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Cytotoxicity, antitumoral and antimycobacterial activity of tetrazole and oxadiazole derivatives

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In this study, the antimycobacterial activity of mono and di-substituted tetrazole and oxadiazole derivatives and their precursors was assayed on *Mycobacterium tuberculosis* H37Rv, and cytotoxicity was evaluated on J774 macrophages and on tumoral cell lines. Structure Activity Relationship (SAR) analysis was performed using Principal Component Analysis (PCA) to determine the relationship between these compounds and their biological activities.

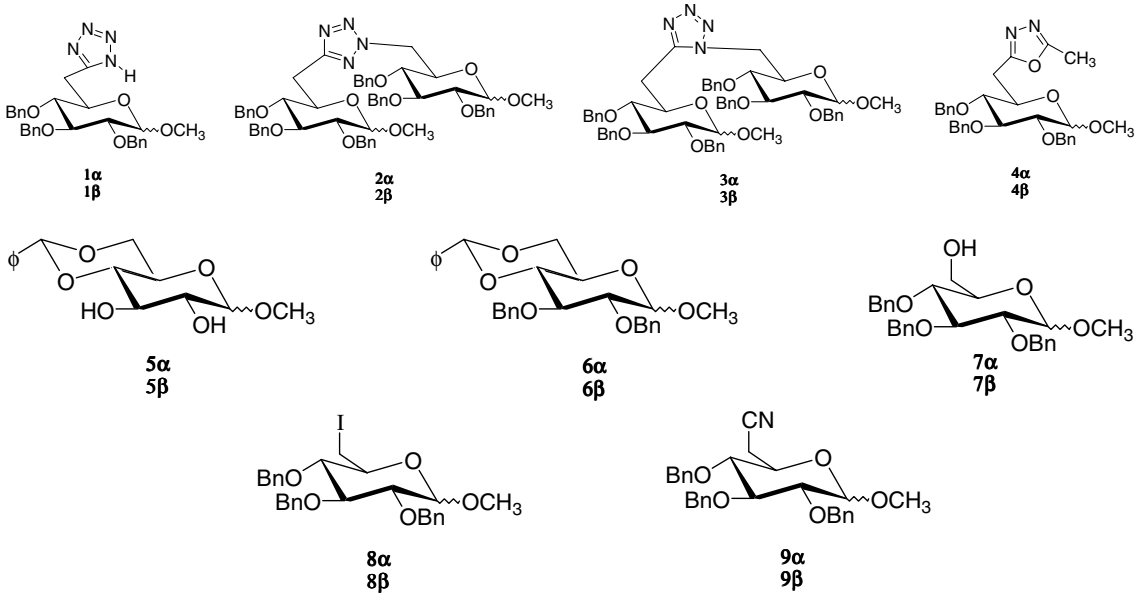
Tetrazole and oxadiazoles derivatives are compounds associated with important biological activities (Singh et al. 1980; Butler 1984; Herr 2002; Mehta and Parekh 1988), which has been inspired the synthesis of new derivatives. In this study, mono (**1**α–**1**β) and di-substituted (**2**α, **2**β, **3**α, **3**β) tetrazoles and oxadiazoles **4**α and **4**β and some of their precursors **5**α, **5**β–**9**α/**9**β (Pedrosa et al. 2003) were assayed on *Mycobacterium tuberculosis* H37Rv.

Compounds **4**α, **7**α and **7**β exhibited the highest efficiency on *M. tuberculosis* H37Rv, with MICs of 62.5, 62.5, and 31.25 μmol L^{-1}, respectively, while the other samples were not effective with MICs higher than 1,000 μmol L^{-1}. Due to the importance of a balance between antimycobacterial activity and cytotoxicity to mammalian cells, the cytotoxicity of the compounds was evaluated on J774 macrophages. Compounds **4**α and **7**α showed IC_{50} values of 74.0 and 105.0 μmol L^{-1} respectively and **7**β was not toxic to the cells.

In an assay on tumoral cell lines, compound **4**α reduced the growth of cell lines MCF7, NCI-H460, and SF-268 to 38%, 51%, and 23%, respectively and was subsequently assayed on 55 tumoral cell lines. The other samples were considered as inactive, since the growth of the cells was reduced to values higher than 32%. Among the cell lines assayed with **4**α, SR (leukemia), EKVX (non-small cell lung cancer), KM12 (colon cancer), SF-295 (CNS cancer), SK-MEL-2 (melanoma), IGROV1 (ovarian cancer), RXF 393 (renal cancer), and MDA-MB-435 (breast cancer) were the most susceptible, with GI50 of 13.2, 12.7, 15.8, 18.9, 14.5, 13.9, 18.3, and 19.7 μmol L^{-1}, respectively. At 100 μmol L^{-1} several cell lines died, while cell lines K-562, RPMI-8226, HOP-62, HOP-92, HCT-116, HCT-15, SW-620, SF-539, SNB-19, SNB-75, U251, MDA-MB-231-ATCC, HS578T, and T-47D were insensitive with higher GI50 values.

The exact numerical values of biological activity are unknown, and therefore a method capable of classifying the compounds into distinct categories, such as low and highly active compounds, is necessary. Principal Component Analysis (PCA) is a useful method to determine the relationship between compounds and their biological activities (Costa et al. 1997; De Souza et al. 1999).

The 18 compounds investigated can be classified according to their antimycobacterial activity. Compounds **4**α, **7**α, and **7**β are highly active, while the remaining compounds exhibit low activity. The classification process of the 18 derivatives was performed by successive PCA on different sets of descriptors. The first two principal components, regarding anti-



mycobacterial classification, are defined by eqs. (1) and (2):

$$PC1 = 0.465\varepsilon_{HOMO} - 0.397N^{EE} - 0.521RPCSA - 0.595Q_p \quad (1)$$

$$PC2 = 0.490\varepsilon_{HOMO} - 0.613N^{EE} + 0.522RPCSA + 0.334Q_p \quad (2)$$

In these equations, ε_{HOMO} is the HOMO energy, N^{EE} is the number of electronic levels occupied/number of atoms, and RPCSA is the relative positive charged surface area. $Q_p = Q_{max} - Q_{min}$ represents the polarity parameter, where Q_{max} and Q_{min} are the maximum and minimum atomic charges. Values for these descriptors are displayed in the Table.

The classification obtained by PCA was able to promote separation between low and highly active compounds and only derivative **4**α was incorrectly classified.

It is clear that reactivity parameters (ε_{HOMO}, N^{EE}), along with RPCSA and Q_p descriptors, conduct the classification process. These descriptors, associated with reactivity characteristics of the derivatives and the solvation process (RPCSA and Q_p), stress the importance of both orbital and electrostatic effects for the antimycobacterial activity of the tetrazoles and oxadiazoles studied in this work.

The antitumoral activity of tetrazole and oxadiazole derivatives was evaluated for 8 compounds and only derivative **4**α can be classified as active. PCA correctly classified this compound as an active derivative. The first two principal components, regarding antitumoral classification, are defined by eqs. (3) and (4):

$$PC1 = 0.7071f_{max}^{elec} - 0.7071RNCSA \quad (3)$$

$$PC1 = 0.7071f_{max}^{elec} + 0.7071RNCSA \quad (4)$$

In this case, only two descriptors are necessary for the classification process. These descriptors are displayed in the Table, where f_{max}^{elec} and RNCSA denote the maximum electrophilic reactivity index for a carbon atom and the relative negative charged surface area. Again, PCA stresses the importance of orbital (f_{max}^{elec}) and electrostatic (RNCSA) effect in the determination of antitumoral activities of the derivatives studied in this report.

Table: Descriptors selected by Principal Component Analysis (PCA) to biological activities of tetrazole and oxadiazole derivatives: antimycobacterial and antitumoral activities

Compd.	Descriptors to antimycobacterial activity				Descriptors to antitumoral activity	
	ε_{HOMO}	N^{EE}	RPCSA	Q_p	f_{max}^{elec}	RNCSA
1α	−9.418	14.143	0.7613	0.2472	0.038000	0.0465
1β	−9.500	14.143	0.4200	0.2472	0.037900	0.0000
2α	−9.205	13.910	0.4158	0.2335	0.027200	0.0233
2β	−9.302	13.910	0.0450	0.2335	0.009270	0.1384
3α	−9.237	13.910	0.3844	0.2334	0.031600	0.0239
3β	−9.298	13.910	0.0113	0.2334	0.036400	0.0000
4α	−9.341	13.973	0.1929	0.2393	0.032700	0.2888
4β	−9.449	13.973	0.2250	0.2393	0.032300	0.0410
5α	−9.518	14.474	13.881	0.2581		
5β	−9.545	14.474	17.450	0.2581		
6α	−9.250	13.906	0.6078	0.2325		
6β	−9.302	13.906	0.3907	0.2325		
7α	−9.299	13.636	20.073	0.2611		
7β	−9.337	13.636	19.446	0.2611		
8α	−9.349	13.846	0.5933	0.2335		
8β	−9.439	13.846	0.4509	0.2335		
9α	−9.411	13.788	0.5271	0.2334		
9β	−9.442	13.788	0.4354	0.2334		

Experimental

Tetrazole and oxadiazole derivatives were obtained as previously described (Pedrosa et al. 2003) and were assayed against *M. tuberculosis* H37Rv ATCC 27294 in order to obtain the Minimal Inhibition Concentration (MIC). The tests were performed as previously reported (Collins and Franzblau 1997) and rifampicin was used as a reference drug. MICs were defined as the lowest drug concentration that prevented mycobacterial growth.

Cytotoxicity of the compounds was determined on J774 cells by the tetrazolium reduction assay (MTT), (Denizot and Lang 1986; De Souza et al. 2004). Cells were exposed to the drugs at concentrations ranging from 31.25 to 1,000 μmol L^{-1} or to medium without drugs (control) for 24 h. Results are expressed by comparison between cellular viability of cells treated with drugs and controls and the IC_{50} was defined as the drug concentration required to reduce the cellular viability in 50%.

Antitumoral assays were carried out by the National Institutes of Health – National Cancer Institute (NCI). In an initial assay, mono and di-substituted tetrazoles and oxadiazoles were evaluated at a single dose of 50 or 100 μmol L^{-1} on tumoral cell lines MCF7 (breast), NCI-H460 (lungs) and SF-268 (central nervous system). Samples were classified as active or inactive according with the percentage of growth of treated cells compared to untreated control cells. Compounds which reduced the growth of the cells lines to approximately 32% or less were considered as active and submitted to a second evaluation. This second step involved a full panel of 55 cell lines: 6 leukemia, 8 non-small cell lung, 6 colon cancer, 6 CNS cancer, 8 melanoma, 5 ovarian cancer, 8 renal cancer, 1 prostate cancer and 7 breast cancer. Only compound **4**α progressed to the second step, in concentrations ranging from 0.01 to 100 μmol L^{-1}.

Structure activity relationship (SAR) analysis was performed using Principal Component Analysis (PCA). A total of 40 chemical quantum descriptors obtained by the AM1 semiempirical orbital method (Dewar et al. 1985) were used to perform the classification of the antitumoral and antimycobacterial activities of 18 derivatives. The geometry of the compounds was submitted to total optimization, and all reported properties were calculated for the optimized geometry. The process of classification of the derivatives was carried out using mean centered data, since this procedure allowed a better clustering of the objects. Several trials were performed with different sets of descriptors, until the set most capable of separating the derivatives into low and highly active was obtained.

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