Research Article

A Green Approach to the Production of Hybrid Diindolylmethane-Phenylboronic Acids via a 3MCR: Promising Antineoplastic Molecules

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The current role of the “Green Chemistry Protocol” in multicomponent reactions is first highlighted. Then, the green approach to the production of three novel hybrid diindolylmethanes-phenylboronic acids via a 3MCR is discussed, which features the following: solventless conditions, the use of microwave irradiation to activate the reactions, the absence of catalyst, and an efficient atom economy. The products were achieved with moderate yields (41–61%) within a short time frame (5 min) and appropriately characterized by elemental analysis and spectroscopic methods (NMR: 1H, 13C, 11B; MS: EI, CI, FAB+, HRMS). During the FAB+ MS determinations, various artifacts that are associated with the boron atom via an interaction with thioglycerol from the matrix were noted in the corresponding FAB+ mass spectra; in addition, the accurate mass determination of these adducts unequivocally confirmed the presence of the target molecules. Moreover, the activity of these target molecules was evaluated in the presence of six cancer cell lines (U251 = glia of the central nerve system, PC-3 = prostate, K562 = leukemia, HCT-15 = colon, MCF-7 = breast, and SKLU-1 = lung), which resulted in the meta-regioisomer being the most active. Finally, the products were also analyzed using computational chemistry in order to determine their most stable geometries and reactivities by computing the respective molecular electrostatic potentials.

1. Introduction

Cell growth and division are highly regulated, although a notable exception is seen in the cancer cell, a cell that has lost its usual proliferation control pathways. Consequently, there is growing interest in the search for novel anticancer substances with high efficacy, low toxicity, and minimal side effects [1].

In recent years, cancer prevention by means of natural products has received considerable attention. The potential protective role of cruciferous vegetables and certain active components present in these vegetables, such as isothiocyanates and indole-3-carbinol (I3C), has been extensively studied in experimental in vitro and in vivo carcinogenesis models. The results of these experiments indicate that some chemoprotective agents derived from vegetables of the Cruciferae family influence carcinogenesis, both during the initiation and the promotion of cancer development [2–6]. The major in vivo product derived from the acid-catalyzed condensation of I3C is 3,3'-diindolylmethane (DIM), Scheme 1, a favorable antitumor agent; in particular, several studies have revealed its clinical efficacy against various epithelial cancers, including endometrial and mammary tumors [7, 8]. In addition, I3C and DIM are currently among the most popular adjunct therapies for recurrent respiratory papillomatosis because of their effectiveness and low levels of toxicity [9, 10].
Certain compounds containing B–N bonds possess broad biological activity including insecticidal, fungicidal, herbicidal, antibacterial, calcium channel-blocking and antineoplastic activities [11–14]. Recently, the proteosome inhibitor PS341, an α-amidoboronic acid, was approved as an effective antineoplastic agent [15]. Moreover, several amino boron compounds have been used in boron neutron capture therapy (BNCT) and in chemotherapeutic forms of cancer treatment [16, 17].

On the other hand, one of the main objectives of green chemistry is to carry out reactions using conditions that are not detrimental to the environment [18]. Under such a protocol, an ideal synthesis would be that by which a target molecule is produced quantitatively in one step from available and inexpensive starting compounds in an environmentally sustainable process; in this sense, the proposal and application of novel sustainable processes are among the major challenges in modern organic synthesis. The multicomponent reaction (MCR) is a significant subclass of tandem reactions, which must be considered as a favorable green chemistry procedure because at least three or more components react to directly yield a unique product, incorporating the atoms of the starting materials with great atomic efficiency [19]. In other words, MCRs are highly flexible, chemoselective, convergent, and atom-efficient processes of high exploratory influence [20–22]. It is important to note that it is not necessary to isolate the reaction intermediates, making the complete procedure sustainable. In addition, the multicomponent reaction, also known as a “one-pot” process, is a valuable tool in green organic synthesis, with several advantages: reducing the number of steps and consequently simplifying the purification procedure, enhancing the corresponding synthetic efficiency, and conserving solvents and resources. Furthermore, these features are in general agreement with several of the twelve principles (protocols) of green chemistry [23] such as prevention (principle 1), where the formation of by-products in the reaction is minimized; atom economy (principle 2), where the target methodology is designed in order to maximize the incorporation of atoms added to the reaction into the final product; safer solvents and auxiliaries (principle 5), where there is no need to use solvents for intermediary purification, thereby avoiding the generation of waste; design for energy efficiency (principle 6), where the use of alternative modes of reaction activation such as microwave or infrared irradiation leads to shorter reaction times and consequently decreased energy consumption compared to a typical thermal activation (mantle heating); reduced derivatization (principle 8), where unnecessary derivatization is avoided (use of blocking groups, protection/deprotection, and temporary modification of physical/chemical processes); and catalytic processes (principle 9), where, in many cases, ecofriendly catalysts are employed.

As part of our ongoing research program, we are interested in green chemistry protocols for the production of novel hybrid heterocyclic molecules in order to synergize or modify the pharmacological activity of the prototypes, mainly by using microwave or near infrared irradiation as the activating source in the absence of solvent and by using natural nontoxic catalysts [24–29], with particular attention to Tonsil Actisil FF (TAFF) [30, 31].

In accordance with our research interests, in this work, we discuss the use of a 3MCR in the presence of green reaction conditions (Scheme 2) for three new hybrid molecules introduced in this paper (5–7). We used two moles of indole as substrates and ortho-, meta-, or paraformylphenylboronic acid (2–4) in the absence of solvent, in the presence or absence of a bentonitic clay catalyst, and with microwave irradiation. We evaluated the products in the presence of six different cancer cell lines and analyzed these products using a density functional theory computational analysis in order to generate a complete characterization of products 5–7.

2. Materials and Methods

All solvents and reagents were purchased from Sigma-Aldrich Chemical Co. and were used without further preparation. The reactions were monitored by TLC on percolated Merck silica-gel 60–F~254~ aluminum sheets. In general, the product visualization was achieved using a UV lamp or I. The employed catalyst, bentonitic clay was purchased commercially as Tonsil Actisil FF (TAFF) from Tonsil Mexicana S.A. of C.V. Km 7 High road Puebla-Tlaxcala, Puebla, México. Melting points, uncorrected, were determined using a Fisher-Jones Scientific apparatus. Reactions were run in a chemical microwave oven MIC-1 SEV. Elemental analysis was carried out using a Vario ELIII ELEMENTAR analyzer. The IR spectra were obtained from a Perkin-Elmer 100 spectrophotometer spectrum with zinc selenide ATR by direct measurement. The NMR spectra were recorded using a Varian VNMRS-500 spectrometer at 500 and 125 MHz for $^1$H and $^{13}$C, respectively, using TMS as the internal standard and CO(CD$_3$)$_2$ as the solvent. The multiplicities are reported as singlet (s), broad singlet (bs), doublet (d), and triplet (t). For $^{11}$B NMR, the corresponding spectra were obtained using a Varian Unity 300 spectrometer at 96 MHz using BF$_3$-Et$_2$O as the internal standard and CO(CD$_3$)$_2$ as the solvent. The mass spectrometry analyses (EI, FAB’, CI, and HRMS) were performed with a JEOL MSStation JMS-700 mass spectrometer. The HRMS-FAB and FAB measurements by positive mode ionization (+) were performed at 25°C, 6 KeV of FAB energy, 10 mA of emission current, and 10 kV of acceleration voltage, using Xe as the ionization gas and thioglycerol as the matrix and PEG (poly (ethylene glycol)) mixtures from 200 to 800 as internal mass references at resolutions of 10 000, 20 000, 30 000, and 45 000. The accurate mass was calculated as the mean value of the data measured in 5-6 scans as determined from the top and centroid mass of the ion signals. The elemental compositions

![Scheme 1: Formation of 3,3'-diindolylmethane (DIM).](image-url)
were calculated within a mass range of ±10 ppm from the measured accurate mass.

2.1. General Procedure for the Synthesis of Borated DIMs. A mixture of 1 mmol (120 mg) of indole and 0.5 mmol (80 mg) of ortho-, meta-, or paraformylphenylboronic acid (100 mg) with or without TAFF was placed in round bottom flask compatible with MWS. The mixture was subjected to MW-irradiation at 504 W and agitated at 80 ppm at a temperature of 100°C over 5 minutes. The reactions were monitored by TLC using n-hexane/ethyl acetate (80:40) as eluent. The purification of the products was carried out with column chromatography using the same mobile phase as the TLC.

2-(Di(1H-indol-3-yl)methyl)phenylboronic acid (5): yield: 46%; mp: 140–146°C; elemental analysis calculated for C23H18N2O2 B, C, 75.02%; H, 5.75%; N, 7.61%; O, 8.69%; B, 2.34%. Found C, 76.32%; H, 6.21%; N, 7.73%; O, 6.49%*; B, 3.246% (values determined by the differences between two oxygens and one boron in approximately 10% of the remainder).1H NMR (acetone-d6/TMS) (δ/ppm): 9.91 (s, 2H, NH), 7.42 (d, 2H, H-4,4′, J = 8 Hz), 7.35 (d, 2H, H-7,7′, J = 8 Hz), 7.26 (d, 1H, H-16, J = 7.5 Hz), 7.18 (t, 1H, H-15, J = 8 Hz), 7.14 (t, 1H, H-14, J = 7.5 Hz), 7.05 (t, 2H, H-6,6′, J = 7 Hz), 6.86 (2H, H-5,5′, J = 7 Hz), 6.82 (s, 2H, H-2,2′), 6.69 (s, 1H, H-10), 6.62 (d, 1H, H-13), 7.63-6.70 (s, 2H, OH, over posed for all ArH). 13C NMR (acetone-d6/TMS) (δ/ppm): 149.9 (C-11), 137.2 (C-9,9′), 133.6 (C-13), 128.6 (C-15), 128.1 (C-16), 127.4 (C-8,8′), 124.8 (C-14), 124.0 (C-2,2′), 121.1 (C-6,6′), 119.8 (C-3,3′), 119.7 (C-4,4′), 118.3 (C-5,5′), 111.1 (C-7,7′), 38.5 (C-10), C-ipsos to boron not observed.11B NMR (acetone-d6/BF3-Et2O) (δ/ppm): 30.19 (bs), trivalent boron. IR (KBr) cm−1: 3405 (OH), 1731 (C=O), 1456 (C≡N), 1362 (B-O), 1092 (B-C). FAB′MS (6 KeV) (thioglycerol as matrix): artifact m/z 437(61) [M + C2H5S]+, m/z 366 M* not observed, m/z 322(100) [M–BOH]+, m/z 245(15) [M–C6H5BO2]+, m/z 206(34) [M–C6H5BO2]+, m/z 117(17) [M–C15H12BN2O]+; CIMS (methane as carrier gas): m/z 366 M* not observed, m/z 322(9) [M–BOH]+, m/z 245(3) [M–C6H5BO2]+, m/z 206(17) [M–C6H5BO2]+, m/z 117(100) [M–C15H12BN2O]+, EIMS (70 eV): m/z 366 M* not observed, m/z 338(3) [M–BOH]+, m/z 245(69) [M–BOH]+, m/z 245(10) [M–C6H5BO2]+, m/z 206(24) [M–C6H5BO2]+, m/z 117(100) [M–C15H12BN2O]+; HRMS-FAB′, m/z 437 [M + C6H5S]+, C26H24N2O8BS: observed 437.1489 Da/calculated 437.1495 Da (+1.3 ppm error). Other assignments validated by HRMS-EI: m/z 338, for C23H18N2O3: observed 338.1414 Da/calculated 338.1419 Da (+0.3 ppm error); m/z 322 for C23H18N2: observed 322.1464 Da/calculated 322.1470 Da (−0.8 ppm error); m/z 245 for C12H12N2: observed 245.1073 Da/calculated 245.1079 Da (+3.8 ppm error); m/z 206 for C12H12N2: observed 206.0964 Da/calculated 206.0970 Da (−1.4 ppm error); m/z 117 for C6H12N2: observed 117.0573 Da/calculated 117.0631 Da (+4.9 ppm error).

Scheme 2: Production of borondiindolylmethanes.
245.1073 Da/calculated 245.1079 Da (+3.8 ppm error); m/z 206 for C_{15}H_{12}N_{1}: observed 206.0964 Da/calculated 206.0970 Da (−1.4 ppm error); m/z 117 for C_{6}H_{5}N_{1}: observed 117.0573 Da/calculated 117.0631 Da (+4.9 ppm error).

4-(Di(1H-indol-3-yl)methyl)phenylboronic acid (7): yield: 61%; mp: 190–195 °C; elemental analysis calculated for C_{23}H_{21}O_{3}N_{4}B C, 75.02%; H, 5.75%; N, 7.61%; O, 6.89%; B, 2.34%. Found C, 76.32%; H, 6.21%; N, 7.73%; O, 6.49%. B: 3.24% (values determined by the differences between two oxygens and one boron in approximately 10% of the remainder). \(^1\)H NMR (acetone-d$_6$/TMS) (δ/ppm): 9.98 (s, 2H, NH), 7.80 (d, 2H, H-13, H-14, J = 8 Hz), 7.38 (m, 6H, H-12, H-16, H-4', and H-7', J = 7 Hz), 7.06 (t, 2H, H-6', J = 7 Hz), 6.89 (t, 2H, H-5,5', J = 8 Hz), 6.71 (s, 2H, H-2,2'), 5.93 (s, 1H, H-10), 5.90 (s, 1H, H-10), 7.81–6.81 (s, 2H, OH, signal over -N=), 6.89 (t, 2H, H-5,5', J = 8 Hz), 7.06 (t, 2H, H-6', J = 7 Hz), 6.89 (t, 2H, H-5,5', J = 8 Hz), 6.71 (s, 2H, H-2,2'), 5.93 (s, 1H, H-10), 7.81–6.81 (s, 2H, OH, signal over -N=), 6.89 (t, 2H, H-5,5', J = 8 Hz), 7.06 (t, 2H, H-6', J = 7 Hz), 6.89 (t, 2H, H-5,5', J = 8 Hz), 6.71 (s, 2H, H-2,2').

Other peaks validated by HRMS-EI (70 eV): 3407 (OH), 1720 (C=C), 1456 (C=N), 117 (100) [M–C=O] m/z 117, 113 (C≡N), 110 (C≡N), 100 (C≡N), 99 (C≡N). HRMS-MS (6 KeV) (thioglycerol as matrix): artifact [M + C_{6}H_{5}Si]⁺, m/z 366 M⁺ not observed, m/z 322(100) [M–BO_{2}H]⁺, m/z 245(85) [M–C_{6}H_{5}BO]⁺, m/z 206(25) [M–C_{6}H_{5}BNO]⁺, m/z 117(10) [M–C_{6}H_{5}BNO_{2}]⁺. CIMS (methane as carrier gas): m/z 366 M⁺ not observed, m/z 322(83) [M–BO_{2}H]⁺, m/z 245(57) [M–C_{6}H_{5}BO]⁺, m/z 206(29) [M–C_{6}H_{5}BNO]⁺, m/z 117(100) [M–C_{6}H_{12}BNO_{2}]⁺. EIMS (70 eV): m/z 366 M⁺ not observed, m/z 338(18) [M–BOH]⁺, m/z 322(100) [M–BO_{2}H]⁺, m/z 245(56) [M–C_{6}H_{5}BO]⁺, m/z 206(9) [M–C_{6}H_{5}BNO]⁺, m/z 117(20) [M–C_{6}H_{12}BNO_{2}]⁺. HRMS-FAB⁺, m/z 437 [M + C_{6}H_{5}Si]⁺, C_{26}H_{29}N_{2}O_{8}BS: observed 437.1489 Da/calculated 437.1495 Da (+1.3 ppm error). Other peaks validated by HRMS-El (70 eV): m/z 338 for C_{23}H_{21}O_{3}N_{4}B observed 338.1414 Da/calculated 338.1419 Da (+3.0 ppm error); peak m/z 322 for C_{23}H_{21}O_{3}B_{2}N_{2} observed 322.1464 Da/calculated 322.1470 Da (−0.8 ppm error); m/z 245 for C_{17}H_{12}N_{2} observed 245.1073 Da/calculated 245.1079 Da (+3.8 ppm error); m/z 206 for C_{15}H_{12}N_{1} observed 206.0964 Da/calculated 206.0970 Da (−1.4 ppm error); m/z 17 C_{6}H_{3}N_{1} observed was 117.0573 Da/calculated 117.0631 Da (+4.9 ppm error).

Then, aqueous tris base [tris(hydroxymethyl)aminoethane] (100 mM, 10 mM) was added to each well to solubilize the cell-bound dye, and the absorbance at 515 nm was measured. The results are expressed as a percentage of control cell growth.

2.3. Computational. All the calculations reported here were carried out using density functional theory [32, 33] employing Becke’s three parameters with Lee-Yang-Parr correlation functional (B3LYP) [34, 35] in conjunction with 6-311++G(d, p) basis sets [36–39] using a Gaussian computational program [40]. The same level of wave function analysis was used to calculate the molecular electrostatic potential (MESP). For the estimation of the probable reactive sites in the systems studied, the MESP descriptor was employed; sites corresponded to the electric density, which can thus be regarded as another fundamental determinant of atomic and molecular properties. MESP has proven to be useful in rationalizing interactions between molecules and in molecular recognition processes because the electrostatic forces are primary responsible for long-range interactions [41]. MESP has largely been used as a molecular descriptor of the chemical reactivity of a number of biological systems which take part in both electrophilic and nucleophilic reactions, in addition to hydrogen bonding interactions [41, 42]. The MESP was rigorously calculated using (1), where \( \rho \) is the charge on the nucleus A, located at \( R_A \), and \( \rho(r) \) is the electron density [43]:

\[
V(r) = \sum_A \frac{Z_A}{|r-R_A|} - \int \frac{\rho(r') \, d^3r'}{|r-r'|}. \tag{1}
\]

In general, electron-dense regions are expected to show high negative MESPs, whereas electron deficient regions are characterized by positive (\( V_{\text{Max}} \)) MESPs [44]. The most negatively (\( V_{\text{Max}} \)) valued point in electron-rich regions can be obtained from the MESP topography calculation [45, 46].

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield (%) with catalyst(^b)</th>
<th>Yield (%) without catalyst(^b)</th>
<th>Melting point (°C)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>20</td>
<td>46</td>
<td>140–146</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>41</td>
<td>180–184</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>61</td>
<td>190–195</td>
</tr>
</tbody>
</table>

\(^{a}\)These results are the averages of five experiments using 504 Watts of power over five minutes at 100°C. \(^{b}\)The reactions were carried out without solvent. \(^{c}\)These values are uncorrected.

2.2. Cytotoxicity Assays of Borated DIMs. One hundred microliters of each cell line was added in duplicate to each of two 96-well plates marked as cell line or control cell. A third plate was used as another control (zero time evaluation), in which was placed equal volumes of cell line and control cell. Each plate was incubated for 24 hours at 37°C under an atmosphere containing 5% CO\(_2\). Subsequently, 100 µL of reaction products, dissolved in DMSO (0.5%) as was added to the first two plates. The plates were incubated for 48 hours under the same conditions as above. After 48 h, cell culture was fixed in situ by adding 50 µL of cold 50% trichloro acetic and incubated 60 min at 4°C. The supernatant was discarded, and the plates were washed with water. Cultures fixed were stained for 30 min with 100 µL of 4% SRB solution.

Table 1: Production of boron-diindolylmethanes assisted by microwave\(^a\).

3. Results and Discussion

3.1. Synthesis. Table 1 summarizes the main results corresponding to the production of novel hybrid molecules 5–7 in moderate yields (41–61%) within very low reaction time (5 minutes). The TAFF was shown to be unnecessary because higher yields were obtained without it. This is likely due to the presence of a boronic acid moiety that must promote a 3MCR between two moles of indole and a mole
of ortho-, meta-, or paraformylphenylboronic acid (2–4) in the absence of solvent. Additionally, several attempts to activate the reaction using typical mantle heating or near infrared irradiation were found to be ineffective, using the same temperature and longer reaction times and also in the absence of solvent. To our knowledge, this is the first time that this mode to produce diindolylmethanes is considered as a multicomponent reaction.

3.2. Spectroscopic Attributions. Nuclear Magnetic Resonance. In general, the $^1$H NMR spectra of the target molecules 5–7 exhibited an expected singlet between 5.91 and 6.69 ppm, corresponding to the benzylic proton, and a singlet (9.91–9.98 ppm) unequivocally assigned to the proton attached to the nitrogen of the indole moiety. In addition, a signal (two hydrogens) between 6.70 and 8.00 ppm was appropriately assigned to the protons of the hydroxyl groups of the boronic moiety; these signals are overlapped by the aromatic signals of both the indole and phenyl rings; complementarily, see, for example, Figure 1, where complete $^1$H assignments of 5 are involved. An interesting observation was the absence of signals between 10.02 and 10.18 ppm, indicating the disappearance of the hydrogen of aldehyde groups of 2–4. In regard to the corresponding $^{13}$C NMR spectra, the signal between 38.5 and 41.3 ppm was assigned to the respective benzylic carbon. It is worth noting that the signal corresponding to the carbon ipso to the boron atom was not observed, perhaps as a result of a quadrupole effect. In addition, the absence of a signal between 193.6 and 194.4 ppm clearly indicated the disappearance of the aldehyde groups of 2–4;

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**Figure 1:** $^1$H NMR (acetone-$d_6$/TMS) of 5 determined at 500 MHz.

**Figure 2:** $^{13}$C NMR (acetone-$d_6$/TMS) of 5 determined at 125 MHz.
moreover, the expected $^{13}$C NMR patterns for the acrylic and indolic moieties were also observed in the experimental data for these novel molecules, as it can be seen in Figure 2, an example where complete assignments are offered for the target compound 5. In the $^{11}$B NMR spectra, compounds 5–7 exhibited broad signals corresponding to trivalent boron at 30.19, 28.66, and 28.60 ppm, respectively.

**Infrared Spectrophotometry.** The respective IR spectra exhibited interesting bands at 1362, 1336, and 1337 cm$^{-1}$, arising from B–O. In addition, bands from B–C at 1092 cm$^{-1}$ for 5, 1090 cm$^{-1}$ for 6, and 1092 cm$^{-1}$ for 7 correspond to the boronic moiety in the target compounds. In addition, the expected vibrations arising from C=O and C=N functions are overlapped, as suggested by the corresponding experimental data.

**Mass spectrometry.** Several attempts to determine the expected molecular ions of the target molecules 5–7 using EI, CI, and FAB$^+$ (glycerol, m-nitrobenzylalcohol, and octyloxybenzoinene) were unsuccessful; however, common and appropriate fragmentation patterns were detected, as mentioned in the experimental section. Therefore, due to our recent knowledge that the thioglycerol matrix could yield interesting interactions [47, 48], we considered that the sulfur atom inside the matrix could produce artifacts by means of a Lewis interaction, with the empty orbital of the boron atom in the molecules of interest (sometimes known as quasimolecular ions). In this case, m/z 437 [M + $\text{C}_2\text{H}_5\text{S}$]$^+$ corresponds to the molecules; many other common peaks were also observed: m/z 338 [M–BOH]$^{+1}$, for $\text{C}_{23}\text{H}_{18}\text{N}_2\text{O}_4$, observed 338.1419 Da/calculated 338.1419 Da (+3.0 ppm error), m/z 322 [M–BO$_2$H]$^{+1}$, for $\text{C}_{23}\text{H}_{18}\text{N}_2$, observed 322.1464 Da/calculated 322.1470 Da (−8.0 ppm error), m/z 245 [M–$\text{C}_8\text{H}_8\text{BO}_2$]$^+$, for $\text{C}_{17}\text{H}_{13}\text{N}_2$, observed 245.0737 Da/calculated 245.1079 Da (+3.8 ppm error), m/z 206 [M–$\text{C}_8\text{H}_8\text{BNO}_2$]$^+$, for $\text{C}_{15}\text{H}_{12}\text{N}_1$: observed 206.0964 Da/calculated 206.0970 Da (−1.4 ppm error) and m/z 117 [M–$\text{C}_{13}\text{H}_{12}\text{BNO}_2$]$^{+1}$; for $\text{C}_9\text{H}_7\text{N}_1$, observed 117.0573 Da/calculated 117.0631 Da (+4.9 ppm error). Consequently, the predicted structures were unequivocally confirmed by obtaining the elemental compositions using high resolution mass spectrometry, as shown in Figure 3.

### Figure 3: FAB$^+$ mass spectra and accurate mass data of adduct from 6.

3.3. Cytotoxicity of Borated DIMs. The target borated-DIMs compounds 5–7 were evaluated against six cell lines, and these results are summarized in Table 2. Only those molecules that inhibited more than 50% of cell growth were considered active. Thus, the corresponding inhibition growth values of 5 indicate that it is active only in the presence of cell lines PC-3 and MCF-7; on the other hand, 6 displayed activities in the cell lines PC-3, HCT-15, and SKLU-1 and an even higher activity for MCF-7; conversely, 7 was inactive in all cell lines studied. Additionally, it is worth noting that 5 and 7 were entirely inactive against cell line K562.

When comparing the results of percentage of growth inhibition of compounds 5, 6, and 7 versus the control (5-fluorouracil), it was observed that the percentage of growth inhibition by compound 5 was higher only in cell lines MCF-7 (60.27%) and PC-3 (62.79%); on the other hand, 6 displayed activities in the cell lines PC-3, HCT-15, and SKLU-1 and an even higher activity for MCF-7; conversely, 7 was inactive in all cell lines studied. Additionally, it is worth noting that 5 and 7 were entirely inactive against cell line K562.
Figure 4: Optimized geometries (electronic, enthalpy, and Gibbs free energy, described in Hartrees and kcal/mol) for the three lowest energy conformers: 5-ct (ortho), 6-ct (meta), and 7-ct (para). MESP of 5-ct, 6-ct, and 7-ct showing the minima ($V_{\text{min}}$: negative) and maxima ($V_{\text{max}}$: positive) of electron densities, in kcal/mol. All calculations were obtained by DFT calculations at the B3LYP/6-311++G** level.

Table 2: Cytotoxicity of 5–7 in cancer lines cellular.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Growth inhibition by cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td>U251</td>
<td>PC-3</td>
</tr>
<tr>
<td>5</td>
<td>57.00</td>
</tr>
<tr>
<td>6</td>
<td>36.29</td>
</tr>
<tr>
<td>7</td>
<td>49.79</td>
</tr>
</tbody>
</table>

U251: gli of central nerve system; PC-3: prostate; K562: leukemia; HCT-15: colon; MCF-7: breast; SKLU-1: lung; WA: without activity.

Changes in the activity of the target molecules in the studied cell lines.

3.4. Computational Chemistry. Figure 4 shows the obtained optimized geometries of the three lowest energy conformers, corresponding to target molecules 5, 6, and 7. A main attribute between each lowest energy conformer is that they could be trans-trans (tt), cis-cis (cc), or trans-cis (tc) with respect to the hydroxyl groups and the B–C bond, as displayed at the top of the figure. The three cis-trans (ct) conformations were found to be the most stable, with both hydrogen atoms in the O–B–O plane. In other words, in the lowest energy conformer of 5–7, the (OH)₂ system is planar, lying in the plane of the benzene ring. Furthermore, for each lowest energy conformer, the corresponding molecular electrostatic potential (MESP) values were calculated in order to estimate the probable reactive sites of the target molecules: electrophilic (+: blue) and nucleophilic (−: yellow). These electrostatic maps, obtained from the equilibrium conformation, display electropositive zones around the hydrogen atoms: those supported on N₁ and N₂ and those linked to the oxygen atoms O₁ and O₂ belonging the boronic acid group. These zones correspond to the electronic deficiency of the hydrogens. It is also worth noting that, in these sites in 5–7, both the boronic and the indolylic moieties are positioned to form hydrogen bonds.

According to the $V_{\text{min}}$ (−26 to −34 kcal/mol) values of 5-ct, 6-ct, and 7-ct determined by using (1) mentioned in the methodology, the nucleophilic portions of 5–7 are located...
in the aromatic rings. Correspondingly, the $V_{\text{max}}$ (+42 to +56 kcal/mol) values of 5–7ct, corresponding to electrophilic centers, are located at the hydrogen atoms. At the bottom of the figure, the electron energy ($E$), enthalpy ($H$), and Gibbs free energy ($G$) values suggest that the most stable conformers occur when both OH groups are cis-trans (ct); comparing these values, the most stable systems correspond to the para-regioisomer.

4. Conclusions

The significance of the multicomponent reaction in the “Green Chemistry Protocol” is emphasized. A green approach to the production of three novel hybrid diindolylmethanes-phenylboronic acids via a 3MCR was performed. The reaction was performed using solventless conditions, microwave irradiation to activate the reaction, and no catalyst. The target molecules were appropriately characterized by spectroscopic methods. During the FAB$^+$ MS determinations, various artifacts appeared in the corresponding spectra; this, in addition to the accurate mass determination of these adducts, unequivocally validates the presence of the target products. The target molecules were evaluated in the presence of six cancer cell lines, and the meta-regioisomer was found to be the most active. Furthermore, the products were analyzed using computational chemistry in order to determine their most stable geometries as well as their reactivities by computing their molecular electrostatic potentials. To our knowledge, this is the first time a multicomponent reaction has been used to produce diindolylmethanes.

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