# Antioxidant Activity of Synthetic Resveratrol Analogs: A Structure-Activity Insight

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***Received March 07, 2012: Revised May 09, 2012: Accepted May 09, 2012***

**Abstract:** This study evaluated the antioxidant activity of five resveratrol analogs using the DPPH method. The molecules activity was related with their chemical structure. Besides descriptive statistics, the analysis of variance (ANOVA) followed by Tukey's post hoc test were performed ($p<0.05$). The antioxidant activity of analogs A and B was statistically similar with each other and from the reference standard resveratrol, possibly due to the presence of 4-hydroxy grouping. The aromatic hydroxyl reduces reactive free radicals and produces phenoxyl radical, stabilized by resonance. Although the analog C has shown $IC_{50}$ value statistically different from the resveratrol ($p<0.001$), its antioxidant activity was considered satisfactory. The other analogs (D and E), which have a 4-acid grouping in place of 4-hydroxy grouping, showed lower antioxidant activity than resveratrol ($p<0.001$). Further studies to address possible advantages of analogs in relation to resveratrol should be conducted in order to make them feasible for therapeutic use.

**Keywords:** Analogs, Antioxidant Activity, Chemical Synthesis, Hydroxylation, Resveratrol, Structure-Activity Relationship.

## INTRODUCTION

The continuous production of free radicals during the metabolic processes resulted in the development of antioxidant defense mechanisms, in order to limit the intracellular levels of these reactive species and to control the damage caused by them [1]. The free radicals toxic effects and the cell damages raised by them are responsible for aging process and oxidative stress of the organism [2]. This oxidative stress is considered a major cause for diseases such as cancer, diabetes, Alzheimer and Parkinson diseases, atherosclerosis and rheumatoid arthritis [1-4].

Resveratrol (3,5,4'-trihydroxystilbene) (Figure 1), a natural polyphenol, is a chemopreventive agent which has attracted interest, especially in the last ten years, due to its pharmacological properties. These include antioxidant, anti-inflammatory, antiaging, cardioprotective, neuroprotective and anticancer effects of this compound [5-8]. The search for effective antiproliferative agents has been growing along with the evidence that higher intake of flavonoids would be associated with lower risk of cancer and that apoptosis induction is an important component of chemoprevention of this disease [5].

Resveratrol can be found in at least 70 species of plants, besides being also present in the human diet components such as grapes, berries, nuts and peanuts. It is believed that the high level of this compound in red wine (0.1 to 14.3 mg/L) is connected to low incidence of heart disease in some regions of France [7-8].

Based on the hypothesis that 4-hydroxy grouping, in trans conformation, is required for cell proliferation inhibition and aiming to develop a compound with antiproliferative potential higher than resveratrol, Savio and colleagues (2009) [6] synthesized the analog 4,4'-dihydroxy-*trans*-stilbene (Figure 1). Compared with resveratrol, this analog inhibited the efficiency of fibroblasts clonogenic cell nine times more, but through a different mechanism. Cheng and colleagues (2006) [8] observed that synthetic analogs of resveratrol, with characteristic di-ortho-hydroxy, had antioxidant and cytotoxicity activities against HL-60 cancer cells, greater than resveratrol. Calil and colleagues (2012) [9] evaluated the antioxidant activity of resveratrol analogs and found that the hydroxyl grouping insertion is directly related to the increase of antioxidant activity of compounds.

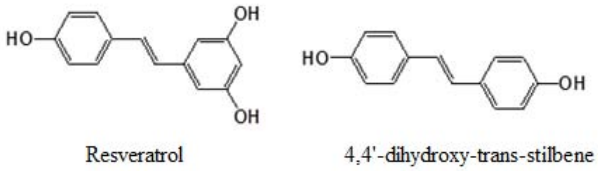


**Fig. (1).** Chemical structure of resveratrol and 4,4'-dihydroxy-*trans*-stilbene.

Thus, the present study aimed to evaluate the antioxidant activity of five synthetic analogs of resveratrol, with or without hydroxyl at position 4 of the aromatic ring, and to relate the molecules activity with their chemical structure.

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1875-628X/12 $58.00+.00 

## MATERIAL AND METHODS

### Chemistry

Five analogs of resveratrol were synthesized in the Laboratory of Chemistry of Federal University of Juiz de Fora (Juiz de Fora, Minas Gerais - Brazil) and their antioxidant activities were evaluated. As a reference standard, resveratrol (*trans*-resveratrol 99.0%, Attivos Magistrais, Brazil) was used (Table 1).

The resveratrol analogs A-E were synthesized by the classical method of imine formation involving condensation between aromatic amine (4-aminophenol and 4-amino-salicylic acid) with a variety of aromatic aldehydes in ethanol [10]. All compounds were characterized by $^{1}H$ and $^{13}C$ NMR I.R. and melting point (Table 1). It was performed in accord with data in the literature [11-12].

The NMR experiments were performed at 300 MHz for $^{1}H$ and 75 MHz for $^{13}C$ in DMSO-$d_6$ (ppm) and I.R. experiments was performed at KBr support ($cm^{-1}$).

## ANTIOXIDANT ACTIVITY

The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DDPH; Sigma-Aldrich, USA) method, described by Sreejayan and Rao (1996) [13], with minor modifications. This method is based on the DPPH reduction in the presence of an antioxidant (AH) a proton donor (H+) for a non-radical (DPPH-H) [14].

A 150 μL of a DPPH ethanolic solution of 0.05 mM were added to 50 μL of ethanol solution of resveratrol analogs at concentrations from 0.97 to 250 μg /mL in 96-well plates. Resveratrol was used as a standard at the same concentrations. Reactions elapsed at room temperature for 30 minutes in the dark and then the absorbance was read in a spectrophotometer (UV mini 1240, Shimadzu®, Japan) (λ= 510 nm). The concentration of DPPH radical inhibition ($IC_{50}$) was calculated using the equation: $IC_{50}(\%) = 100 \times (A_0 - A_s) / A_o$, being $A_o$ absorbance as the negative control and $A_s$ a absorbance of test samples. The $IC_{50}$ value was calculated from the line in the equation of the linear dispersion graph. All tests were performed in triplicate.

## STATISTICAL ANALYSIS

Descriptive statistics and analysis of variance (ANOVA) followed by Tukey's post hoc test were carried out using Statistical Package for Social Sciences (SPSS) version 14.0 for Windows software. It were compared the average values of $IC_{50}$ of resveratrol analogs among themselves and with the standard (resveratrol), assuming a significance level of $p<0.05$.

## CONCLUSION

Results of resveratrol analogs $IC_{50}$ are shown in Table 2. The antioxidant activity of analogs A and B was statistically similar to resveratrol standard ($p=0.109$ and $p=1.000$, respectively) and among themselves ($p= 0.079$).

The use of resveratrol by mammals shows some disadvantages such as low bioavailability and rapid clearance from the circulation. The administration of high doses (above 1g/kg body weight) to improve its effectiveness, can cause an increase of toxic effects risk. According to Baur and Sinclair (2006) [15], some alternatives for improving the resveratrol bioavailability would be the block of its metabolism, the discovery of more potent compounds (which mimic its effects) or the development of analogs with better bioavailability.

Based on previous work in which the olefinic double of resveratrol was replaced by an imino group and the products obtained had significant antioxidant activity [9], it was also changed in such present work the olefinic group by an imino group. Imino groups may be able of forming more stable intermediates that resveratrol [16].

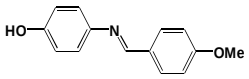
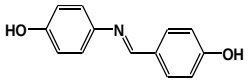
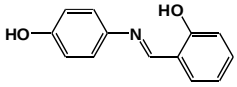
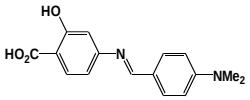
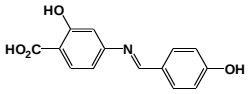
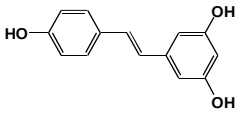
The analogs A and B showed antioxidant activity statistically similar to resveratrol. The activity found is possibly related to the hydroxy grouping insertion as a differential in comparison to other samples tested. According to Cerqueira and colleagues (2007) [17], the aromatic hydroxyl radical has reducing power of reactive free radicals and produces phenoxyl radical, stabilized by resonance. The results found by Calil and colleagues (2012) [9] also confirm that the degree of activity of antioxidant substances is directly related to the number of hydroxyl groups present in the molecule. Among five synthetic analogs of resveratrol, the ones (A and B) which have a hydroxyl group in para position of aromatic ring containing the nitrogen atom were those that presented the best potential for inhibition of DPPH radical.

Although the analog C has shown the $IC_{50}$ value statistically different to resveratrol, its antioxidant activity was considered satisfactory. It is important to study the

**Table 1. Spectral Data of Resveratrol Analogs**

| Analogs | C<u>*H*</u>=N | δ <u>*C*</u>=N | $\bar{\nu}$ C=N | Melting Point (ºC) | Yield (%) |
|---|---|---|---|---|---|
| **A** | 8.51(s,1H) | 161.5 | 1609 | 189.0 | 61 |
| **B** | 8.43(s,1H) | 160.2 | 1607 | 203.7 | 53 |
| **C** | 8.89(s,1H) | 160.2 | 1616 | 141.4 | 55 |
| **D** | 9.66(s,1H) | 172.0 | 1605 | 191.5 | 59 |
| **E** | 9.78(s,1H) | 172.2 | 1602 | 182.4 | 57 |

**Table 2. Radical Scavenging Activities of Resveratrol and Analogs**

| Sample | Chemical Structure | $IC_{50}$ (µg/mL)* |
|---|---|---|
| **A** |  | 24.79 ± 0.40 [a] |
| **B** |  | 7.41 ± 0.11 [b] |
| **C** |  | 55.78 ± 1.51[a,b,c] |
| **D** |  | 442.80 ± 15.63 [a,b,c] |
| **E** |  | 539.41 ± 6.21[a,b,c] |
| **Resveratrol** |  | 8.53 ± 0.12 [c] |

*Results expressed as mean ± standard deviation (n=3). Means followed by same letter differ by ANOVA followed by Tukey's post hoc test (a,b,cp<0.001 for comparison of average values of $IC_{50}$ among samples). $IC_{50}$ = concentration of drugs that inhibits 50% of DPPH radicals.

hydroxyl group elsewhere in this structure in order to find some improve of the antioxidant capacity. Analogs D and E, which presented a 4-acid group in place of 4-hydroxy group, did not show relevant result in the antioxidant test, possibly due to the presence of a electron withdrawing group such as carboxylic acid.

The efficiency of the phenolic antioxidant is determined not only by the functional groups present in chemical structure of the molecule as well as the position which they take on the aromatic ring [18].

In such study, analogs A and B proved to be bioactive molecules with antioxidant activity statistically similar to the natural compound resveratrol. The changes of chemical structures showed that the hydroxyl group is important for the activity. Further studies about the bioavailability of such analogs, which may demonstrate some potential advantages in relation to resveratrol, must be performed to make them feasible for therapeutic use.

## CONFLICT OF INTEREST

Declared none.

## ACKNOWLEDGEMENTS

The authors would like to thank CAPES, CNPq and FAPEMIG for scholarships.

## REFERENCES

[1] Barbosa, K.B.F.; Costa, N.M.B.; Alfenas, R.C.G.; Paula, S.O.; Minim, V.P.R.; Bressan, J. Estresse oxidativo: conceito, implicações e fatores modulatórios. *Rev. Nutr.*, **2010**, *23*(4), 629-643.

[2] Rizvi, S.I.; Jha, R. Current perspectives on anti-aging interventions. *Lett Drug Des Discov*, **2010**, *7*(5), 379-388.

[3] Gottlieb, M.G.V.; Morassutti, A.L.; Cruz, I.B.M. Transição epidemiológica, estresse oxidativo e doenças crônicas não transmissíveis sob uma perspectiva evolutiva. *Sc. Me.*, **2011**, *21*(2), 69-80.

[4] Shami, N.J.I.E.; Moreira, E.A.M. Licopeno como agente antioxidante. *Rev. Nutr.*, **2004**, *17*(2), 227-236.

[5] Shukla, Y.; Singh, R. Resveratrol and cellular mechanisms of cancer prevention. *Ann N Y Acad Sci.*, **2011**, *1215*, 1-8.

[6] Savio, M.; Coppa, T.; Bianchi, L.; Vannini, V.; Maga, G.; Forti, L.; Cazzalini, O.; Lazzè, M.C.; Perucca, P.; Prosperi, E.; Stivala, L.A. The resveratrol analogue 4,4'-dihydroxy-*trans*-stilbene inhibits cell proliferation with higher efficiency but different mechanism from resveratrol. *Int J Biochem Cell Biol.*, **2009**, *41*, 2493-2502.

[7] Shang, Y.J.; Qian, Y.P.; Liu, X.D.; Dai, F.; Shang, X.L.; Jia, W.Q.; Liu, Q.; Fang, J,G, Zhou, B. Radical-scavenging activity and mechanism of resveratrol-oriented analogs: influence of the solvent, radical, and substitution. *J Org Chem.*, **2009**, *74*, 5025-5031.

[8] Cheng, J.C.; Fang, J.G.; Chen, W.F. Zhou, B.; Yang, L.; Liu, Z.L. Structure–activity relationship studies of resveratrol and its analogs by the reaction kinetics of low density lipoprotein peroxidation. *Bioorg Chem.*, **2006**, *34*, 142-157.

[9] Calil, N.O.; Carvalho, G.S.G.; Franco, D.C.Z.; Silva, A.D.; Raposo, N.R.B. Antioxidant activity of resveratrol analogs. *Lett Drug Des Discov*, **2012**, *9*(1) 8-11.

[10] Pavan, F.R.; Carvalho, G.S.G.; Da Silva, A.D.; Leite, C.Q.F. Synthesis and anti–*Mycobacterium tuberculosis* evaluation of Aza-Stilbene derivatives. *ScientificWorldJournal*, **2011**, *11*, 1113-1119.

[11] Wadher, S.J.; Karande, N.A.; Sonawane, S.D.; Yeole, P.G. Synthesis and biological evaluation of Schiff base and 4-thiazolidinones of amino salicylic acid and their derivatives as an antimicrobial agent. *Int.J. ChemTech Res*., **2009**, *1*(4) 1303-1307.

[12] Cheng, L.; Tang, J.; Luo, H.; Jin, X.; Dai, F.; Yang, J.; Qian, Y.; Li, X.; Zhou, B. Antioxidant and antiproliferative activities of hydroxyl-substituted Schiff bases. *Bioorg. Med. Chem. Lett.*, **2010**, *20*(8), 2417-2420.

[13] Sreejayan, N.; Rao, M. N. Free radical scavenging activity of curcuminoids. *Arzneimittelforschung*, **1996**, *46*(2), 169-171.

[14] Koleva I.I.; Beek Van, T.; Linssen, J.P.H.; Groot, A.; Evstatieva, L.N. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem Anal*., **2002**, *13*, 8-17.

[15] Baur, J.A.; Sinclair, D.A. Therapeutic potencial of resveratrol: the *in vivo* evidence. *Nat Rev Drug Discov.*, **2006**, *5*, 493-506.

[16] Lange, M.K.; Heberlé, G.; Milão, D. Avaliação da estabilidade e atividade antioxidante de uma emulsão base não-iônica contendo resveratrol. *Braz. J. Pharm. Sci.*, **2009**, *45*(1), 145-151.

[17] Cerqueira, F.M.; Medeiros, M.H.G.; Augusto, O. Antioxidantes dietéticos: controvérsias e perspectivas. *Quím. Nova*, **2007**, *30*(2), 441-449.

[18] Luzia, D.M.M.; Bertanha, B.J.; Jorge, N. Sementes de pitanga (*Eugenia uniflora* L.): potencial antioxidante e perfil de acidos graxos. *Rev Inst Adolfo Lutz.*, **2010**, *69*(2), 175-180.