Synthesis, characterization of novel isoindolinyl- and bis-isoindolinylphenylboronic anhydrides. Antiproliferative activity on glioblastoma cells and microglial cells assays of boron and isoindolines compounds

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ABSTRACT

Boron compounds importance have been raising due to the development of its synthesis and remarkable biological activities, so in this manner the study here presented is focused on the synthesis of novel isoindolinylphenylboronic anhydrides (2a, 2c and 2e) and bis-isoindolinylphenylboronic anhydrides (2b and 2d), as well as a comparative study of the antiproliferative activity of these and their precursors (a-e), 1(a-e) over glioblastoma cells U373 and the cytotoxic activity over normal microglial cells. All compounds were characterized by spectroscopic methods; 1H, 13C, 11B NMR, IR and HRMS. 1H and 11B NMR data at variable temperature of 2b and 2d showed equilibrium between species that contain a tetrahedral and a trigonal boron atom due to an intramolecular N→B coordination and decoordination bond. The study of frontier molecular orbitals of B-derivatives showed that 2b and 2d′ are harder molecules than 2a, 2c and 2e, therefore can react with a second isoindoline to obtain 2b and 2d′. Whereas, HOMO site of potassium derived 1bk and 1dk are over NCHCO2K moiety, consequently, to react with 2b and 2d′ to obtain 2b′ and 2d′. These results clarify the obtainment of 2a-2d independently of the equimolecular ratio of reagents. On another hand, the biological assay showed that compound 2d possessed the most pronounced effect over U373 cells with no effect over normal microglial cells, providing a new set of boron compounds with important antitumoral activity.

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1. Introduction

The importance of boron compounds have been increasing because of its several applications, the synthetic efforts to provide new molecules and due to their biological properties [1–5]. In fact, boron therapeutics showed different modes of inhibition for a variety of biological targets, the first classes of boron compounds reported antibiotic activity [6], boronic acid analogs of lysine and arginine have been reported as inhibitors for serine protease in the blood coagulation cascade [7], boronic compounds also have exhibited application in boron neutron capture therapy (BNCT) for the treatment of brain tumors like glioblastoma multiforme [8–11], as inhibitors of human Arginases I and II for the treatment of myocardial ischemia reperfusion injury [12], 2-APB as responsible for the store-operated calcium entry (SOCE) potentiation ability [13] and cytotoxic activity [14–18]. In the last few years, boron compounds become more frequent in the treatment of some types of inoperable and high malignancy cancers [19], as glioblastoma (GBM) that is the most common primary malignant tumor of the brain in adults, which is characterized by diffusely silent infiltration in the normal brain [9,20], which makes it impossible to detect it in a timely manner, it is often manifested by a single attack of progressive intensity headache in a few days. The evaluation with
magnetic resonance imaging reveals a large mass that was probably present for months [21]. The histopathological characteristics of GBM are defined by necrosis and endothelial proliferation, which has led to the highest grade assigned to it in the classification of brain tumors by the World Health Organization (WHO) [22]. GBM has been classified into three subtypes according to gene expression, called proneural (PN), mesenchymal (MES) and classic (CL). The GBs of the MES subclass are predominantly primary tumors that arise de novo and in some studies, they show poorer prognosis compared to PN tumors [23]. It has been described that gliomas develop within a mechanically challenging microenvironment, where the dense extracellular matrix (ECM) compromises the vascular integrity and induces hypoxia and with it the activation of the hypoxia-inducible factor 1α (HIF1α). Therefore, the aggression of the glioma has been correlated with the prognosis of the patient with HIF1α increased levels and the stiffness of the ECM enriched with tenasin C (TNC) [24]. Multiple genetic and epigenetic alterations have been identified in GBM cells [25] suggesting that such epigenetic aberrations result in the inactivation of critical genes that not only participate in tumorigenesis but may also precede genetic changes [26], therefore, for its treatment, HDAC, chemotherapy or radiotherapy have been explored [27,28]. Despite these treatments, the prognosis for patients with glioblastoma also is poor, since the average duration of survival for newly diagnosed patients is 8–15 months [29] and is 3–9 months for patients with recurrent disease [30]. In 2018 it was reported that despite advances in the surgical and oncological treatment of glioblastoma, the prognosis continues being poor, with a 5-year survival between 0.05 and 4.7% [31].

On the other hand, microglia are neuroglial cells, which constantly monitor the microenvironment and respond quickly to stress, infection and other lesions, their presence is induced by molecules expressed or secreted by adjacent neurons and astrocytes. It is characterized by its fibroblastic morphology, however, it has the ability to adapt morphologically, so there is branched microglia that examines the microenvironment and ameboid microglia that phagocytes and eliminates waste particles, dead cells, redundant synapses and microorganisms that endanger to the SNC [32,33]. Microglia can strongly influence the pathological outcome or the response to a stressor due to the release of a large number of substances, including cytokines, chemokines and growth factors, making it an important modulator of neuro-inflammatory responses [34]. It has been reported that the proper development and function of the central nervous system (CNS) of mammals depend fundamentally on the sentinel activity of microglia and its deregulation can lead to neurological and neurodegenerative diseases [35]. Therefore, in glioblastoma multifforme, a large proportion of the tumor microenvironment consists of an inflammatory infiltrate in which microglia and macrophages predominate, which have been proposed to be subverted by glioblastoma cells for tumor growth [36].

On the other hand, isoindolines have shown diverse biological properties, between these, they have been reported as selective antagonists of endothelin-1 (ETA-1) in endothelin-1 receptor A [37], α-adrenergic receptor blockers [38], as multidrug resistance reversal agents [39], and modulators of autoimmune diseases [40]. Our group has worked in the interest of the synthesis, characterization, structural analysis and reactivity of boron compounds derived of ethanoloamines, diethanolamines, hydroxyaminos, iminodiacetic acids, and N-substitutedimino- and aminodiacetic acids with N–B coordination bond [41–50] and also, we have synthesized various isoindolines derived from α-α-amino acids [31] as antitumor agents [52], inhibitors of COX-1 and COX-2 [53,54], Ca2+ channel blockers [55,56] and as inhibitors of HDAC8 [57].

In this context, we interest is the synthesis of new boron compounds with an isoindoline moiety had lead us to develop the synthesis of a novel series of boron compounds and to investigate their antiproliferative activity on glioblastoma cells, one of the most frequent and lethal brain tumor in adults [9], which generation and progression have been associated to epigenetic changes together with genetic modifications regulated by HDAC inhibition [27]. Based on these research, the current work aims to synthesis of novel isoindolinylphenylboronic anhydrides 2a, 2c and 2e and isoindolinylphenylboronic anhydrides 2b and 2d, characterized by spectroscopic methods of 1H, 13C, 11B NMR, infrared spectroscopy and high-resolution mass spectra (HRMS), and evaluation of their antiproliferative activity on glioblastoma cells U373, and the cytotoxicity on normal microglial cells. The 1H and 13B NMR data showed the existence of intramolecular N–B bond coordination in compounds 2b and 2d. The reaction pathway was analyzed by means of theoretical calculation of frontier molecular orbitals (FMO) of 1a–1e, their potassium carbonate derivatives, boron compounds 2a, 2b, 2c, 2d and 2e in order to explain the synthesis of isoindolinylphenylboron 2a, 2c and 2e and bis-isoindolinylphenylboron 2b and 2d independently of the ratio 1:1 or 2:1 isoindolines 1a–1e and PhB(OH)2, respectively. Meanwhile, the comparative study of antiproliferative activity on glioblastoma cells 6–207T cell lines and microscopical cells U373, characterized by their antiproliferative activity on glioblastoma cells U373, and the cytotoxicity on normal microglial cells 1a–1e and 2a–2e described their antitumoral activity dependence on the isoindoline ring, and an exacerbation of cytotoxicity by addition of the boron moiety, with exception of compound 2d, which showed a cytotoxic selectivity over tumoral cells.

2. Experimental
Chemical synthesis reagents were purchased from Sigma Aldrich Co®. NMR spectra were recorded on JEOL 500 ECA and JEOL Eclipse 400. DMSO-d6 and D2O used were as solvents. All chemical shifts of 1H and 13C are reported relative to TMS and 11B chemical shift are reported relative to BF3·OEt2. The high-resolution mass spectra (HRMS) were obtained on an Agilent Technologies Model LC/MSD-TOF spectrometer, coupled to HPLC 1100 with ESI as ionization source. The Infrared spectra were obtained on Varian FT-IR-640 series. The melting points were measured in open capillary tubes on a Gallenkamp equipment MFB-595. Isoindolines 1a–1e and 1a–e were prepared by the method described previously by the present authors, all of them were identified by 1H and 13C NMR spectra, and the resulting data agree with those previously reported [51,52].

2.1. Synthesis and characterization of boron compounds 2(a–e)

2.1.1. The procedure outline below is general used for the synthesis of boron compounds 2(a–e)

**Synthesis of 2-(isoindolin-2-yl)acetic phenylboronic anhydride (2a):** Compound 1a (0.16 g, 0.90 mmol), phenylboronic acid [PhB(OH)2] (0.11 g, 0.90 mmol) and potassium bicarbonate (KHCO3) (0.90 g, 0.90 mmol) in 75 mL of toluene were placed in a 250 mL balloon fitted with a Stark trap. The reaction mixture was refluxed and vigorous stirring was maintained for 10 h. After cooling to room temperature under nitrogen and filtered. The solvent was evaporated under vacuum and the remaining solid was treated with 5 mL of methylene chloride and 5 mL of methanol and allowed stirring for 20 min and it was filtered under vacuum in a Buckner funnel to give 2a (0.17 g, 67.8%) as a white solid; m.p.: 206–207°C (decomp.). All NMR spectra were obtained at 50°C because of its low solubility at room temperature, 1H NMR (400 MHz, D2O); δ 4.23 (s, [2H], 2CH2), 4.99 (s, [4H], 3CH2), 6.68 (s, [4H], 5.6 CH), 8.02 (d, 3JHH = 6.7 Hz, [2H], 4CH), 7.71–7.73 (2H, m, mCH), 7.79 (t, 3JHH = 7.0 Hz, [1H], 3CH); 11B NMR (128 MHz, D2O);
δ = +29.13 (s); 13C NMR (100 MHz, D2O): δ = 70.9 (C), 58.2 (C), 59.9 (C), 133.7 (C), 123.4 (C), 129.4 (C), 134.1 (C), 128.5 (C) and 131.5 (C). FT-IR (ATR solid) 3049, 2991, 1603, 1321 cm⁻¹. HRMS-ESI: C10H14N2O2 [M + H-PhBO]⁺, calc. 178.0862 and found 178.0863.

Synthesis of (N-B) [Bis-(S)-2-(isoindolin-2-yl)-4-methylpentanoic]phenylboronic anhydride (2b): Compound 1b (0.25 g, 1.07 mmol), PhB(OH)2 (65.4 mg, 0.53 mmol) and KHCO3 (0.250 g, 0.936 mmol), PhB(OH)2 (0.057 g, 0.468 mmol) and KHCO3 (0.0981 g, 0.98 mmol), give 2b (0.26 g, 88%) as a brown solid; m.p.: 139.0°C. 1H NMR (400 MHz, DMSO-d6): δ = 2.80 (t, 3JHH = 7.3 Hz, [2H], 7CH2), 7.24 (m, [4H], 5,6CH), 3.2 (dd, 2JHH = 3.7, 5.7 Hz, [2H], 8CH), 7.37-7.40 (3H, m, 9,10CH), 7.77 (d, 3JHH = 6.0 Hz, [2H], 7CH), 7.30-7.32 (m, [2H], 4,5CH); 13C NMR (100 MHz, DMSO-d6): δ = 28.27, 13C NMR (100 MHz, DMSO-d6): δ = 172.2 (C), 72.4 (C), 57.4 (C), 139.8 (C), 123.9 (C), 127.9 (C), 127.3 (C), 127.9 (C), 127.9 (C), 130.5 (C), FT-IR (ATR solid) 3049, 2991, 1603, 1321 cm⁻¹. HRMS-ESI: C16H16NO2 [M + H-PhBO]⁺, calc. 254.1175 and found 254.1174.

Synthesis of (S)-2-(isoindolin-2-yl)-3-propylenylacetic phenylboronic anhydride (2c): Compound 1c (0.35 g, 1.38 mmol), Ph(OH)2 (0.168 g, 1.38 mmol) and KHCO3 (138.4 g, 1.38 mmol), give 2c (0.276 g, 56%) as white solid, m.p.: 153.0°C. 1H NMR (400 MHz, DMSO-d6): δ = 2.82 + 2.85 (m, [4H], 5,6CH), 1.31 (t, 3JHH = 7.3 Hz, [3H], 7CH3), 0.91, 0.88 (d, 3JHH = 7.3, [2H], 8CH); 13C NMR (100 MHz, DMSO-d6): δ = 28.20, 13C NMR (100 MHz, DMSO-d6): δ = 173.9 (1C), 65.5 (2C), 55.5 (3C), 139.7 (4C), 122.8 (5C), 127.2 (6C), 26.9 (7C), 111.0 (8C), 123.9 (9C), 127.8 (10C), 111.9 (11C), 118.80 (12C), 121.40 (13C), 118.80 (14C), 136.60 (15C), 134.6 (16C), 127.2 (17C), 130.5 (C). FT-IR (ATR solid) 3057, 3060, 2850, 2919, 1602, 1131 cm⁻¹. HRMS-ESI: C16H14B3NO4 [M + H-PhBO]⁺, calc. 307.1441 and found 307.1445.

2.2. Computational procedure

The geometries of the isoidolines a-e, 1(a-e), their potassium carboxylates derivatives (1aK-1eK) and boron compounds 2a, 2b, 2c, 2d and 2e have been optimized based on molecular mechanics, semi-empirical, ab-initio and DFT calculations, using the molecular modeling program Spartan’14. Molecular mechanics calculations were carried out using MM + force field. For semi-empirical calculations, the routine PM3 was used. Ab-initio calculations were performed with the Hartree-Fock 6-31G+ + method. The optimization of structures was performed by single point calculations to obtain the HOMO and LUMO energies, dipole moments and free energy.

2.3. Primary mixed glial cell culture

The primary cell culture of mixed glial cell (MGC) was performed according to the Tamashiro protocol [58], first with the dissection of neonatal rat brain tissue as followed: The Wistar neonatal rap pups were rinsed with 70% ethanol. (Neonatal rat pups ages were between 1-5 day born). After a quick decapitation, the rat pup heads were transferred into 70% ethanol. Whole brains were removed from the heads and placed into a Petri dish of L-15 solution (Leibowitz L-15 solution + 0.1% BSA + 1% of antibiotic (10,000 units penicillin and 10 mg of streptomycin per mL) on ice. The meninges from the brain were removed and transfer the desired rest of the brain tissue into a new Petri dish with 4-5 mL of L-15 solution. Immediately the preparation of MGC population was performed by the transference of the tissue with a 10 mL pipette to a sterile 50 mL conical tube and centrifuged at 2500 RCF for 5 min at 4°C. The supernatant was aspirated and added 4-5 mL of fresh L-15 media. The suspension was placed in a cell strainer (100 μm pores) onto a fresh 50 mL conical tube and the centrifuged once again at 2500 RCF for 5 min at 4°C. The suspension was cultured into a 75 cm² culture flask with 12 mL of culture media (DMEM + 10% FBS + 1% Penicillin/Streptomycin) and incubated in a 5% CO2 incubator at 37°C for a total of 1–3 weeks. Cells were characterized by using a primary antibody anti-fibrillar glial acid protein (product number Z0334 Laboratories DAKO).

2.4. Cell culture

The U373 cell line was donated by the National Cancer Institute of México. Human U373 and rat primary astrocyte cells groups were grown into 25 cm² Corning culture bottles at 1.0 × 10⁶ cells/well in DMEM-F12 and DMEM culture medium supplemented with 10% of FBS and 1x of antibiotic in a 5% CO2 incubator.

2.5. Cancer cell antiproiferative assays and normal cell cytotoxic assays

The antiproliferative evaluation was carried out for compounds by the MITT assay using human U373 cells. The MITT assay was used to measure metabolic active cells. Cells were seeded into 96-well microtiter Corning® plates at 1.0× 10⁵ cells/well in 100 μL of DMEM-F12 medium supplemented with 10% of SBF + 1% Penicillin/Streptomycin. 72-hour confluence cells were treated for 24 h with compounds at concentrations from 0.0005 mM to 10 mM, SAHA concentration was 10 μM. Post-treatment cell viability was measured by conventional MITT dye-reduction assay. For MITT assay,
20 μL of 5 mg/mL MT reagent in PBS 1X was added to each well. Viable cells with active mitochondria reduced the MTT to an insoluble purple formazan precipitate that is solubilized with the subsequent addition of 50 μL of DMSO. The formazan dye was measured spectrophotometrically using an ELISA reader. The cytotoxic effect of each treatment was expressed as a percentage of cell viability relative to untreated control cells (percentage of control) and is defined as [(A595-or A570-nm-treated cells)/A595-or A570-nm-nontreated cells)] x 100 [59,60].

The evaluation of cytotoxicity over rat microglial cell culture was carried out by the neutral red (NR) assay. The NR is a dye that correlates with the decrease in the number of viable cells [61]. The NR uptake was only with the solvent and plotted against the Log of compound concentration. A decrease in the NR uptake by the microglia cells was replaced with 100 μM of acid alcohol (1%, v/v, acetic acid in 50% ethanol), which elutes the NR from the cells. Absorbance was read at 545 nm on an ELISA reader The mean absorbance of the NR in the control) and is defined as [(A595-or A570-nm-nontreated cells)].

3. Results and discussion

3.1. Synthesis

Isoindolines (a- and b) and (1-a-e) derived from 1-α-amino acids were synthesized by the method described previously by the present authors [51,52]. The reaction of isoindolines (1-a-e) and phenylboronic acid independently of the ratio 1:1 or 2:1, respectively, led to the synthesis of novel boron compounds 2a-2e (Scheme 1).

3.2. Spectroscopy

Compounds were characterized by 1H, 13C, 11B NMR, IR and HRMS spectroscopic methods, resulting data of compound are given in the materials and methods section. The integral of the spectra of 2a, 2c, and 2e show a 1:1 ratio between protons belong to isoindolinyl group and protons of the phenyl group attached to the boron atom, whereas spectra of 2b and 2d show a 2:1 ratio, respectively. Proton H-2 of 2a and 2c exhibit a single signal at 4.23 and 4.49 ppm, respectively as it is expected.

However, H-2, H-7 and H-8 of 2b and 2d displays broad signals instead of complex pattern due to the coupling between these. Therefore, their H-3 diastereotopic protons did not show the AB coupling pattern as is observed for the isoindolines (1a-1e) [51]. This suggests the existence of an intramolecular N→B coordination bond, as it has been demonstrated for boron compounds derived from amino alcohols, diethanolamines and iminodiacids [41–43]. In order to obtain evidence of trigonal or tetrahedral environment of the boron atoms for 2a-2e, their 11B NMR spectra were obtained. Thus, δ(11B) value for 2a, 2c and 2e are in the range for trigonal environment of the boron nucleus, since they lie in the range reported for analogous boron heterocycles [62]. Whereas, 2b and 2d containing two isoindolinyl groups exhibit two signals in a ratio of 7:93 for 2b, at δ = 28.2 and δ = 0.58, respectively and for 2d in a 6:94 ratio, at δ = 28.0 and δ = 0.60, respectively, which give evidence of tri- and tetracoordinate boron, where the chemical shift of boron atom at lower frequency corresponds to the tetrahedral boron atom [62], showing the existence of an intramolecular N→B coordination bond, this result and the fact that the signals of H-2, H-3, H-7 and H-8 in 1H NMR display broad signals, suggest the existence of a species-equilibrium that contain a trigonal and a tetrahedral boron atom (Fig. 1), such phenomena have been observed for analogous boron derived of amino alcohols and amino acids derived [41,43,49]. To demonstrate this equilibrium, variable temperature of 1H and 11B NMR experiments were achieved by increasing the
temperature at intervals of 10 °C starting from room temperature until 120 °C. In this way, the change was observed for 2b at 50 °C and for 2d at 70 °C showing the splitting of the signals H-2, H3, H-7 and H-8, indicating the dissociation of N→B coordination bond, which was confirmed from their 11B NMR, which exhibited an increase of the signal assigned to trigonal boron atom and decrease of those for tetrahedral boron atom. Thus, 2b display two signals at δ +28.11 and +0.61 in a ratio 75:25, while 2d at δ +28.05 and +0.65 in a ratio 70:30.

13C NMR data for compounds 2(a-e) are C-1 chemical shifts are in the range of 168.35 to 174.80 ppm, for C-2 are between 54.64 y 72.25 ppm, the C-3 is in the range of 55.03–58.85 ppm and the signals for C-4, C-5 y C-6 are between 122.72 and 139.87 ppm. The infrared spectra of 2(a-e), show stretching absorption bands C–H aromatic and aliphatic in the range of 3006–3060 cm⁻¹ and 2850-2995 cm⁻¹ respectively, carbonyl group C(=O)O in the range of 1600–1607 cm⁻¹ and B–O are in the range of 1311–1366 cm⁻¹, respectively. The high resolution mass spectra of boron compounds 2b and 2d exhibit the ion of the protonated molecules [M+H]⁺ at m/z = 553 and 621, respectively. However, compounds 2a, 2c, 2e display the [M + H-PhBO]⁺ fragment ion and it was assigned as the corresponding protonated isoindoline at m/z = 178, 254 and 307, respectively.

3.3. Frontier molecular orbitals

As stated above, isoindolinylphenylboronic anhydrides 2a, 2c, 2e and bis-isoindolinylphenylboronic anhydrides 2b and 2d are obtained independently of the ratio of isoindolines 1(a-e) and phenylboronic acid 1:1 or 2:1, respectively. In order to find an explanation for this result, theoretical calculations of frontier molecular orbitals (HOMO and LUMO) were carried out for boron compounds 2a, 2c, 2e (Scheme 1) and 2b’, 2d’, which were not obtained experimentally, (Fig. 2), isoindolines (1a-1e) and their potassium carboxylate derivatives (1aK-1eK) with the purpose of evaluate the chemical reactivity using semi-empirical and Density

![Fig. 2. Isoindolinylphenylboronic anhydrides 2b and 2d' precursors of 2b and 2d.](image)

![Fig. 3. Nodal patterns of HOMO and LUMO energy in eV of 2a, 2b', 2c, 2d' and 2e.](image)

![Fig. 4. Nodal patterns of HOMO and LUMO energy in eV of 2a, 2b', 2c, 2d' and 2e, with intramolecular hydrogen bond.](image)
Functional Theory (DFT) methods [63]. Theoretically, is expected that the boron atom of 2b’ and 2d’ shows a major Lewis acid character than of 2a, 2c, 2e, in order to react with a second isoindoline as a nucleophile. Therefore, an evaluation of the HOMO and LUMO energies of 2a, 2b’, 2c, 2d’ and 2e, was carried out to explore the contribution of the LUMO, which indicates the most likely site to undergo by a nucleophilic attack. In addition, the molecules with intramolecular hydrogen bond (ihb) between B–OH and C=O groups were evaluated to examine if this bond is involved in the increase of the acidity of the boron atom.

Fig. 3 shows the frontier molecular orbitals of 2a, 2b’, 2c, 2d’ and 2e. Fig. 4 shows those with intramolecular hydrogen bond (HOMOihb and LUMOihb) and their energy in eV; as shown, HOMO is located on the isoindolinyl group, except in 2e, which is located on the indolyl group. Whereas, LUMO is located on the C(O)OB(OH) Ph group, so it is expected that all molecules could undergo by a nucleophilic reaction in that site [64]. Although, HOMO-LUMO energy gap in eV (Fig. 5) shows that in both cases (without and with intramolecular hydrogen bond) 2b’ and 2d’ have a greater gap than 2a, 2c and 2e. this suggests that the first ones are harder molecules than the second ones in order to react with another isoindoline to obtain 2b and 2d. Whereas, since HOMO describes the most likely site for electrophilic attack. Therefore, an evaluation of the frontier molecular orbitals of isoindolines 1a-1e (Fig. 6) and its carboxylate potassium derived compounds 1aK-1eK (Fig. 7) was carried out, in order to know their site and hardness to react towards boron compounds 2a, 2b’, 2c, 2d’ and 2e. The HOMO site differs in the structures, thus for 1a it is observed over the whole molecule, this is also observed for 1b and 1c, with the exception of the R substituent of the amino acid. HOMO site for 1d is over the boron group and the R substituent, meanwhile for 1e, it can be observed over the indolyl group. In relation to 1aK-1eK, the HOMO site of 1bK and 1dK is over NCHCO2K, whereas, for 1aK and 1cK, it is over the isoindolinyl group, and for 1eK is over the indolyl group. These results suggest that only 1bK and 1dK are able to react with 2b’ and 2d’ in order to obtain 2b and 2d. Fig. 8, shows the HOMO donor (d) of 1bK, 1dK, LUMO acceptor (a) of 2b’, 2d’ energies and the HOMO-LUMO energy gap in eV.

3.4. Antiproliferative activity on glioblastoma cells and microglial cells assays of boron and isoindolines compounds

Tested compounds exerted concentration-dependent anti-proliferative effects on tested tumor cells and microglial cells (Table 1). Selective effect at micromolar concentrations (IC50 in the range of 0.04–20.24 μM) was observed for isoindolines b-e, 1c and 1d, as well for the boron compound derivatives 2a-2e on U373 cell line.

Results for both series of isoindolines demonstrated that compound e owns the most pronounced effect on U373 cell line, while 1a, 1b, and 1e were the less active. Based on these results, there is a selective and strongest effect of isoindolines b-e series in comparison with 1(a-e) observed on U373 cells and normal rat microglial cells, evidencing the importance of the methyl group of the ester moiety as an essential clue for the antitumoral activity. For these series, almost all of the compounds tested, did not show effect on normal microglial cells, except for 1e.

On the other hand, boron compounds 2(a–e), exhibit the effect on U373, in particular, 2c and 2d display the major effect in comparison with c-d and 1c-1d, however, 2(a–c), and 2e also show effect normal rat microglial cells. So probably the addition of the
The inhibition effects of compounds a–e, 1(a–e) and 2(a–e) on the growth of tumor cells U373 and normal rat microglial cells in vitro.

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* Margin of error; N = 8.

4. Conclusion

Novel isoindolinylphenylboronic anhydrides 2a, 2c and 2e and bis-isoindolinylphenylboronic anhydrides 2b and 2d were synthesized, where the last one showed by \(^1\)H and \(^{11}\)B NMR at variable temperature the existence of an equilibrium between species containing a tetrahedral and a trigonal boron atom due to an intramolecular N→B coordination and decoordination bond. The theoretical calculations of frontier molecular orbitals (HOMO and LUMO) showed that 2b’ and 2d’ are harder molecules than 2a, 2c and 2e due to the fact that they have a greater HOMO-LUMO gap, in consequence, they can react with another isoindoline to obtain 2a and 2b. Whereas, HOMO site of carboxylate potassium derived 1bk and 1dk are on NCHCO\(_2\)K moiety, even so, for 1ak and 1ck is on the isoindoliny group, for 1ek is on indolyl group. Thus, only 1bk and 1dk are able to react with 2b’ and 2d’ in order to obtain 2b and 2d. These theoretical results clarify why isoindolinylphenylboronic anhydrides 2a, 2c, 2e and bis-isoindolinylphenylboronic anhydrides 2b and 2d are obtained independently of the ratio of isoindolines 1(a–e) and phenylboronic acid 1:1 or 2:1. Regarding the biological activity of the compounds tested, the isoindolines and boron compounds display concentration-dependent anti-proliferative effects on tested tumor cells and normal rat microglial cells. The selective effect at micromolar concentrations (IC\(_{50}\) values in the range of 0.04–20.24 µM) was observed for isoindolines b–e, 1c and 1d, as well for the boron compound derivatives 2a–2e on U373 cell line, from these, compound e exhibited the most pronounced effect. Therefore, isoindolines b–e showed a selective and strongest effects in comparison with 1(a–e) observed on U373 cells and normal rat microglial cells, revealing the importance of the methyl group of the ester moiety for the antitumoral activity. Also, the lack of activity over the normal rat microglial cells, excepting 1e, show the low cytotoxicity of isoindolines over normal cells, therefore for boron compounds 2c and 2d displayed the major effect on U373 in comparison with c–d and 1c–1d. However, 2(a–c), and 2e also showed effect on normal rat microglial cells. Thus, between the boron compound, 2d emerge as a good candidate to further assays in vivo due to its major activity on U373 cell line and lack of activity on microglial cells.

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