highly active against all cell lines. **BA20** and **BA33** were as potent as the reference drug doxorubicin against adriamycin-resistant ovarian and prostate cancer cells, respectively. These results highlight some monastrol analogs as lead compounds for the design of new free radical scavengers and anticancer agents.

1. Introduction

A multicomponent reaction (MCR) is defined as the process in which three or more reactants are combined in a single step to form a product that incorporates structural characteristics of each reagent. Besides providing structures more complex than the starting materials, MCRs offer simplicity and efficiency to synthetic approaches.[1,2] Despite the spectacular growth of combinatorial chemistry in the 80s and 90s, only few MCRs have been developed by companies (especially pharmaceutical industries) or research laboratories.[1–4] The Biginelli reaction, discovered by Pietro Biginelli in 1893, is an MCR that involves the cyclocondensation of acetoacetic esters, aromatic aldehydes and (thio)urea.[5] The products of this three-component synthesis were identified as 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs). Special attention was given to this type of MCR in the late 20th century with the discovery of the potential biological activities of compounds bearing a DHPM moiety.[6–9] Indeed, batzeladine B (**1**) (Fig. 1), a marine natural product isolated from *Batzella* sp. was found to inhibit the binding of HIV envelop protein gp-120 to human CD4 cells.[10] Monastrol (**BA2**) (Fig. 1) was identified as a potent anticancer agent due to its ability to freely cross cell membrane.[11] Such Biginelli adduct affects mitosis independently of tubulin binding, a mechanism of action presented by drugs such natural taxanes, vinca alkaloids and epothilones.[12–16] Other DHPMs, such as SQ 32926 (**3**) and SQ 32547 (**4**) (Fig. 1), were reported to possess antihypertensive properties.[17,18] The antioxidant properties of DHPMs were also described by Stefani's[19] and Kumar's[8] groups. Adducts **5** and **6** (Fig. 1) were able to scavenge 46% of reactive oxygen species (ROS) produced in mice liver, when used at 100 μM.[19] The DHPMs **7** and **8** (Fig. 1) were able to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals by 50% when employed at 1 mM.[8]

The diverse biological profile exhibited by Biginelli adducts brought new perspectives for the development of new drugs based on DHPM core. Herein, we report the synthesis of twenty-six Biginelli adducts and their potential as scavengers of reactive nitrogen and oxygen species (RNS and ROS, respectively) and inhibitors of cancer cells proliferation.

2. Results and discussion

2.1. Synthesis of Biginelli adducts

Twenty-six Biginelli adducts (**BA2**, **BA9**-**BA33**; Table 1) were synthesized from MCRs that consisted of aldehydes, ethyl acetoacetate, (thio)urea and *p*-sulfonic acid calix[4]arene as a catalyst according to an approach developed by our group.[20] DHPMs derived from aromatic aldehydes bearing electron-donating or electron-withdrawing groups were obtained in good yields. Non-aromatic aldehydes, however, were less reactive, affording moderate yields of Biginelli adducts. Urea furnished better yields when compared with thiourea (Table 1).

* Corresponding authors. Tel.: +55 31 3409 6373; fax: +55 31 3409 5700 (Â.d.F.); tel./fax: +55 31 3409 3008 (L.V.M.).
E-mail addresses: lvmodolo@icb.ufmg.br (L.V. Modolo), adefatima@qui.ufmg.br (Â. de Fátima).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2012.02.036

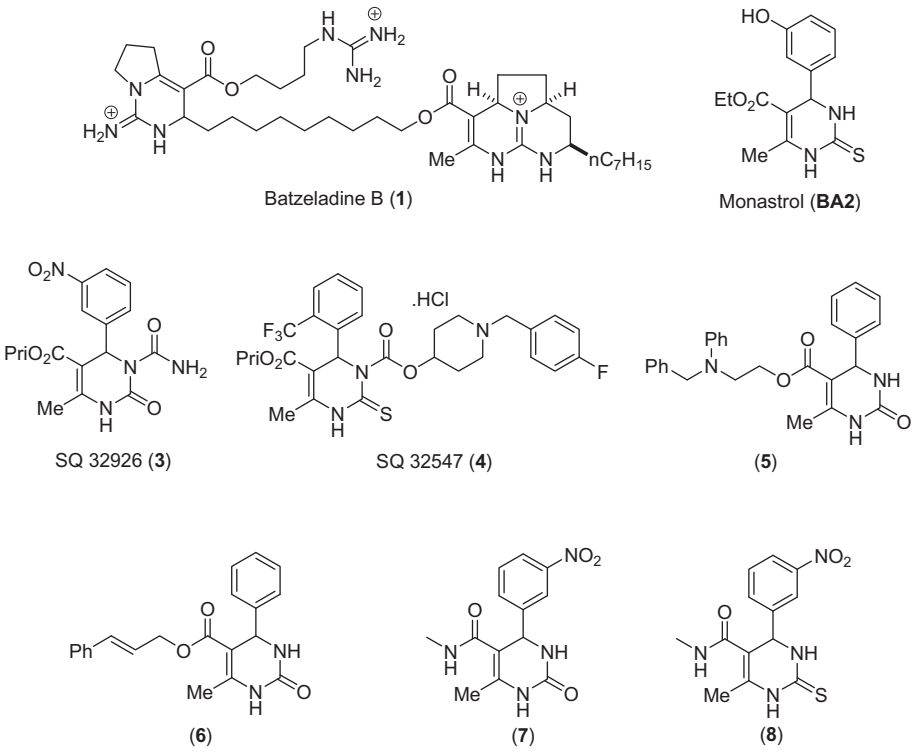
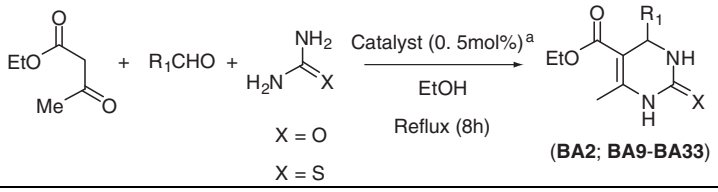


Figure 1. Biginelli adducts of interesting biological profiles.

Table 1
Synthesized Biginelli adducts using *p*-sulfonic acid calix[4]arene as catalyst



Product	R_1	X	Yield[b] (%)
BA2	3-OH–C_6H_4	S	52
BA9[c]	C_6H_5	O	69
BA10	C_6H_5	S	63
BA11[c]	4-OH–C_6H_4	O	81
BA12	4-OH–C_6H_4	S	77
BA13[c]	3-OH–C_6H_4	O	79
BA14[c]	3,4-OH–C_6H_3	O	56
BA15	3,4-OH–C_6H_3	S	49
BA16[c]	4-OCH_3–C_6H_4	O	89
BA17[c]	4-OCH_3–C_6H_4	S	78
BA18[c]	3-OCH_3–C_6H_4	O	78
BA19[c]	3-OCH_3–C_6H_4	S	74
BA20[c]	4-OH–3-OCH_3–C_6H_3	O	76
BA21[c]	4-OH–3-OCH_3–C_6H_3	S	72
BA22	4-OH–3,5-OCH_3–C_6H_2	O	65
BA23	4-OH–3,5-OCH_3–C_6H_2	S	80
BA24[c]	4-SMe–C_6H_4	O	92
BA25[c]	4-SMe–C_6H_4	S	78
BA26[c]	3,4-(OCH_2O)–C_6H_3	O	71
BA27[c]	3,4-(OCH_2O)–C_6H_3	S	64
BA28[c]	4-F–C_6H_4	O	91
BA29	4-F–C_6H_4	S	83
BA30[c]	$CH_3CH_2CH_2$	O	34
BA31	$CH_3CH_2CH_2$	S	31
BA32[c]	C_6H_{11}	O	38
BA33	C_6H_{11}	S	34

[a] *p*-Sulfonic acid calix[4]arene.
[b] Yields of isolated and purified products.
[c] Data previously reported by our group[20] are listed for comparison.

2.2. Scavenging of reactive nitrogen species (RNS)

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as a source of reactive nitrogen species (RNS). An initial screening was performed with all synthesized compounds to select potential candidates able to capture free radicals. The reaction media consisted of an ethanolic solution of DPPH at 100 μM and the Biginelli adduct at 160 μM. Each reaction medium was analyzed at 517 nm after 30 min of incubation under stirring and darkness at 25 °C. Resveratrol, a known antioxidant derived from plants, was used as a positive control. The percentage of DPPH radicals scavenged by (thio)urea-derived Biginelli adducts is shown in Figure 2.

The screening revealed the adducts **BA14**, **BA15**, **BA22** and **BA23** as the best candidates for further studies of DPPH scavenging activity since such DHPMs were as potent as resveratrol (Fig. 2). Adducts **BA20** and **BA21** were also considered promising compounds as they scavenged 65% of DPPH radicals present in the reaction medium. No significant activity was exhibited by other analogs. A phenolic hydroxyl group at *para* position and oxygenated groups (hydroxyl or methoxyl) at *meta* position in the aromatic ring (Table 1) contributed relevantly to the RNS scavenging activity of **BA14**, **BA15**, **BA22** and **BA23**. The absence of second methoxyl group at *meta* position diminished at some degree the adducts potency as attested by the results obtained for **BA20** and **BA21**. In general, thiourea-derived Biginelli adducts (Fig. 2B) seemed to be more effective than the corresponding *oxo*-analogs (Fig. 2A) in sequestering DPPH radicals. These results prompted us to further investigate the concentration of adducts **BA14**, **BA20** and **BA22** (*oxo*-derivatives) and **BA15**, **BA21** and **BA23** (*thio*-derivatives) necessary to scavenge 50% of DPPH radicals (SC_{50}) present in the reaction medium. A linear behavior of DPPH scavenging was observed when the DHPMs were used at concentrations up to 40 μM, with exception of **BA20** and **BA21** (Fig. S1, Supplementary data). The Biginelli adducts **BA14**, **BA15**, **BA22** and **BA23** presented SC_{50} values lower than that of resveratrol (Table 2). Adducts **BA20** and **BA21**, however, were about 4-fold less potent than **BA14**, **BA15**, **BA22** and **BA23**. Moreover, these last four adducts were 37- to 40-fold more potent in scavenging DPPH radicals than the monastrol (**BA2**) analogs reported elsewhere.[8]

A kinetic study was then performed with **BA14**, **BA15**, **BA22** and **BA23** at the corresponding SC_{50}. In the first minutes of reaction, the tested Biginelli adducts were more efficient in scavenging DPPH radicals than was resveratrol (Fig. 3). The adduct **BA14** was twice as effective as resveratrol after the first minute in contact with DPPH radicals. The capture of DPPH radicals was linear up to the third minute of reaction for all adducts tested, from which a plateau was reached. In contrast, DPPH scavenging by resveratrol was almost constant along with 10 min of reaction (Fig. 3), which resulted in failure to determining the scavenging velocity. The urea-derivatives **BA14** and **BA22** were able to scavenge 488.5 ± 14.5 and 527.3 ± 25.7 pmol of DPPH/min, respectively, while the thiourea-derivatives **BA15** and **BA23** sequestered 412.8 ± 23.8 and 369.8 ± 6.4 pmol of DPPH/min, respectively (Fig. 3). Thus, *oxo*-derived Biginelli adducts were faster than the corresponding *thio*-analogs in the scavenging of DPPH radicals. Combined, these results indicate the great potential of **BA14**, **BA15**, **BA22** and **BA23** to scavenge RNS.

Table 2
Concentration of Biginelli adducts necessary to scavenge DPPH radicals by 50% (SC_{50})

Biginelli adduct	SC_{50}[a] (μM)
BA14	20.3
BA15	29.7
BA20	105.4
BA21	103.9
BA22	23.3
BA23	24.2
Resveratrol	34.4

[a] SC_{50} values were obtained from three independent experiments performed with 100 μM DPPH, each done in triplicate.

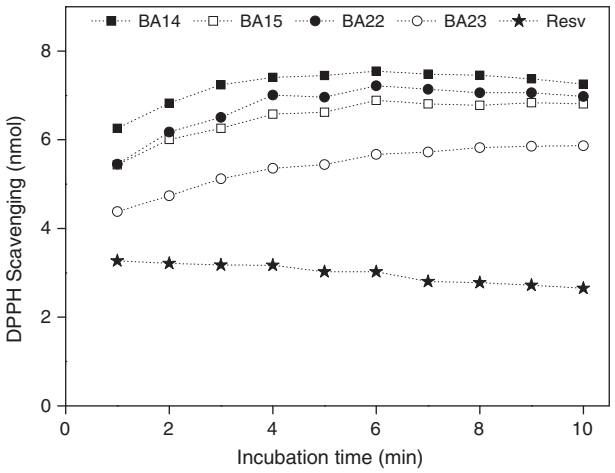


Figure 3. Kinetics of DPPH radical scavenging by Biginelli adducts. The reaction medium consisted of 10 nmol of DPPH radicals and Biginelli adducts at their SC_{50} as it follows: **BA14** (20.3 μM), **BA15** (29.7 μM), **BA22** (23.3 μM), **BA23** (24.2 μM) and resveratrol (Resv, 34.4 μM). Standard deviations were lower than 0.6 nmol. Data are from three independent experiments, each done in triplicate.

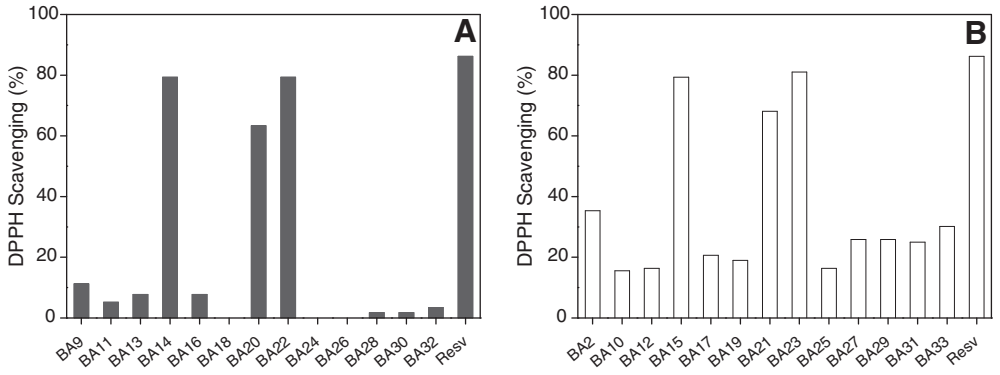


Figure 2. Percentage of reactive nitrogen species scavenged by Biginelli adducts (**BA**) derived from urea (A) or thiourea (B). The reaction medium consisted of compound-test (160 μM) and DPPH radical (100 μM). Resveratrol (Resv) was employed as a positive control. Data are from three independent experiments, each done in triplicate.

2.3. Scavenging of reactive oxygen species (ROS)

The ability of Biginelli adducts **BA14**, **BA15**, **BA22** and **BA23** to scavenge ROS was also investigated. The profile of $^{\cdot}O_2^-$ scavenging as a function of Biginelli adducts concentration is presented in Figure 4. Resveratrol scavenged $^{\cdot}O_2^-$ in a concentration-dependent manner. The scavenging of $^{\cdot}O_2^-$ was linear when Biginelli adducts were employed at concentrations up to 50 μM, except for **BA22**. The **BA15** was the most potent among the tested compounds, exhibiting an SC_{50} value of 25.7 μM. **BA14**, **BA22**, **BA23** and resveratrol sequestered 50% of $^{\cdot}O_2^-$ radicals when used, respectively, at 33.0, 122.3, 78.0 and 121.4 μM (Fig. 4). Then, the *thio*-derived Biginelli adducts were more effective than the corresponding *oxo*-derivatives.

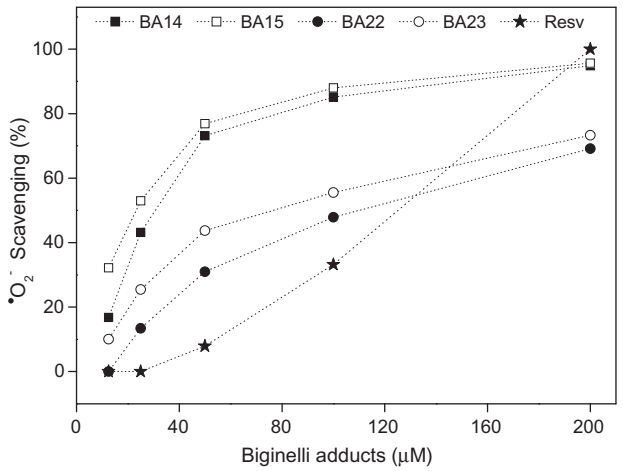


Figure 4. Percentage of reactive oxygen species scavenged by Biginelli adducts (BA). The production of $^{\cdot}O_2^-$ was induced as described in Section 4. Each compound-test was employed at concentrations that ranged from 12.5 to 200 μM and the free radical scavenging determined after 10 min of reaction. Standard deviations were lower than 2%. Data are from three independent experiments, each done in triplicate.

2.4. Antiproliferative activities

Since the disclosure of monastrol (**BA2**) effect on cancer cells, only few works explored the potential of **BA2** analogs.[9,21,22] Based on this, the effect of a series of synthesized Biginelli adducts (0.25–250 μg/mL) were investigated on glioma (U251), 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) and colon cancer (HT-29) cells. Cell proliferation was determined by the sulforhodamine B method and doxorubicin used as a positive control.[23] The concentration of Biginelli adducts that elicited the inhibition of cell growth by 50% (GI_{50}) is summarized in Table 3.

U251, NCI-ADR/RES, PC-3 and OVCAR-03 were the most sensitive cancer cells as attested by the number of Biginelli compounds that negatively affected cells growth at $GI_{50} \leqslant 10$ μg/mL (Table 3). HT-29 cells were the least sensitive to the tested adducts (Table 3). Adducts **BA14** and **BA23** were the most active compounds against U251 cells (GI_{50} <1 μg/mL). **BA11**, **BA13**, **BA20**, **BA24** and **BA25** were also promising against U251 cells by exhibiting GI_{50} around 5 μg/mL (Table 3). **BA20** was roughly as potent as doxorubicin against NCI-ADR/RES cells. Biginelli compounds **BA11**, **BA14**, **BA16**, **BA17**, **BA24** and **BA28** were also effective on NCI-ADR/RES cells by presenting GI_{50} between 2 and 6 μg/mL. **BA33** was the most potent adduct against PC-3 cells; its GI_{50} value was found to be in the same order of magnitude of that for doxorubicin (Table 3). PC-3 cells were also sensitive to **BA2**, **BA17**, **BA19**, **BA24**, **BA28** and **BA29**. OVCAR-03 cells were highly sensitive to **BA13**, **BA14**, **BA16**, **BA17**, **BA19**, **BA20**, **BA23-25** and **BA33** (Table 3). Compounds **BA2**, **BA24**, **BA25** and **BA33** compromised 786-0 cells growth by 50% when used at concentrations lower than 6 μg/mL

Table 3
Concentration of Biginelli adducts (GI_{50}[a] in μg/mL) that elicits cancer cells[b] growth inhibition by 50%

Compound	U251	NCI-ADR/RES	786-0	NCI-H460	PC-3	OVCAR-03	HT29
BA2	24.0	22.2	4.0	10.6	5.6	14.4	29.6
BA9	>250.0	>250.0	>250.0	28.9	>250.0	>250.0	>250.0
BA10	26.6	27.4	27.7	28.0	42.2	27.7	29.8
BA11	4.2	3.2	25.8	40.6	135.9	27.3	28.7
BA12	27.2	83.6	27.5	>250.0	47.6	37.7	>250.0
BA13	5.1	11.7	26.3	42.0	34.7	6.0	27.1
BA14	0.25	2.1	25.7	34.0	18.2	1.6	37.8
BA15	26.5	24.3	28.3	31.7	25.0	25.0	33.7
BA16	11.6	5.7	24.8	>250.0	26.5	8.0	28.3
BA17	29.0	3.2	25.9	31.4	5.5	0.99	30.6
BA18	>250.0	>250.0	>250.0	>250.0	>250.0	>250.0	>250.0
BA19	28.1	25.6	24.9	29.2	10.2	1.3	28.3
BA20	4.5	0.25	17.5	125.6	46.3	1.7	28.1
BA21	88.3	59.7	30.0	180.4	72.6	11.0	34.9
BA22	>250.0	>250.0	129.2	>250.0	131.7	39.0	>250.0
BA23	0.77	25.0	13.4	65.6	19.0	1.9	28.2
BA24	8.0	2.7	2.5	3.6	9.5	10.0	10.2
BA25	6.0	35.7	5.3	21.6	23.6	8.5	17.2
BA26	34.9	15.5	26.3	48.9	83.1	32.4	29.9
BA27	25.9	57.1	27.0	27.0	13.4	55.3	31.4
BA28	23.5	3.6	24.7	28.1	3.1	27.1	25.3
BA29	28.3	26.1	24.5	14.2	1.7	24.7	26.5
BA30	35.4	20.9	18.8	49.7	25.0	52.4	17.5
BA31	29.2	40.3	27.7	22.5	42.4	38.1	50.4
BA32	141.9	>250.0	>250.0	>250.0	>250.0	>250.0	>250.0
BA33	25.2	24.1	4.2	3.5	0.46	2.2	25.2
Doxorubicin[c]	0.068	0.14	0.18	<0.025	0.24	0.27	0.27

[a] GI_{50} values were obtained from two independent experiments, each done in triplicate.
[b] U251, glioma 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.
[c] Reference drug.

while NCI-H460 cells were sensitive to three adducts (**BA2**, **BA24** and **BA33**). Only 786-0, NCI-H460 and PC-3 cells were sensitive to monastrol (**BA2**), a known anticancer agent, at a concentration lower than 10 μg/mL. Interestingly, the adduct **BA33** was 12-fold more potent than monastrol against PC-3 cells while **BA24** and **BA33** were threefold more potent on NCI-H460 cells (Table 3). Among the tested compounds, **BA24** exhibited a large spectrum of action affecting all the cell lines at concentrations lower than 10 μg/mL (Table 3). The Biginelli adducts in this study were more active against cancer cells than those previously reported by Russowsky et al.[21] Overall, the potency of Biginelli adducts was dependent on the histological origin of cancer cells.

3. Conclusion

The potential to scavenge reactive nitrogen/oxygen species (RNS and ROS) and the ability to inhibit the growth of cancer cells were investigated for 26 Biginelli adducts. Among them, **BA14**, **BA15**, **BA22** and **BA23** were found to be promising RNS and ROS scavengers, being indeed more potent than the known antioxidant agent resveratrol. The uncontrolled production of RNS/ROS leads to cellular damages that may result in a series of diseases.[24] Thus, such adducts may be used as lead compounds for the design of effective antioxidant agents. Sixteen monastrol-analogs had their antiproliferative activity against cancer cells evaluated for the first time. The capacity to inhibit cancer cells growth of the Biginelli adducts was dependent on the histological origin of cells. The adduct **BA24** was the only exception, being active against all cell lines at concentrations lower than 10 μg/mL. For each cancer cell studied, at least two adducts were more potent than monastrol (**BA2**). Notably, **BA20** and **BA33** were as potent and the reference drug doxorubicin against NCI-ADR/RES and PC-3, respectively. These results disclose some monastrol analogs as lead compounds for obtaining new anticancer agents.

4. Experimental

4.1. Chemistry

4.1.1. General procedures

All chemicals were obtained from commercially available sources and used without further purification. Anhydrous conditions were not required for the reaction. Melting points (uncorrected) were determined on Mettler FP 80 HT apparatus. Elemental analyses were performed on CHN Perkin-Elmer 2400 apparatus. Infrared spectra were recorded on Perkin Elmer, Spectrum One spectrophotometer (ATR). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE DPX-200 spectrometry at 200 MHz and 50 MHz respectively, in DMSO-d_6.

4.1.2. Synthesis of DHPMs

Aldehydes (3 mmol), ethyl acetoacetate (4.5 mmol) and (thio)urea (4.5 mmol) were dissolved in 3 mL of ethanol containing *p*-sulfonic acid calix[4]arene (0.5 mol %). The mixture was heated under reflux and stirred for 8 h.[20] All DHPMs were characterized by NMR (^{1}H and ^{13}C), infrared, melting point and elemental analysis. Characterization data for compounds **BA9**, **BA11-BA14**, **BA16-BA21**, **BA24-BA28**, **BA30** and **BA32** were recently reported by da Silva et al.[20] Data for compounds **BA2**, **BA10**, **BA15**, **BA22**, **BA29**, **BA31** and **BA33** are listed as Supplementary data.

4.1.2.1. Ethyl 4-(4-hydroxy-3,5-dimethoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (BA23). Yield: 80%. Yellow solid. Mp 189–191 °C. IR (ATR, cm^{-1}): 3473, 3326, 3193, 2945, 2840, 1663, 1619, 1516, 1454, 1432, 1371, 1330, 1278, 1217, 1184, 1148, 1119, 859, 821, 789, 753, 672. ^{1}H NMR (DMSO-d_6): δ 1.14 (t, 3H, J = 7.0 Hz, CH_3), 2.28 (s, 3H, CH_3), 3.71 (s, 6H, 2 × OCH_3), 4.04 (q, 2H, J = 7.0 Hz, CH_2), 5.11 (d, 1H, $J_{H\text{-}NH}$ = 3.2, C*H*), 6.46 (s, 2H, CH_{AR}), 8.42 (s, 1H, O*H*), 9.58 (s, 1H, N*H*), 10.29 (s, 1H, N*H*); ^{13}C NMR (DMSO-d_6): δ 14.1 (*C*H_3), 17.1 (*C*H_3), 53.9 (*C*H), 56.0 (2 × O*C*H_3), 59.6 (*C*H_2), 100.9 (*C*), 103.9 (2 × *C*H_{AR}), 133.7 (*C*$_{AR}$), 135.3 (*C*$_{AR}$), 144.7 (*C*), 147.9 (2 × *C*H_{AR}), 165.3 (*C*OO), 174,3 (*C*(S)). Anal. calcd. for $C_{16}H_{20}N_2SO_5$: C, 54.53; H, 5.72; N, 7.95. Found: C, 54.16; H, 5.63; N, 7.49.

4.2. Biological activities

4.2.1. Scavenging of reactive nitrogen species

The ability of Biginelli adducts **BA2** and **BA9**-**BA33** to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, a reactive nitrogen species (RNS), was determined according to Gülcin,[25] with modifications. The screening of potential candidates was done by incubating each compound-test (160 μM) in an ethanolic medium containing 100 μM DPPH. The systems were maintained under stirring and absence of light for 30 min and the absorbance recorded at 517 nm. Those compounds with potential scavenging activity were then tested in the range of 0–160 μM to determine the concentration necessary to scavenge DPPH radicals by 50% (SC_{50}). The best compounds were subjected to kinetic studies at the respective SC_{50} values. Results presented are from three independent experiments, each done in triplicate.

4.2.2. Scavenging of reactive oxygen species

The capacity of Biginelli adducts to scavenge superoxide anions ($^{\cdot}O_2^-$) was evaluated in 50 mM phosphate buffer (pH 7.8) containing 13 mM L-metionine, 75 μM nitroblue tetrazolium, 100 μM EDTA, 2 μM riboflavin and compound-test at 0–200 μM. Reaction mixtures were incubated for 10 min at 25 °C in the presence of fluorescent light to induce $^{\cdot}O_2^-$ formation. Controls consisted of reaction mixtures kept at 25 °C for 10 min in absence of light. The percentage of $^{\cdot}O_2^-$ scavenged by each compound-test was determined through spectrophotometric analysis at 575 nm. Results presented are from three independent experiments, each done in triplicate.

4.2.3. Antiproliferative assay

Human tumor cell lines U251 (glioma), NCI-ADR/RES (multiple drugs-resistant ovarian), 786-0 (renal), NCI-H460 (lung, non-small cells), PC-3 (prostate), OVCAR-03 (ovarian) and HT-29 (colon) were kindly provided by 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).[26–28] Cells in 96-well plates (100 μL cells/well) were exposed to Biginelli adducts (0.25–250 μg/mL) for 48 h at 37 °C and 5% of CO_2. Afterward cells were fixed with 50% trichloroacetic acid, submitted to sulforhodamine B assay for cell proliferation quantitation at 540 nm.[23] 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. Results presented are from two independent experiments, each done in triplicate.

Acknowledgments

Authors are thankful to the financial support provided by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível

Superior (CAPES). Ângelo de Fátima and Luzia Valentina Modolo are supported by research fellowships from CNPq.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2012.02.036.

References and notes

1. Weber, L. *Curr. Med. Chem.* **2002**, *9*, 2085.
2. Ganen, G. *Acc. Chem. Res.* **2009**, *42*, 463.
3. Hulme, C.; Gore, V. *Curr. Med. Chem.* **2003**, *10*, 51.
4. Touré, B. B.; Hall, D. G. *Chem. Rev.* **2009**, *109*, 4439.
5. Biginelli, P. *Gazz. Chim. Ital.* **1893**, *23*, 360.
6. Kappe, C. O. *Eur. J. Med. Chem.* **1043**, *2000*, 35.
7. Ashok, M.; Holla, B. S.; Kumari, N. S. *Eur. J. Med. Chem.* **2007**, *42*, 380.
8. Kumar, B. R. P.; Sankar, G.; Baig, R. B. N.; Chandrashekaram, S. *Eur. J. Med. Chem.* **2009**, *44*, 4192.
9. Azizian, J.; Mohammadi, K. M.; Firuzi, O.; Mirza, B.; Miri, R. *Chem. Biol. Drug Discov.* **2010**, *75*, 375.
10. Patil, A. D.; Kumar, N. V.; Kokke, W. C.; Bean, M. F.; Freyer, A. J.; Brosse, C.; Mai, S.; Truneh, A.; Faulkner, D. J.; Carte, B.; Breen, A. L.; Hertzberg, R. P.; Johnson, R. K.; Westley, J. W.; Potts, B. C. M. *J. Org. Chem.* **1995**, *60*, 1182.
11. Mayer, T. U.; Kapoor, T. M.; Haggarty, S. J.; King, R. W.; Schreiber, S. L.; Mitchison, T. J. *Science* **1999**, *286*, 971.
12. Sakowicz, R.; Finer, J. T.; Beraud, C.; Crompton, A.; Lewis, E.; Fritsch, A.; Lee, Y.; Mak, J.; Moody, R.; Turincio, R.; Chabala, J. C.; Gonzales, P.; Roth, S.; Weitman, S.; Wood, K. W. *Cancer Res.* **2004**, *64*, 3276.
13. Brier, S.; Lemaire, D.; DeBonis, S.; Forest, E.; Kozielski, F. *Biochemistry* **2004**, *43*, 13072.
14. Heald, R. *Cell* **2000**, *102*, 399.
15. Peters, T.; Lindenmaier, H.; Haefeli, W. E.; Weiss, J. *Arch. Pharmacol.* **2006**, *372*, 291.
16. Cochran, J. C.; Gatial, J. E.; Kapoor, T. M.; Gilbert, S. P. *J. Biol. Chem.* **2005**, *280*, 12658.
17. Atawal, K. S.; Swanson, B. N.; Unger, S. E.; Floyd, D. M.; Moreland, S.; Hedberg, A.; O'Reilly, B. C. *J. Med. Chem.* **1991**, *34*, 806.
18. Rovnyak, G. C.; Atwal, K. S.; Hedberg, A.; Kimball, S. D.; Moreland, S.; Gougoutas, J. Z.; O'Reilly, B. C.; Schwartz, J.; Malley, M. F. *J. Med. Chem.* **1992**, *35*, 3254.
19. Stefani, H. A.; Oliveira, C. B.; Almeida, R. B.; Pereira, C. M. P.; Braga, R. C.; Cella, R.; Borges, V. C.; Savegnago, L.; Nogueira, C. W. *Eur. J. Med. Chem.* **2006**, *41*, 513.
20. da Silva, D. L.; Fernandes, S. A.; Sabino, A. A.; de Fátima, A. *Tetrahedron Lett.* **2011**, *52*, 6328.
21. Russowsky, D.; Canto, R. F. S.; Sanches, S. A. A.; D'Oca, M. G. M.; de Fátima, A.; Pilli, R. A.; Kohn, L. K.; Antônio, M. A.; Carvalho, J. E. *Bioorg. Chem.* **2006**, *34*, 173.
22. Tawfik, H. A.; Bassyouni, F.; Gamal-Eldeen, A. M.; Abo-Zeid, M. A.; El-Hamouly, W. S. *Pharmacol. Rep.* **2009**, *61*, 1153.
23. Monks, A.; Scudeiro, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbelli, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757.
24. Halliwell, B.; Gutteridge, J. M. C. *Methods Enzymol.* **1990**, *186*, 1.
25. Gülçin, I. *Innovat. Food Sci. Emerg. Tech.* **2010**, *11*, 210.
26. Euzébio, F. P. G.; dos Santos, F. J. L.; Piló-Veloso, D.; Alcântara, A. F. C.; Ruiz, A. L. T. G.; de Carvalho, J. E.; Foglio, M. A.; Ferreira-Alves, D. L.; de Fátima, A. *Bioorg. Med. Chem.* **2010**, *18*, 8172.
27. Euzébio, F. P. G.; dos Santos, F. J. L.; Piló-Veloso, D.; Ruiz, A. L. T. G.; de Carvalho, J. E.; Ferreira-Alves, D. L.; de Fátima, A. *Bioorg. Chem.* **2009**, *37*, 96.
28. Marquissolo, C.; de Fátima, A.; Kohn, L. K.; Ruiz, A. L. T. G.; de Carvalho, J. E.; Pilli, R. A. *Bioorg. Chem.* **2009**, *37*, 52.