Investigation on Quantitative Structure–Activity Relationships of 1,3,4-Oxadiazole Derivatives as Potential Telomerase Inhibitors

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Abstract: A series of 1,3,4-oxadiazole derivatives with significant broad-spectrum anticancer activity against different cell lines, and demonstrated telomerase inhibition, was subjected to Quantitative Structure-Activity Relationships (QSAR) analysis. Validated models with high correlation coefficients were developed. The Multiple Linear Regression (MLR) models, by Ordinary Least Squares (OLS), showed good robustness and predictive capability, according to the Multi-Criteria Decision Making (MCDM = 0.8352), a technique that simultaneously enhances the performances of a certain number of criteria. The descriptors selected for the models, such as electrotopological state (E-state) descriptors, and extended topochemical atom (ETA) descriptors, showed the relevant chemical information contributing to the activity of these compounds. The results obtained in this study make sure about the identification of potential hits as prospective telomerase inhibitors.

Keywords: 1,3,4-oxadiazoles, Anticancer activity, Telomerase inhibitors, QSAR, 2D descriptors, MLR.

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1. INTRODUCTION

Telomerase, a reverse transcriptase, maintains telomere and chromosomes integrity of dividing cells, while it is inactivated in most somatic cells (1,2). In tumor cells, telomerase is highly activated, and works in order to maintain the length of telomeres causing immortality, hence it could be considered as a potential marker to tumorigenesis (3–5). The great advantage of targeting this reverse transcriptase, with respect to other cancer targets, is due to its strict specificity for cancer cells. In fact, it is expressed in up to the 90% of cancers (6,7). Human telomerase consists of two portions: a template-encoding RNA (TER), and a reverse transcriptase part (TERT) which also consists of an essential N-terminal domain (TEN), a telomerase RNA binding domain (TRBD), a reverse transcriptase domain (RT), and a C-terminal domain (8,9). In the past decades, several classes of inhibitors were identified: oligonucleotides targeting the telomerase RNA templates (10), compounds targeting telomeric DNA(11), nucleosidic transcriptase inhibitors (12) and G-quadruplex stabilizing compounds as telomerase inhibitors (13,14). Among this range of compounds' classes, different substituted 1,3,4-oxadiazoles showed potent anti-tumor activities (15–18), and in particular telomerase inhibitory activity (18,19). Moreover, oxazole, bioisoster of 1,3,4-oxadiazole ring, is the scaffold of telomestatin, which is a natural product isolated from *Streptomyces anulatus*, with potent telomerase inhibitory activity (20). The emphasis of recent efforts to develop new telomerase inhibitors has been focused on structure-based design (18,19,21–23). Ligand-based design by means of Quantitative Structure Activity Relationships (QSAR), an important application of chemometrics, revealed in the last years to be useful to obtain information in the design of new molecules against a specific target (24–26). Nevertheless, QSAR modeling is affected by one severe problem: model validation. In fact, in the past many QSAR models have been published as predictive, although not all the validation checks have been done. Therefore, model validation has been subject of many debates in scientific and regulatory communities. To date, to consider a QSAR model as predictive, this latter should be associated to defined OECD principles (27). A QSAR model, for regulatory purposes, and for the identification of new chemical entities in all the field of chemistry, should be associated with the following information: (1) a defined endpoint; (2) an unambiguous algorithm; (3) a defined domain of applicability; (4) appropriate measures of goodness-of-fit, robustness and predictivity; (5) a mechanistic interpretation, if possible. Our interest in the chemistry of oxadiazoles (28), and the alive pharmaceutical interest in this outstanding scaffold (29), have placed our attention to the structures-activity relationships, with the aim of underline the features which could increase anti-tumor activity. Even though other attempts have been carried out (18,19,21,23,30), no validated models have been built according to OECD principles for 1,3,4-oxadiazoles as telomerase inhibitors, making predictive power and mechanistic interpretation not reliable. In this paper, our main aim is to develop validated and predictive models for 1,3,4-oxadiazole derivatives as telomerase inhibitors, according to the OECD principles, exploiting the great amount of available biological data. The models developed are commented by means of the selected descriptors, and some interesting mechanistic interpretations could be stated.

2. MATERIALS AND METHOD

2.1. Dataset

A series of 24 N-benzylidene-2-((5-(pyridine-4-yl)-1,3,4-oxadiazol-2yl)thio)acetohydrazide derivatives as telomerase inhibitors has been considered to carry out QSAR studies (30). The endpoint to build QSAR models is determined by the IC_{50} values for telomerase inhibition, i.e., the concentration (μM) of inhibitor that produces 50% inhibition. These values were converted to pIC_{50} (-log IC_{50}) values. In Table 1 the structure of the 24 compounds are reported together with their biological data related to telomerase inhibition.

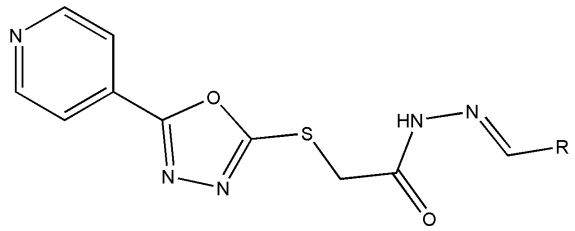

Table 1. Structures of 1,3,4-oxadiazole derivatives with activities

Comp No.	*R*	*Exp pIC$_{50}$*[a]	*QSAR set*[b]
1	Ph-	5.012	Training
2	4-F-C_6H_4-	4.992	Training
3	4-Cl-C_6H_4-	4.925	Prediction
4	4-Br-C_6H_4-	4.907	Training
5	4-O_2N-C_6H_4-	4.778	Training
6	4-HO-C_6H_4-	5.271	Prediction
7	4-MeO-C_6H_4-	5.044	Training
8	4-H_3C-C_6H_4-	5.070	Training
9	3-F-C_6H_4-	4.803	Training
10	3-F_3C-C_6H_4-	5.286	Training
11	3-MeO-C_6H_4-	5.123	Training
12	2-F-C_6H_4-	4.837	Training
13	2-O_2N-C_6H_4-	4.741	Training
14	2-HO-C_6H_4-	5.401	Training
15	2-HO-5-Cl-C_6H_3-	5.504	Training
16	2-HO-5-Br-C_6H_3-	5.369	Training
17	2-HO-3,5-2Cl-C_6H_3-	5.319	Prediction
18	2-HO-3,5-2Br-C_6H_3-	5.148	Training
19	3,4-2HO-C_6H_3-	5.928	Prediction
20	3-MeO-4-HO-C_6H_3-	5.539	Training
21	2,4-2Cl-C_6H_3-	4.749	Prediction
22	2-Furan-	5.016	Training
23	2-Thiophene-	4.871	Training
24	(*E*)-styryl-	5.182	Training

[a] –log IC_{50}; [b] the compounds considered for training and prediction set for QSAR study

2.2. Calculation of Descriptors

A QSAR study requires the calculation of molecular descriptors. In order to have mechanistically interpretable descriptors, we limited the calculation to 1D-2D descriptors, since this study uses a ligand-based approach and 3D descriptors could instead be highly influenced by bound ligand conformations (31,32). A total of 1444 1D and 2D molecular descriptors were calculated using PADEL 2.1 software (33). Constant and semi-constant values (>80%), and correlated pairwise descriptors were excluded in a cleaning preliminary step (one of any two descriptors with a correlation greater than 0.95 was removed to reduce redundant information), and a final set of 195 molecular descriptors were used as input variables for model generation.

2.3. Model generation

Dataset was randomly split into a training set (19 compounds) for model generation, and a prediction set (5 compounds) for the validation of developed models, as reported in Table 1. First, the models were generated by the all-subset procedure with two variables, and subsequently by using genetic algorithm (GA) up to three variables, respecting the objects/descriptors ratio ≥ 5 (27). We used the most common and transparent method, where models are described by clearly expressed mathematical equations: Multiple Linear Regression (MLR) by Ordinary Least Squares (OLS).

2.4. Models validation

The generated models were measured according to appropriate measures of goodness-of-fit, robustness, and predictive capability. Used statistics for goodness-of fit are: $R^2 > 0.7$, concordance correlation coefficient (CCCtr) > 0.85 (34), RMSE, R^2_{adj}, and R^2-R^2_{adj}. Used statistics to measure robustness of the model are: Q^2(eq.1) > 0.7, CCCcv, RMSEcv, Q^2_{LMO}, and R^2 calculated according Y-scrambling procedure.

$$\text{Eq.1} \qquad \boldsymbol{q^2 = 1 - \frac{\sum_{i=1}^{training}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{training}(y_i - \bar{y})^2}}$$

Where $\boldsymbol{y_i}$ $\boldsymbol{\hat{y}_i}$ are the actual and predicted activities of the *i*th molecule, respectively, and $\boldsymbol{\bar{y}}$ is the average activity of all molecules.

Predictive capability of the models generated was assessed by means of the external validation of the prediction set. Used statistics for external validation are: Q^2_{ext} > 0.70(eq.2), Q^2_{F1} > 0.70(35), Q^2_{F2} > 0.70(36), Q^2_{F3} > 0.70(37), Golbraikh and Tropsha parameters k and k'(38), r^2_m metrics >0.65 (39), CCCext > 0.85(40)

$$\text{Eq.2} \qquad \boldsymbol{q^2_{ext} = 1 - \frac{\sum_{i=1}^{test}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{test}(y_i - \bar{y}_{tr})^2}}$$

Where $\boldsymbol{y_i}$ $\boldsymbol{\hat{y}_i}$ are the actual and predicted activities of the *i*th molecule, respectively, and $\boldsymbol{\bar{y}_{tr}}$ is the average activity of all molecules in the training set.

With the aim to choose indeed the best performing model, excluding bias due to evaluating many statistic parameters at the same time, we decided to use the Multi-Criteria Decision Making (MCDM) (41). MCDM is an approach that sums up the performances of many criteria simultaneously. This is realized associating a desirability function, which values range from 0 to 1 (where 0 represents the worst validation criteria value and 1 the best), to every validation

criterion. The MCDM scores reported in this paper are: MCDMfit regarding fitting criteria (maximizing R^2, R^2_{adj}, and CCCtr, and minimizing R^2-R^2_{adj}), MCDMcv regarding internal validation (maximizing Q^2, Q^2LMO, CCCcv, and minimizing R^2yscr), MCDMext regarding external validation (maximizing Q^2_{F1}, Q^2_{F2}, Q^2_{F3}, and CCCext). MCDMall, calculated with all the previous criteria, is able to determine the best compromise models among the selected validating criteria.

2.4. Applicability Domain

Prediction capability of modeled properties for the whole universe of chemicals is still not expected, even if robust and validated models are developed (42). QSAR models must be verified for their applicability domain, the latter having the ability to provide predicted data for compounds that are similar to chemicals used to generate the model. The applicability domain of the model was verified by the leverage approach, and fixed thresholds have been used to define both structural and response outliers. The Williams plot verified the presence of response outliers (compounds with cross-validated standardized residuals greater than 3.0 standard deviation units), and chemicals very structurally influent in determining model parameters. These latters are compounds with a leverage value (h) greater than 3p'/n (h*) where p' is the number of model variables plus one, and n is the number of the objects used to calculate the model.

3. RESULTS AND DISCUSSION

For the development of the QSAR models for 1,3,4-oxadiazole derivatives, MLR with OLS was applied. Initially, we generated models considering only one descriptor, then, we extended the calculation to two variables using the "all-subset" procedure, and finally, we proceeded to the third variable with GA. According to the fitness, robustness and predictive parameters, explained in materials and methods, some statistically significant models have been selected for discussion and mechanistic interpretation.

Model 1: pIC_{50} = +6.50 (±0.74) Intercept

+0.12 (±0.04) naaCH

+25.5 (±3.54) ETA_dEpsilon_D

-3.70 (±0.79) ETA_BetaP_ns

N = 19, R^2 = 0.85, CCCtr = 0.92, RMSEtr = 0.09, R^2_{adj} 0.82, R^2-R^2_{adj}=0.03 fitness

Q^2 = 0.73, CCCcv = 0.86, RMSEcv = 0.12, Q^2_{LMO} = 0.71 R^2_{yscr} = 0.16 robustness

Q^2_{ext} = 0.95, Q^2_{F1} = 0.93, Q^2_{F2} = 0.92, Q^2_{F3} = 0.78, CCCext = 0.78, r^2_m = 0.87, k = 1.01, k' = 0.99 predictive

In Figure 1 is shown the plot of experimental versus calculated endpoint.

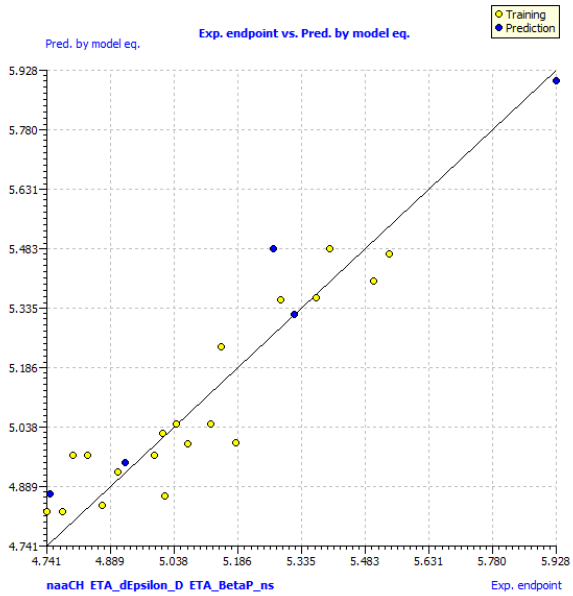

Figure 1 *plot of experimental versus calculated endpoint for model 1*

Model 1 is represented by a three parametric expression. This model, built using the GA-OLS method, has good measures of fitness above the optimal thresholds, and it shows an internal predictive power of 73% (Q^2 = 0.73) with a very low probability of random correlation among activity values and independent variables (R^2_{yscr} = 0.16). The external predictive power on test set is good (Q^2_{ext} = 0.95), and all the other predictive parameters are above the considered significant thresholds. Descriptors are ordered according to their importance, based on their standardized coefficient values, which are reported in brackets after each descriptors symbol: ETA_dEpsilon_D (+0.93) is a measure of contribution of hydrogen bond donor atoms; ETA_BetaP_ns (-0.47) is a measure of electron-richness of the molecule relative to molecular size; naaCH (+0.37) is an electrotopological state index related to aromatic CH group.

In terms of applicability domain, one structural outlier has been identified (compound #10) based on h*=0.632 (Figure 2).

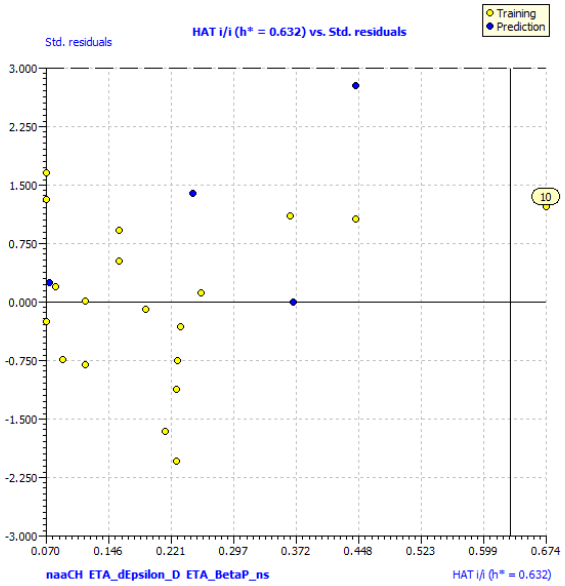

Figure 2. *Applicability domain for model 1*

Model 2: pIC_{50} = +7.67 (±0.74) Intercept

-2.77 (±1.36) VCH-7

+22.49 (±3.46) ETA_dEpsilon_D

-3.55 (±0.87) ETA_BetaP_ns

N=19, R^2 = 0.82, CCCtr = 0.90, RMSEtr = 0.10 R^2_{adj} = 0.78, R^2-R^2_{adj}=0.04 fitness

Q^2 = 0.72, CCCcv = 0.85, RMSEcv = 0.12, Q^2_{LMO} = 0.70 R^2_{yscr} = 0.16 robustness

Q^2_{ext} = 0.96, Q^2_{F1} = 0.94, Q^2_{F2} = 0.93, Q^2_{F3} = 0.80, CCCext = 0.96, r^2_m = 0.87, k = 1.01, k' = 0.99 predictivity

In Figure 3 the plot of experimental versus calculated endpoint for model 2 is shown.

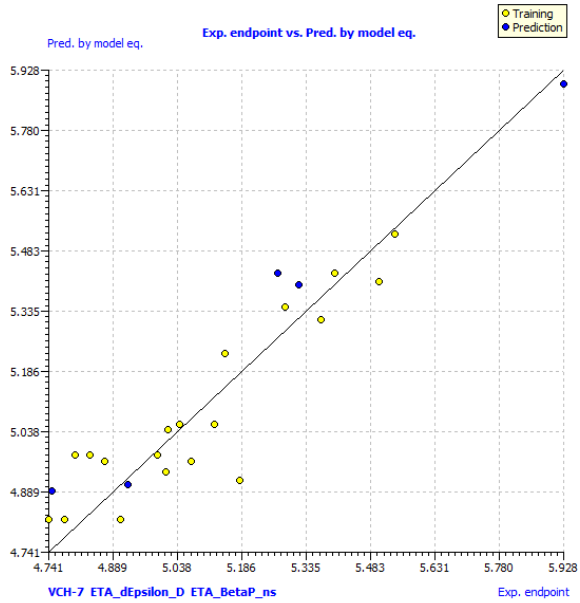

Figure 3 *the plot of experimental versus calculated endpoint for model 2*

Model 2 is also represented by a three parametric expression, and it was obtained using the same method of model 1. Model 2 has measures of fitness above the optimal thresholds too, and it shows an internal predictive power of 72% ($Q^2 = 0.72$), with a very low probability of random correlation among activity values and independent variables (R^2_{yscr} =0.16). The external predictive power on test set is good (Q^2_{ext} = 0.95), and all the other predictive parameters are above the considered significant thresholds. Two of the descriptors correlated with the endpoint, are the same of model 1: ETA_dEpsilon_D (+0.81), and ETA_BetaP_ns (-0.45); the third descriptor is VCH-7 (-0.25), a topochemical descriptor related to Kier-Hall indices (valence chain order 7), which is known for its importance in anticancer drug design (43).

In terms of applicability domain, the same structural outlier of model 1 has been identified (compound #10) based on h*=0.632 (Figure 4).

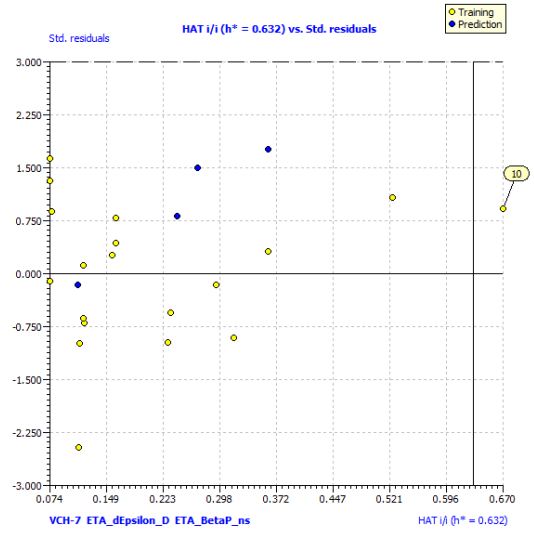

Figure 4. *Applicability domain for model 2*

Model 3: pIC_{50} = +5.96 (±1.17) Intercept

+21.22 (±3.38) ETA_dEpsilon_D

-4.81 (±1.21) ETA_BetaP_ns

+8.68 (±5.10) ETA_EtaP_F_L

N=19, R^2 = 0.81, CCCtr = 0.89, RMSEtr = 0.10 R^2_{adj} = 0.77, R^2-R^2_{adj}=0.04 fitness

Q^2 = 0.70, CCCcv = 0.89, RMSEcv = 0.13, Q^2_{LMO} = 0.68 R^2_{yscr} = 0.17 robustness

Q^2_{ext} = 0.96, Q^2_{F1} = 0.92, Q^2_{F2} = 0.91, Q^2_{F3} = 0.75, CCCext = 0.95, r^2_m = 0.80, k = 1.01, k' = 0.99 predictivity

As the previous models, Model 3 is also represented by a three parametric expression and has measures of fitness above the optimal thresholds. In fact it shows an internal predictive power of 70% (Q^2 = 0.70), with a very low probability of random correlation among activity values and independent variables (R^2_{yscr} = 0.17). The external predictive power on test set is good (Q^2_{ext} = 0.96), and all the other predictive parameters are above the considered significant thresholds. This model comprises two previously retrieved descriptors too: ETA_dEpsilon_D (+0.77), and ETA_BetaP_ns (-0.61); the third descriptor is ETA_EtaP_F_L, another extended topochemical atom descriptor, which correlates local functionality contribution (EtaF_local) with molecular size. The local functionality index was proposed to measure the molecule functionality, intended as the presence of heteroatoms and multiple bonds (44). In Figure 5 the plot of experimental versus calculated endpoint for Model 3

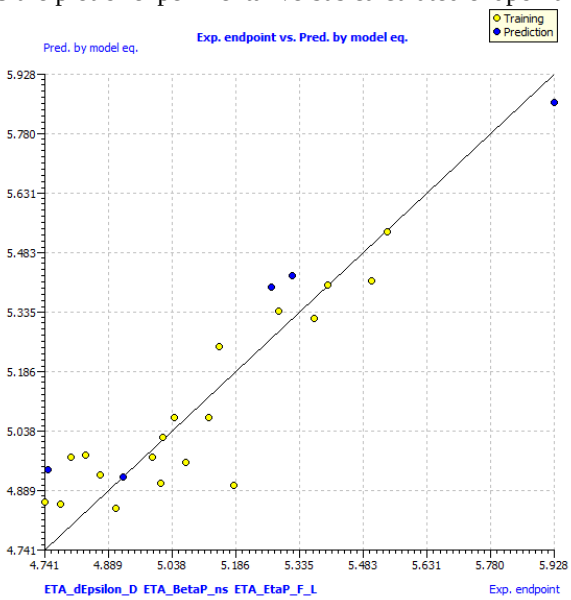

Figure 5 *plot of experimental versus calculated endpoint for Model 3*

In terms of applicability domain, Model 3 is quite similar to the previous two, with only compound #10 as structural outlier (Figure 6).

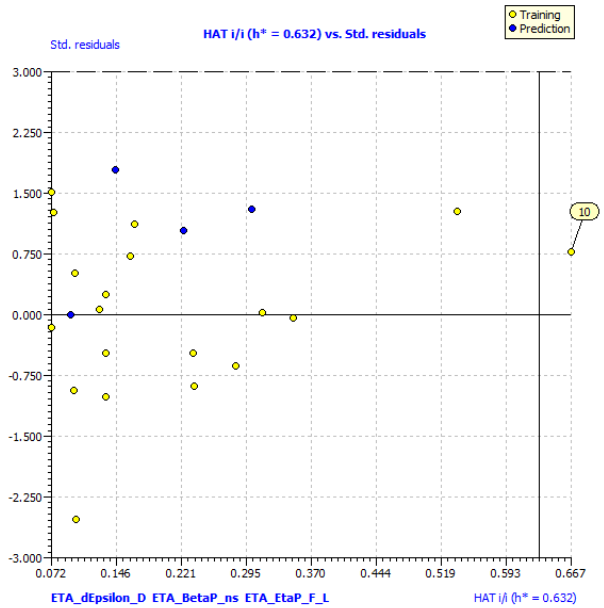

Figure 6. *Applicability domain for model 3*

All the three models identified showed good parameters of fitness, robustness and predictive capability. They differ from each other by a single descriptor, and in terms of applicability domain have a quite similar behavior. Therefore, in such a landscape of QSAR models, the choice of the best performing model to identify new and more potent compounds could be very difficult. For this reason, we decided to entrust the management of the best performing model to the MCDM criteria. In Table 2, predicted and residuals for the three QSAR models are reported. In Table 3, the MCDM values are shown. Model 1 could have, in terms of MCDMall, the best performing capability, even though with a slight difference compared to the other two models. As the last choice criteria, we decided to consider the significance (p-value) of descriptors coefficients in each model. In Model 1, all the descriptor coefficients have p-value<0.05; in model 2, VCH-7 has p-value > 0.05, such as in model 3, ETA_EtaP_F_L has p-value > 0.10. When p-value for descriptors coefficients are under the confidence threshold of 95%, models should be considered with caution. In the light of this latter considerations, Model 1, in virtue of the best MCDMall value, and in virtue of p-values < 0.05, could be definitively considered as the QSAR model of choice for 1,3,4-oxadiazole derivatives as telomerase inhibitors.

Table 2. *predicted and residuals for the three QSAR models*

ID	*STATUS*	*EXP.*	*MODEL 1*		*MODEL 2*		*MODEL 3*	
			Pred.	Res.	Pred.	Res.	Pred.	Res.
1	Training	5.012	5.023	0.011	4.939	-0.073	4.910	-0.102
2	Training	4.992	4.968	-0.024	4.981	-0.011	4.974	-0.018
3	Prediction	4.925	4.951	0.026	4.908	-0.017	4.925	0.000
4	Training	4.907	4.927	0.020	4.823	-0.084	4.845	-0.062
5	Training	4.778	4.828	0.050	4.824	0.046	4.856	0.078
6	Prediction	5.271	5.483	0.212	5.431	0.160	5.399	0.128
7	Training	5.044	5.046	0.002	5.057	0.013	5.072	0.028
8	Training	5.070	4.998	-0.072	4.966	-0.104	4.960	-0.110
9	Training	4.803	4.968	0.165	4.981	0.178	4.974	0.171
10	Training	5.286	5.358	0.072	5.346	0.060	5.339	0.053
11	Training	5.123	5.046	-0.077	5.057	-0.066	5.072	-0.051
12	Training	4.837	4.968	0.131	4.981	0.144	4.980	0.143
13	Training	4.741	4.828	0.087	4.824	0.083	4.861	0.120
14	Training	5.401	5.483	0.082	5.431	0.030	5.404	0.003
15	Training	5.504	5.404	-0.100	5.408	-0.096	5.414	-0.090
16	Training	5.369	5.361	-0.008	5.315	-0.054	5.321	-0.048
17	Prediction	5.319	5.319	0.000	5.400	0.081	5.427	0.108
18	Training	5.148	5.239	0.091	5.233	0.085	5.250	0.102
19	Prediction	5.928	5.904	-0.024	5.895	-0.033	5.859	-0.069
20	Training	5.539	5.472	-0.067	5.525	-0.014	5.536	-0.004
21	Prediction	4.749	4.873	0.124	4.894	0.145	4.943	0.194
22	Training	5.016	4.866	-0.150	5.044	0.028	5.023	0.007
23	Training	4.871	4.843	-0.028	4.967	0.096	4.929	0.058
24	Training	5.182	4.999	-0.183	4.921	-0.262	4.903	-0.280

Table 3. *MCDM values for the QSAR models*

	MCDM FIT	*MCDM CV*	*MCDM EXT*	*MCDM ALL*
MODEL 1	0.871	0.753	0.888	0.835
MODEL 2	0.843	0.742	0.901	0.826
MODEL 3	0.831	0.726	0.871	0.807

CONCLUSION

In this paper, we have successfully developed robust and predictive QSAR models for 1,3,4-oxadiazole derivatives as telomerase inhibitors. The results obtained in this study suggests that QSAR models developed with 1D and 2D molecular descriptors can be used for the design of new analogs with more potent telomerase inhibitory activity as anticancer drugs. In particular, Model 1 revealed to be the most reliable. *A posteriori* mechanistic interpretation of descriptors included in the model suggests important structural information. The ETA (Extended topochemical atom) descriptor ETA_dEpsD, which takes into account the contribute of H-donor atoms, increases according to the presence of donor atoms into the aromatic ring. At the same time, the Electrotopological-state descriptor naaCH, which considers the aromatic CH, has a positive coefficient: this suggests that too many substitutions on the aromatic ring could lead to non-active compounds. The last descriptors, the ETA descriptor ETA_dEpsD, which takes into account the electron-richness of the molecule relative to molecular size, slightly decreases related to the endpoint, so this suggests that electron-rich substituents do not have to be in excessive number related to dimension of the compounds. Therefore, we hope that this theoretical approach, and obtained structural information, could be an important aid in the design of novel compounds, to boost the identification of lead compounds to be tested *in vitro* and *in vivo*.

LIST OF ABBREVIATIONS

OECD, Organisation for Economic Co-operation and Development; QSAR, Quantitative Structure-Activity Relationships; MLR, Multi Linear Regression; OLS, Ordinary Least Squares; MCDM, Multi Criteria Decision Making; TER, template-encoding RNA; TERT, telomerase reverse transcriptase; TRBD, telomerase RNA binding domain, E-state, Electro topological state; ETA, Extended topochemical atom.

CONFLICT OF INTEREST

The authors confirm the article content has not conflict of interest.

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All listed as authors must have contributed equally to the design, performance, and analysis of the work.

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