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Short communication

Synthesis and anticancer activity of (*E*)-2-benzothiazole hydrazones

Eric B. Lindgren [a, 1], Monique A. de Brito [b], Thatyana R.A. Vasconcelos [a], Manuel O. de Moraes [c], Raquel C. Montenegro [d], Julliane D. Yoneda [e], Kátia Z. Leal [a, *]

[a] Programa de Pós-Graduação em Química, Instituto de Química, Universidade Federal Fluminense, Outeiro de São João Batista s/n°, 24020-141 Niterói, RJ, Brazil
[b] Faculdade de Farmácia, Universidade Federal Fluminense, Rua Mário Viana 523, 24241-000 Niterói, RJ, Brazil
[c] Laboratório de Oncologia Experimental, Universidade Federal do Ceará, Rua Coronel Nunes de Melo 1127, 60430-270 Fortaleza, CE, Brazil
[d] Laboratório de Citogenética Humana, Instituto de Ciências Biológicas, Universidade Federal do Pará, Rua Augusto Corrêa 01, 66075-110 Belém, PA, Brazil
[e] Departamento de Química, Instituto de Ciências Exatas, Universidade Federal Fluminense, Rua Des. Ellis Hermydio Figueira 783, 27213-415, Volta Redonda, RJ, Brazil

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ABSTRACT

Benzothiazole hydrazones have been synthesized and evaluated for their *in vitro* antiproliferative activity against three human cancer cell lines: HL-60 (leukemia), MDAMB-435 (breast) and HCT-8 (colon). The good cytotoxicity for the three cancer cell lines and theoretical profile of compounds **3o** and **3p** pointed them as promising lead molecules for anticancer drug design.

1. Introduction

Cancer still remains a threat to humanity's health, figuring among the leading causes of death worldwide. In 2012, cancer was responsible for 8.2 million deaths and it is expected that annual cases will rise from 14 million in 2012 to 22 within the next two decades [1,2]. In the last years, many efforts have been made to develop new strategies for finding safe and effective ways of treating this disease, which includes a better understanding of biological processes involved in cancer cell survival, and also the search for more selective and potent chemotherapeutic agents [3].

In this context, benzothiazoles represent an important class of heterocyclic compounds that have attracted special attention due to their diverse biological activities, including antitumor [4], antimicrobial [5], antidiabetic [6], anticonvulsant [7] and anti-inflammatory [8]. Recently, our research group has published a review article highlighting aspects of the chemistry and biological properties of this heterocyclic core during the past few years [9].

For some time, we have been interested in structures of aryl–NH–N=CH–aryl, especially those having some pharmacologically important heterocycles [10–15]. In a previous work, we have reported the synthesis and *in vitro* anticancer activities of eleven (*E*)-2-benzothiazole hydrazones (**3a**–**k**) [13]. In addition, we have performed structural determinations and conformational analysis of some of the synthesized compounds [14–15]. In the course of our investigation on the anticancer properties of this class of compounds, we report herein the synthesis of six hydrazones (**3l**–**q**), and the anticancer activities against three cancer cell lines: HL-60 (leukemia), MDAMB-435 (breast) and HCT-8 (colon), for all benzothiazole hydrazones obtained from these studies (**3a**–**q**).

2. Results and discussion

2.1. Chemistry

The target compounds **3a**–**q** were synthesized from reactions between 2-hydrazinyl-1,3-benzothiazole **1** with an appropriate arenealdehyde **2a**–**q** in ethanol at room temperature (1–2 h) in good yields (Scheme 1). Spectral data of all compounds (^{1}H NMR,

* Corresponding author.
E-mail addresses: julianeyoneda@id.uff.br (J.D. Yoneda), kzleal@gmail.com (K.Z. Leal).
[1] Present address: School of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom.

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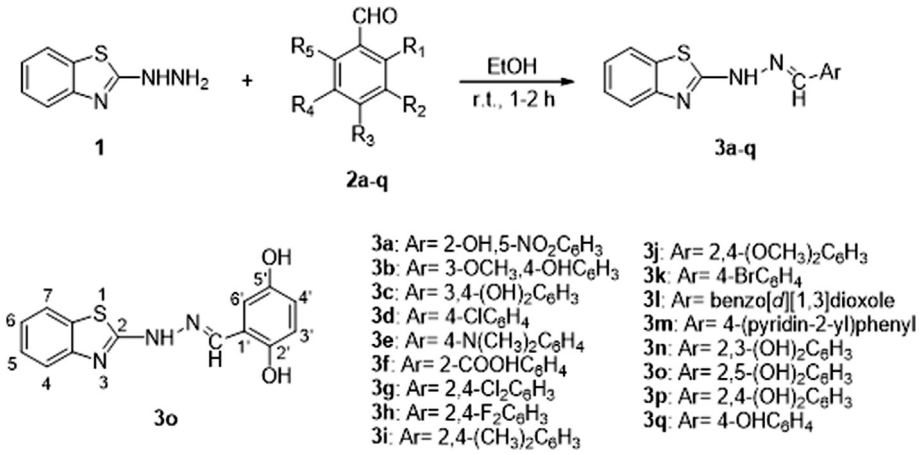


Scheme 1. Synthetic route for preparing the (*E*)-2-benzothiazole hydrazones.

^{13}C NMR, IR and ESI-MS) are in full agreement with the proposed structures. As an example, the IR spectra for compound **3o** shows the C=N peak at 1624 cm^{-1} and the N–H stretching vibrations at 3358 cm^{-1}. The ^{1}H NMR spectrum of compound **3o** exhibits four singlets at 12.13, 9.66, 8.95 and 8.36 ppm for NH, OH, OH and N=C–H, respectively. For the benzothiazole core, the hydrogens H5 and H6 are shown as double–double doublets at 7.29 and 7.09 ppm and H4 and H7 as doublets at 7.75 and 7.38 ppm. On the aromatic region we assigned phenyl hydrogens at 7.04, 6.74 and 6.68 for H6′, H3′ and H4′, respectively. The ^{13}C NMR spectra exhibits the N=C–S signal at 166.4 ppm and C=N signal at 126.1 ppm.

2.2. Biological activity

The *in vitro* anticancer activities of the title compounds were assessed against three human cancer cell lines in comparison to doxorubicin (**Dox**), the positive control, by using MTT assay [16]. The concentrations that induce 50% inhibition of cell growth (IC_{50}) in μM are reported in Table 1. Results showed that compounds **3a**, **3c**, **3l**–**q** exhibited good cytotoxicity against HL-60 leukemia cell line, especially the substance **3n** that showed a IC_{50} value of 0.35 μM. The novel compound **3o** exhibited activity against all the three cell lines, with IC_{50} ranging from 0.59 to 11.18 μM. Based on data collected from three independent experiments, compounds **3a**, **3l**, **3m** and **3q** were found to be selective to leukemia cell line. It should also be noted the significant cytotoxic activity of compounds **3n** and **3p** against HCT-8 cell line with IC_{50} values of 1.29 and 3.43 μM, respectively. **Dox** exhibited IC_{50} ranging from 0.01 to 0.48 μg/mL (Table 1). These results are in agreement with the National Cancer Institute (NCI) protocols, where compounds exhibiting IC_{50} values <10 μM or 15 μM are considered active [17].

It is noteworthy that the most active compounds, for at least two cancer cell lines, **3n** and **3p**, bear two hydroxyl groups in its structure. It was also found a significant activity improved against the three cancer cell lines from compound **3a**, which bears a hydroxyl group at position 2′ and a nitro group at position 5′, to compound **3o**, with two hydroxyl groups in the same positions. This fact suggests the importance of the hydroxyl for the biological activity for this series.

The mechanical stability of red blood cells is a good parameter for *in vitro* screening of unspecific cytotoxicity, since the membrane of erythrocyte can suffer significant changes in its structural properties [18]. The absence of hemolytic activity (EC_{50} > 200 μg/mL) observed for all compounds suggests that the mechanism involved in cytotoxicity against cancer cell is not be related to membrane damage (Table 1). In addition, none of the most active compounds **3a**, **3c**, **3l**–**q** exhibited cytotoxicity against the human lung fibroblast normal cells (MRC5).

2.3. Molecular modeling and Lipinski's rule of five

Calculations on the isomeric forms of the active compound **3o** were performed by using the Gaussian 09W program [19]. The two isomers were fully optimized at the B3LYP/6-311++G(d,p) level of theory. Since no imaginary frequency was found, all the optimized geometries were characterized as minima energy structures. As shown in Table 2, the *E*-isomer is more stable than the *Z*-isomer, which concurs with the isomeric form obtained synthetically.

In order to assess the potential oral bioavailability of the studied compounds, they were submitted to the Lipinski's rule of five analysis (Table 1), which states that an orally active molecule should respect a molecular weight (MW) ≤ 500 g/mol, a clogP ≤ 5, a number of hydrogen bond acceptors (HBA) ≤ 10 and a number of hydrogen bond donors (HBD) ≤ 5 [20]. The related criteria Polar Surface Area (PSA) ≤ 140 A^2, lately added by Veber et al. [21] was also included in the analysis. Compounds **3a**–**c**, **3e**–**f**, **3h**, **3j**–**l**, **3n**–**q** (encompassing all most active compounds) fulfilled all the parameters which suggest that they may have a good oral biodisponibility (Table 1). Compounds **3d**, **3g**, **3i**, **3k** and **3m** presented clogP higher than 5, which indicates high hydrophobicity. Consequently, they may have problems for penetrating the membrane of the cancer cell. It is worth noting that the control compound **Dox** does not fulfill three parameters: MW, PSA and HBA, which can explains its poor bioavailability after oral administration and also why **Dox** chemotherapy is limited to intravenous administration or Dox-liposomes [22,23].

Additionally, the druglikeness and the drugscore factor were obtained for all compounds in the Osiris Property Explorer (Fig. 1) [24]. The results show that only compounds **3a**, **3f** and **3i** presented negative druglikeness score. Even though compound **3a** has shown antineoplastic activity, the negative value is probably due to the nitro substituent, which is not an ordinary group found in currently traded drugs. Regarding compounds **3f** and **3i**, the carboxyl group is responsible for the negative value in compound **3f**, while in compound **3i** it is probably due to the methyl groups as substituents. The control compound, which is also the marketed and most active molecule, presented the highest druglikeness value.

Table 1
Cytotoxic activity expressed by IC_{50} in μM of compounds for cancer cell lines,[a] hemolytic activity and theoretical oral biodisponibility (Lipinski's rule of 5) for compounds **3a**–**q**.

Compound	IC_{50}[a] μM			EC_{50}[b] (μg/mL)	PSA (A^2)	Lipinski RO5			
	HL-60	MDAMB-435	HCT-8			Mw (Da)	HBA	HBD	cLogP
3a	1.65	14.00	13.52	>200	81.13	314.33	7	1	4.31
3b	>16.70	>16.70	>16.70	>200	52.29	299.35	5	1	4.15
3c	13.60	>17.52	>17.52	>200	67.00	285.33	5	2	3.89
3d	>17.37	>17.37	>17.37	>200	27.88	287.77	3	0	5.23
3e	>16.87	>16.87	>16.87	>200	28.91	296.40	4	0	4.95
3f	>16.81	>16.81	>16.81	>200	58.92	297.34	4	1	4.23
3g	>15.52	>15.52	>15.52	>200	27.71	322.22	3	0	5.78
3h	>17.28	>17.28	>17.28	>200	27.83	289.31	3	0	4.98
3i	>17.77	>17.77	>17.77	>200	27.77	281.38	3	0	5.64
3j	>15.95	>15.95	>15.95	>200	40.59	313.38	5	0	4.42
3k	>15.05	>15.05	>15.05	>200	27.89	332.23	3	0	5.50
3l	5.51	>16.81	>16.81	>200	44.25	297.34	5	0	4.45
3m	11.47	>15.13	>15.13	>200	34.19	330.42	4	0	5.43
3n	0.35	>17.52	1.29	>200	64.43	285.33	5	2	3.89
3o	0.59	6.27	11.18	>200	66.34	285.33	5	2	3.89
3p	1.89	14.44	3.43	>200	66.28	285.33	5	2	3.89
3q	4.60	>18.56	>18.56	>200	47.59	269.33	4	1	4.28
Dox	0.04	0.88	0.02	>200	156.88	543.53	12	5	−0.68

[a] Data are presented as IC_{50} values and 95% of confidence interval for leukemia (HL-60), breast (MDA-MB-435) and colon (HCT-8). Doxorubicin (**Dox**) was used as positive control. Experiments were performed in triplicate.
[b] EC_{50} = effective concentration.

The drugscore, which combines druglikeness score, clogP, logS, molecular weight and toxicity risks in one value, may be used to predict the overall potential as a drug candidate of a particular compound [24]. Results showed that **Dox** exhibited the highest drugscore value, followed by three active compounds (**3c**, **3o** and **3p**) which have in common hydroxyl groups as substituents. The low drugscore value observed for compound **3a** is also probably due to the presence of the nitro group (Fig. 1).

3. Conclusion

The synthesis of (*E*)-2-benzothiazole hydrazones was described and their *in vitro* anticancer activities were evaluated against leukemia (HL-60), breast (MDA-MB-435) and colon (HCT-8). The cytotoxicity against all the three cancer cells lines of the dihydroxyl compounds **3o** and **3p** suggests that they might be possible antineoplastic lead molecules for further investigation.

4. Experimental

4.1. Chemistry

Melting points were determined on a Fisatom 430 instrument and are uncorrected. IR spectra were recorded on a Perkin–Elmer 1420 spectrometer using KBr pellets. Negative mode ESI-MS was done on a ZQ-4000 single quadrupole mass spectrometer. NMR spectra were recorded on a Varian Unity Plus 300 spectrometer operating at 300.00 MHz (^{1}H) and 75.0 MHz (^{13}C) and on a Bruker Avance 500 operating at 500.13 MHz (^{1}H) and 125.75 MHz (^{13}C) in DMSO-d_6. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane. Elemental analysis was performed at CA IQ-USP, São Paulo, Brazil on a Perkin Elmer – CHN 2400 analyzer.

4.1.1. General procedure for the synthesis of 2-benzo[d]thiazole hydrazones (*3a*–*q*)

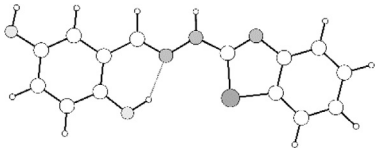
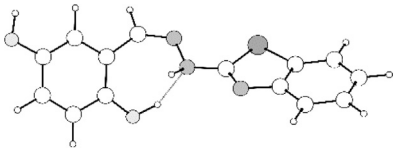
1,3-Benzothiazole hydrazones **3a**–**q** were prepared from reactions between 2-hydrazinyl-1,3-benzothiazole **1** (1 mmol) and appropriate arenealdehydes **2a**–**q** (1 mmol) in ethanol (10 ml) at room temperature. After completion of reaction, 1–2 h (checked by TLC), the resulting precipitate was collected by filtration and washed successively with cold alcohol and ethyl ether to give the pure product **3**. The spectral data of **3a**–**k**, **3m** and **3q** had been already reported in our previous papers [13–15].

*4.1.1.1. (E)-2-(2-(benzo[d][1,3]dioxol-5-ylmethylene)hydrazinyl) benzo[d]thiazole (**3l**).* Yield: 73%, m.p. 219–220 °C, IR (ν cm^{-1}, KBr): 3433 (NH), 1610 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6, δ ppm): 12.15 (s, 1H, NH), 8.04 (s, 1H, N=C–H), 7.74 (d, 1H, J = 7.8 Hz, H_4 or H_7), 7.41 (d, 1H, J = 7.3 Hz, H_7 or H_4), 7.29 (dd, 1H, J = 7.3 and 1.2 Hz, H_5 or H_6), 7.26 (d, 1H, J = 1.6 Hz, $H_{4'}$), 7.15 (dd, 1H, J = 8.2 and 1.6 Hz, $H_{2'}$), 7.09 (td, 1H, J = 7.8 and 1.2 Hz, H_6 or H_5), 6.98 (d, 1H, J = 8.0 Hz, $H_{3'}$), 6.09 (s, 2H, CH_2); ^{13}C NMR (75.0 MHz DMSO-d_6, δ ppm): 167.0, 148.8, 148.0, 128.9, 126.0, 122.6, 121.5, 108.6, 104.9, 101.6; ESI-MS: m/z [M–H]$^-$: 296.3; Anal. Calcd. For $C_{15}H_{11}N_3O_2S$: C, 60.59; H, 3.73; N, 14.13%, Found: C, 60.35; H, 3.83; N, 13.96%.

*4.1.1.2. (E)-3-((2-(benzo[d]thiazol-2-yl)hydrazono)methyl)benzene-1,2-diol (**3n**).* Yield: 78%, m.p. 260–261 °C, IR (ν cm^{-1}, KBr): 3248 (NH), 3051 (OH), 1614 (C=N); ^{1}H NMR (500.13 MHz, DMSO-d_6, δ ppm): 12.12 (s, 1H, NH), 9.99 (s, 1H, OH), 9.30 (s, 1H, OH), 8.44 (s, 1H, N=C–H), 7.73 (d, 1H, J = 7.7 Hz, H_4 or H_7), 7.35 (d, 1H, J = 7.1 Hz, H_7 or H_4), 7.31–7.27 (m, 1H, H_5 or H_6), 7.11–7.05 (m, 2H, H_6 or H_5 and $H_{6'}$), 6.84 (dd, 1H, J = 7.8 and 1.5 Hz, $H_{4'}$), 6.73 (dd, 1H, J = 7.8 and 7.8 Hz, $H_{5'}$); ^{13}C NMR (125.75 MHz DMSO-d_6, δ ppm): 166.2, 145.5, 145.3, 126.2, 121.8, 121.5, 119.8, 119.2, 116.7; ESI-MS: m/z [M–H]$^-$: 284.6; Anal. Calcd. For $C_{14}H_{11}N_3O_2S$: C, 58.93; H, 3.89; N, 14.73%, Found: C, 58.59; H, 4.06; N, 14.36%.

*4.1.1.3. (E)-2-((2-(benzo[d]thiazol-2-yl)hydrazono)methyl)benzene-1,4-diol (**3o**).* Yield: 73%, m.p.: 265–266 °C, IR (ν cm^{-1}, KBr): 3358 (NH), 3034 (OH), 1624 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6, δ ppm): 12.13 (s, 1H, NH), 9.66 (s, 1H, OH), 8.95 (s, 1H, OH), 8.36 (s, 1H, N=C–H), 7.75 (d, 1H, J = 7.6 Hz, H_4 or H_7), 7.38 (d, 1H, J = 7.4 Hz, H_7 or H_4), 7.29 (ddd, 1H, J = 7.2; 6.4 and 1.2 Hz, H_5 or H_6), 7.09 (ddd, 1H, J = 7.2; 6.1 and 1.2 Hz, H_6 or H_5), 7.04 (d, 1H, J = 2.2 Hz, $H_{6'}$), 6.74 (d, 1H, J = 8.7 Hz, $H_{3'}$), 6.68 (dd, 1H, J = 8.7 and 2.7 Hz, $H_{4'}$); ^{13}C NMR (75.0 MHz DMSO-d_6, δ ppm): 166.4, 149.9, 149.5, 126.1, 121.6, 121.4, 119.9, 118.4, 116.9; ESI-MS: m/z [M–H]$^-$: 284.3. Anal. Calcd. For $C_{14}H_{11}N_3O_2S$: C, 58.93; H, 3.89; N, 14.73%, Found: C, 58.64; H, 3.96; N, 14.39%.

Table 2
Gibbs free energy for *E*- and *Z*-isomers of compound **3o** calculated at the B3LYP/6-311++G(d,p) level of theory [19].

E-isomer	*Z*-isomer	Δ*G* (kcal/mol)
		−9.7

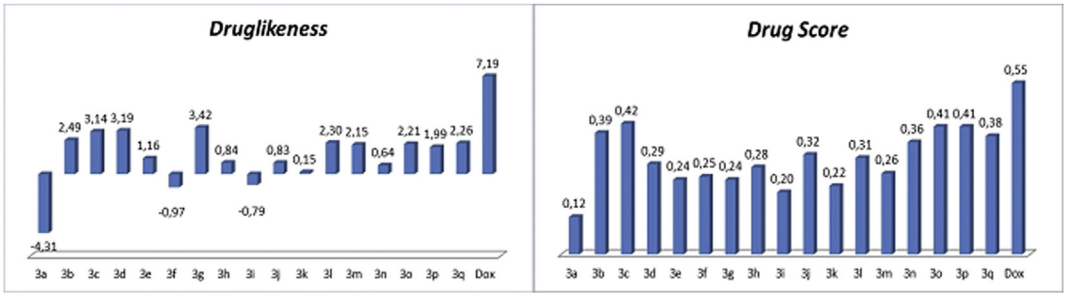


Fig. 1. Druglikeness and drugscore values for compounds **3a**–**q** compared to **Dox**.

*4.1.1.4. (E)-4-((2-(benzo[d]thiazol-2-yl)hydrazono)methyl)benzene-1,3-diol (**3p**).* Yield: 77%, m.p.: 219–220 °C, IR (ν cm^{-1}, KBr): 3336 (NH), 3067 (OH), 1622 (C=N); ^{1}H NMR (300.00 MHz, DMSO-d_6, δ ppm): 9.99 (s, 1H, OH), 8.33 (s, 1H, N=C–H), 7.70 (d, 1H, J = 7.5 Hz, H_4 or H_7), 7.38 (d, 1H, J = 8.2 Hz, H_7 or H_4), 7.29–7.24 (m, 2H, H_5 or H_6 and $H_{3'}$), 7.09–7.02 (m, 1H, H_6 or H_5), 6.37–6.32 (m, 2H, $H_{5'}$ and $H_{6'}$); ^{13}C NMR (75.0 MHz DMSO-d_6, δ ppm): 160.3, 158.6, 126.1, 121.8, 121.2, 111.2, 107.8, 102.4; ESI-MS: m/z [M–H]$^-$: 284.5. Anal. Calcd. For $C_{14}H_{11}N_3O_2S$: C, 58.93; H, 3.89; N, 14.73%, Found: C, 58.56; H, 3.94; N, 14.38%.

4.2. Cytotoxicity against cancer cell lines

Compounds (0.312–20 μM) were tested for cytotoxic activity against three cancer cell lines: HL-60 (leukemia), MDAMB-435 (breast) and HCT-8 (colon). All cell lines were maintained in DMEM medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μM streptomycin at 37 °C with 5% CO_2. Each compound was dissolved with DMSO and diluted with water to obtain a concentration of 20 μM. They were incubated with the cells for 72 h. The negative control received the same amount of DMSO (0.005% in the highest concentration). Doxorubicin was used as a positive control. The cell viability was determined by reduction of the yellow dye 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a blue formazan product after 48 h as described by Mosmann [16].

4.3. Cell membrane disruption

The test was performed in 96-well plates using a 2% mouse erythrocyte suspension in 0.85% NaCl containing 10 mM $CaCl_2$. The compounds diluted as mentioned above were tested at concentrations ranging from 1.5 to 200 μg/mL. After incubation at room temperature for 30 min and centrifugation, the supernatant was removed and the liberated hemoglobin was measured spectrophotometrically at 540 nm. DMSO was used as a negative control and Triton X-100 (1%) was used as positive control. EC_{50} is the calculated effective dose that induced lysis on 50% that of the Triton X-100.

4.4. Calculations

The Gibbs free energy for the two isomeric forms of compound **3o** were obtained by using the Gaussian 09W program [19] at the B3LYP/6-311++G(d,p) level of theory. The druglikeness and drug-score values were obtained by using the Osiris Property Explorer [24]. The Lipinski's rule of five parameters – molecular weight (MW), clogP, number of hydrogen bond acceptors (HBA), number of hydrogen bond donors (HBD), and the related criteria polar superficial area (PSA) – were determined in the SPARTAN'10 program [25].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.08.039.

References

[1] http://www.who.int/mediacentre/factsheets/fs297/en/index.html (accessed 03.06.14).
[2] http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx (accessed 03.06.14).
[3] I. Caleta, M. Krali, M. Marjanovic, B. Bertosa, S. Tomic, G. Pavilovic, K. Pavelic, G. Karminski-Zamola, J. Med. Chem. 52 (2009) 1744–1756.
[4] M. Yoshida, I. Hayakawa, N. Hayashi, T. Agatsuma, Y. Oda, F. Tanzana, S. Iwasaki, K. Koyama, H. Furukawa, S. Kurakata, Y. Sugano, Bioorg. Med. Chem. Lett. 15 (2005) 3328–3332.
[5] S. Bondock, W. Fadaly, M.A. Metwally, Eur. J. Med. Chem. 45 (2010) 3692–3701.
[6] R. Jeon, Y. Kim, Y. Cheon, J. Ryu, Arch. Pharm. Res. 29 (2006) 394–399.
[7] N. Siddiqui, A. Rana, S.A. Khan, M.A. Bhat, S.E. Haque, Bioorg. Med. Chem. Lett. 17 (2007) 4178–4182.
[8] D. Shashank, T. Vishawanth, A. Pasha, V. Balasubramaniam, A. Nagendra, P. Perumal, S. Suthakaran, Int. J. ChemTech Res. 1 (2009) 1224–1231.

[9] V. Fachinetti, R.R. Reis, C.R.B. Gomes, T.R.A. Vasconcelos, Mini-rev. Org. Chem. 9 (2012) 44–53.
[10] I.N. Junior, M.C.S. Lourenço, M.G.M.O. Henriques, B. Ferreira, T.R.A. Vasconcelos, M.A. Peralta, P.S.M. de Oliveira, S.M.S.V. Wardell, M.V.N. de Souza, Lett. Drug Des. Discov. 2 (2005) 563–566.
[11] S.M.S.V. Wardell, M.V.N. de Souza, M.L. Ferreira, T.R.A. Vasconcelos, J.N. Low, C. Glidewell, Acta Crystallogr. Sect. C. Cryst. Struct. Commun. 61 (2005) 617–620.
[12] M.C.S. Lourenço, M.L. Ferreira, M.V.N. de Souza, M.A. Peralta, T.R.A. Vasconcelos, M.G.M.O. Henriques, Eur. J. Med. Chem. 43 (2008) 1344–1347.
[13] A.F. Nogueira, E.C. Azevedo, V.F. Ferreira, A.J. Araújo, E.A. Santos, C. Pessoa, L.V. Costa-Lotufo, R.C. Montenegro, M.O. Moraes, T.R.A. Vasconcelos, Lett. Drug Des. Discov. 7 (2010) 551–555.
[14] A.F. Nogueira, T.R.A. Vasconcelos, J.L. Wardell, S.M.S.V. Wardell, Z. Krist. – Cryst. Mater. 226 (2011) 846–860.
[15] E.B. Lindgren, J.D. Yoneda, K.Z. Leal, A.F. Nogueira, T.R.A. Vasconcelos, J.L. Wardell, S.M.S.V. Wardell, J. Mol. Struct. 1036 (2013) 19–27.
[16] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.
[17] http://dtp.nci.nih.gov/branches/btb/handlingprep.html (accessed 03.06.14).
[18] L.V. Costa-Lotufo, G.M.A. Cunha, P.A.M. Farias, G.S.B. Viana, K.M.A. Cunha, C. Pessoa, M.O. Moraes, E.R. Silveira, N.V. Gramosa, V.S.N. Rao, Toxicon 40 (2002) 1231–1234.
[19] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery Jr., J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, O. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Revision D.01, Gaussian, Inc., Wallingford CT, 2013.
[20] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Adv. Drug Deliv. Rev. 23 (1997) 3–25.
[21] D.F. Veber, S.R. Johnson, H. Cheng, B.R. Smith, K.W. Ward, K.D. Kopple, J. Med. Chem. 45 (2002) 2615–2623.
[22] W. Ke, Y. Zhao, R. Huang, C. Jiang, Y. Pei, J. Pharm. Sci. 97 (2008) 2208–2216.
[23] D.M. Benival, P.V. Devarajan, Int. J. Pharm. 423 (2012) 554–561.
[24] Osiris Property Explorer. Available at: <http://www.organic-chemistry.org/prog/peo/> (accessed 13.11.13).
[25] Spartan'10, Wavefunction Inc., Irvine, CA 92612, U.S.A, 2011.