Quantitative Structure-Activity Relationship of Morita-Baylis-Hillman Adducts with Leishmanicidal Activity

Rodrigo Octavio M. A. de Souza*, José C. Barros, Joaquim F. M. da Silva, and Octavio A. C. Antunes

E-mail: rodrigosouza@iq.ufrj.br

* Author for correspondence and reprint requests

Z. Naturforsch. 66c, 136–142 (2011); received June 18/November 23, 2010

Dedicated to the memory of Professor Octavio Antunes and his family

A quantitative structure-activity relationship model for Morita-Baylis-Hillman adducts with leishmanicidal activities was developed which correlates molecular orbital energy and dipole with percentage in the promastigote stage.

Key words: Leishmaniasis, Morita-Baylis-Hillman, QSAR

Introduction

Leishmaniasis are parasitic diseases in humans that are caused by 20 different pathogenic species belonging to the genus *Leishmania*, a protozoon transmitted by the bite of phlebotomine sand flies. They are among the “most neglected diseases” (Trouiller et al., 2002; Yamey, 2002), currently threatening 350 million people in 88 countries around the world, and are considered to be a major public health problem in many developing countries with an estimated 12 million people presently infected (Feasey et al., 2010; Reithinger, 2008; WHO, 2010). Leishmaniasis disproportionately hit poor and marginalized populations (Morel et al., 2009) and display a wide range of clinical symptoms that depend upon the form of the disease including skin ulceration, damage to the internal organs, and anemia. Because of this, the diseases have traditionally been classified in four different clinical forms, visceral (VL), cutaneous (CL), diffuse cutaneous (DCL), and mucocutaneous (MCL) leishmaniasis, respectively, which have different immunopathologies and degrees of morbidity and mortality (Herrwaldt, 1999). Disfigurement, disability, social and psychological stigma are all severe consequences of the disease (WHO, 2007), and so far there no vaccine has been approved for clinical use.

Amastigotes are obligate intracellular parasites of macrophages (and rarely of other cell types), where they survive and multiply within the phagolysosome compartment. Toxicity and resistance to the pentavalent antimonials, which have been the mainstay of treatment of both VL and CL during the last 60 years, are critical problems (Croft et al., 2006). Although new drugs have become available in recent years, including lipid formulations of amphotericin B, the oral drug miltefosine for VL, and topical paromomycin for CL, these are not entirely satisfactory due to high cost, reported side effects, ineffectiveness, and HIV-coinfection (Croft and Coombs, 2003; Valderrama et al., 2005; Chappuis et al., 2007; Le Pape, 2008; Kedzierski et al., 2009; Cavalli and Bolognesi, 2009). As the currently available chemotherapy for this neglected disease is far from ideal, the search for new safe, affordable, and effective drugs is strongly necessary.

Carbon-carbon bond formation is one of the most important and powerful reactions in synthetic organic chemistry and, therefore, has been a challenging area of major interest in chemistry. The Morita-Baylis-Hillman (MBH) reaction is one such interesting reaction, which involves the selective atom-economical construction of a carbon-carbon bond, between an electrophile and the α-position of an activated alkene under catalysis of a tertiary amine providing densely functionalized molecules (Scheme 1) (Basavaiah et al., 2007; Ma et al., 2009).

Recently several examples of Baylis-Hillman adducts with biological activity have appeared in the literature among which we highlight: antifun-
gal activity, antimicrobial activity, enzyme inhibition activity, chloramphenicol-like activity, and leishmanicidal activity. We have shown that these compounds can act as selective leishmanicidal drugs by evaluating their pharmacological activities with respect to the percentage of amastigote inhibition (AMAST), percentage of promastigote inhibition (PROMAST), and nitric oxide production (NITRITE) (Souza et al., 2007). These compounds discussed in our previous study (Fig. 1) were not new but they could be prepared by a simple and efficient one-pot reaction, and even though some high-throughput screening (Siqueira-Neto et al., 2010) and quantitative structure-activity relationship (QSAR) studies of antileishmanicidal compounds have been reported so far (Costa et al., 2003; Gerpe et al., 2006; Oliveira et al., 2003; Sarciron et al., 2005; Bhattacharjee et al., 2002; Hemmateenejad et al., 2007; Guido et al., 2008; Oliveira and Takahata, 2008; Andrighetti-Fröhner et al., 2009); molecular modeling studies for MBH adducts are still absent. Here, we wish to disclose our results concerning a QSAR study of our previously synthesized adducts as leishmanicidal agents.

Scheme 1. General route to MBH adducts studied.

EWG = COOCH$_3$ or CN
R$^1$ = aryl or heteroaryl
R$^2$ = H or Ac

Fig. 1. Compounds studied.
**Results and Discussion**

Firstly, we evaluated several parameters including physico-chemical, electronic, steric, and quantum mechanical properties of the synthesized compounds (Fig. 1). As we have three different pharmacological activities, we screened several correlations between the logarithm or negative logarithm of the measured biological activities and then computed parameters in a stepwise multiple linear regression (MLR) using SPSS v.16. We could not find correlations between AMAST and NITRITE and the evaluated parameters.

When the pharmacological data pPROMAST \((p = -\log)\) was used as dependent variable, we succeeded in obtaining correlation with computed properties. However, a simple screening of the experimental activities of the compounds indicated that compound 9 was the least active compound and was located outside the range of the others. So we discharged this compound before the model fit. We attributed this difference to the presence of an acidic phenolic hydrogen atom in this compound.

An MLR analysis of pPROMAST resulted in three models, 1–3. As we had prepared 15 compounds \((n)\) we chose models comprising up to three parameters in order to minimize coincidental correlations (Van De Waterbeemd, 1995):

\[
p\text{PROMAST} = 0.526 \pm 0.569 + 5.868 \pm 1.577 \text{HOMO Energy}_{-}\text{PM3} \\
(1)
\]

\[
n = 14; R = 0.732; R^2 = 0.536; R^2_{\text{adj}} = 0.497; \\
s = 0.13006; F = 13.839; p = 0.0029; \\
Q^2 = 0.5459; Q^2_{\text{adj}} = 0.5459; S_{\text{PRESS}} = 0.0371; \frac{\text{PRESS}}{\text{SSY}} = 0.4541.
\]

\[
p\text{PROMAST} = 0.314 \pm 0.481 + 5.551 \pm 1.318 \text{HOMO Energy}_{-}\text{PM3} + 0.045 \pm 0.018 \text{Jurs-RPCS} \\
(2)
\]

\[
n = 14; R = 0.840; R^2 = 0.706; R^2_{\text{adj}} = 0.652; \\
s = 0.10818; F = 13.176; p = 0.0012; \\
Q^2 = 0.7142; Q^2_{\text{adj}} = 0.6904; S_{\text{PRESS}} = 0.0321; \frac{\text{PRESS}}{\text{SSY}} = 0.4368.
\]

\[
p\text{PROMAST} = 0.779 \pm 0.362 + 6.777 \pm 0.988 \text{HOMO Energy}_{-}\text{PM3} + 0.049 \pm 0.013 \text{Jurs-RPCS} + 0.049 \pm 0.014 \mu_s \\
(3)
\]

\[
n = 14; R = 0.932; R^2 = 0.868; R^2_{\text{adj}} = 0.829; \\
s = 0.07584; F = 21.999; p = 0.0001; \\
Q^2 = 0.8733; Q^2_{\text{adj}} = 0.8502; S_{\text{PRESS}} = 0.0235; \frac{\text{PRESS}}{\text{SSY}} = 0.1267.
\]

### Table I. Coefficient correlation matrix for models 1–3.

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
<th>HOMO Energy_PM3</th>
<th>Jurs-RPCS</th>
<th>(\mu_s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HOMO Energy_PM3</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>HOMO Energy_PM3</td>
<td>1.000</td>
<td>-0.096</td>
<td>1.000</td>
</tr>
<tr>
<td>3</td>
<td>HOMO Energy_PM3</td>
<td>1.000</td>
<td>-0.057</td>
<td>1.000</td>
</tr>
</tbody>
</table>

For models 1–3, a coefficient correlation matrix was compiled showing no interdependency between the parameters employed (Table I).

In models 1–3, HOMO Energy_PM3 represents the energy of the highest occupied molecular orbital calculated by semi-empirical PM3 level of theory and measured in a.u. The Jurs-RPCS is a relative positively charged surface area descriptor (Stanton and Jurs, 1990), which can ultimately be connected to hydrophobicity (Leffler and Grunwald, 1963, cited in Cronin and Livingstone, 2004), and \(\mu_s\) is the \(x\)-component of the dipole moment measured in debye. The molecular orbital energies (Costa et al., 2003; Valderrama et al., 1999), the dipole moment (Andrighetti-Fröhner et al., 2009), and several hydrophobicity and steric descriptors like Jurs-RPCS have already been reported in antileishmaniasis QSAR studies. These computed properties can be found in Table II.

The comparison between models 1–3, which reveal different degrees of freedom, can be evaluated by the adjusted squared correlation coefficient \(R^2_{\text{adj}}\), indicating that model 3 provides the best fit and has both the lower standard error of the estimate \(s\) and \(p\)-value (< 0.0001).

In order to determine the predictability of our models, a leave-one-out cross-validation procedure was implemented which generated the predicted variance \(Q^2\) and \(Q^2_{\text{adj}}\) and the predicted residual sum of squares \(\text{PRESS}\). To be a reasonable model, the ratio \(\frac{\text{PRESS}}{\text{SSY}}\) (SSY being the total sum of squares) should be less than 0.4 (Agrawal et al., 2006), the cross-validated \(R^2\) \((Q^2\) and \(Q^2_{\text{adj}}\) should be high (Van De Waterbeemd, 1995), and the standard deviation of the sum of the square of the difference between predicted and observed values \(S_{\text{PRESS}}\) should be low (Gaudio and Zandonade, 2001). All of the statistical parameters tested indicated that model 3 is the best model.

In a search for outliers, we compiled a residues table for models 2 and 3. The results are shown.
in Table III, and compound 12 was considered an outlier for both models as the residual value exceeded twice the standard error of the residues (Jamloki et al., 2006). We attributed this behaviour of 12 to the presence of a bulky halogen atom (Br), having no other analogue in our compound set.

Upon removing the outlier, the models 4 and 5 were obtained:

\[
p\text{PROMAST} = 0.795 \pm 0.334 + 6.626 \pm 0.915 \text{ HOMO Energy_PM3} + 0.029 \pm 0.013 \mu_x \tag{4}
\]

\[
p\text{PROMAST} = 0.730 \pm 0.242 + 6.600 \pm 0.661 \text{ HOMO Energy_PM3} + 0.037 \pm 0.010 \mu_x + 0.031 \pm 0.010 \text{Jurs-RPCS} \tag{5}
\]
The correlation coefficient matrix and the residues tables for the new models were compiled showing no interdependency of the parameters and no outliers (Tables IV and V).

Besides the fact that model 5 possesses the better statistical values, this model requires three parameters for adjustment of 13 compounds, which could introduce coincidental correlations. As model 4 was observed to give good correlations with only two descriptors, this model could be interpreted as the best by implying the principle of parsimony (Ockham's Razor). Therefore, a graphical representation of experimental vs. predicted pPROMAST activities was evaluated (Fig. 2) for model 4.

In order to support future compound synthesis, and aided by model 4, we compared compounds 1 vs. 3, 1 vs. 6, and 7 vs. 15, and concluded that compounds substituted by electron withdrawing groups (EWG) in the aromatic moiety would result in better activities due mainly to a decrease in the HOMO energy. Guided by model 5, it is possible to understand that a change from the hydroxy to the more hydrophobic acetyl group results in a decrease in Jurs-RPCS, thus contributing to lower pPROMAST and conferring better activity (compounds 3 vs. 5). Our model could not be used to decide between nitriles and methyl esters of MBH adducts (compounds 1 vs. 2, 3 vs. 4, or 6 vs. 7), as it was not possible to see a general trend in $\mu_i$ when comparing the two classes of compounds.

**Conclusion**

We have been able to develop a QSAR model for some Morita-Baylis-Hillman adducts show-

---

**Table IV. Coefficient correlation matrix for models 4 and 5.**

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
<th>HOMO Energy_PM3</th>
<th>$\mu_i$</th>
<th>Jurs-RPCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>HOMO Energy_PM3</td>
<td>1.000</td>
<td>0.370</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>$\mu_i$</td>
<td>0.370</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>5</td>
<td>HOMO Energy_PM3</td>
<td>1.000</td>
<td>0.357</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>$\mu_i$</td>
<td>-0.013</td>
<td>0.239</td>
<td>1.000</td>
</tr>
</tbody>
</table>

---

**Table V. Residues table for models 4 and 5.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>(pPROMAST)$_{exp}$</th>
<th>(pPROMAST)$_{pred}$</th>
<th>Residue</th>
<th>(pPROMAST)$_{pred}$</th>
<th>Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1.63</td>
<td>-1.64</td>
<td>0.01</td>
<td>-1.56</td>
<td>-0.07</td>
</tr>
<tr>
<td>2</td>
<td>-1.58</td>
<td>-1.52</td>
<td>-0.05</td>
<td>-1.52</td>
<td>-0.05</td>
</tr>
<tr>
<td>3</td>
<td>-1.67</td>
<td>-1.79</td>
<td>0.12</td>
<td>-1.70</td>
<td>0.03</td>
</tr>
<tr>
<td>4</td>
<td>-1.83</td>
<td>-1.77</td>
<td>-0.05</td>
<td>-1.79</td>
<td>-0.04</td>
</tr>
<tr>
<td>5</td>
<td>-1.77</td>
<td>-1.81</td>
<td>0.04</td>
<td>-1.82</td>
<td>0.05</td>
</tr>
<tr>
<td>6</td>
<td>-1.80</td>
<td>-1.74</td>
<td>-0.06</td>
<td>-1.80</td>
<td>0.00</td>
</tr>
<tr>
<td>7</td>
<td>-1.83</td>
<td>-1.73</td>
<td>-0.10</td>
<td>-1.81</td>
<td>-0.02</td>
</tr>
<tr>
<td>8</td>
<td>-1.41</td>
<td>-1.39</td>
<td>-0.02</td>
<td>-1.42</td>
<td>0.02</td>
</tr>
<tr>
<td>10</td>
<td>-1.48</td>
<td>-1.56</td>
<td>0.08</td>
<td>-1.52</td>
<td>0.04</td>
</tr>
<tr>
<td>11</td>
<td>-1.35</td>
<td>-1.42</td>
<td>0.07</td>
<td>-1.41</td>
<td>0.06</td>
</tr>
<tr>
<td>13</td>
<td>-1.60</td>
<td>-1.62</td>
<td>0.02</td>
<td>-1.63</td>
<td>0.03</td>
</tr>
<tr>
<td>14</td>
<td>-1.51</td>
<td>-1.53</td>
<td>0.02</td>
<td>-1.52</td>
<td>0.01</td>
</tr>
<tr>
<td>15</td>
<td>-1.51</td>
<td>-1.46</td>
<td>-0.05</td>
<td>-1.46</td>
<td>-0.05</td>
</tr>
</tbody>
</table>

2 · SD$_{res}$ = 0.12  2 · SD$_{res}$ = 0.08
ing leishmanicidal activity. The compounds used in this study were readily available through an atom-economic reaction. The best adjustments of pharmacological data were obtained using the parasite in the promastigote stage. The best correlation model used HOMO energy and x-component of dipole moment as descriptors. This study indicates that EWG in the aromatic moiety and acetylated MBH adducts could lead to new compounds with increased activities.

Acknowledgements

The authors are grateful to CAPES, CNPq, FAPERJ, and FINEP for financial support and to Interlab for support in computational facilities.
studies on B-DNA recognition of minor groove bind-
cyclic derivatives. Parasite 12, 251–258.
WHO – World Health Organization (2010), Division of control of tropical diseases. http://www.who.int/leish-