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Quantitative Structure Activity Relationship Studies of A Novel Class of Dual PPAR γ/δ Agonists

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ABSTRACT: The Peroxisome proliferators-activated receptors (PPARs) constitute a highly conserved set of ligand activated transcription factors in the nuclear hormone receptor subfamily. Selective modulation of PPAR could provide significant anti-diabetic activity with the reduction or elimination of side effects. These have increasingly become attractive targets for developing novel anti-type 2 diabetic drugs. In the present study the QSAR analysis was performed employing V-life MDS-3.0 software to retrieve information about structure activity relationships of some 3-{4-[3-(2-aryl-phenoxy)butoxy]-phenyl}propionic acids as novel PPAR γ/δ agonists. 2D QSAR methods were developed using Partial Least Square (PLS) and variable selection methods. Out of the 10 models developed, the two best 2D QSAR models having highest correlation coefficient and cross validated squared correlation coefficient were selected for further study, which are r^2 = 0.9581, q^2 = 0.9262, F test = 160.2108, pred_r^2 = 0.3840, pred_r^2se = 0.6413 and r^2 = 0.9440, q^2 = 0.8987, F test = 118.1064, pred_r^2 = 0.5371, pred_r^2se = 0.5559. 3D QSAR models were developed using kNN-MFA method, combined with simulated annealing selection procedure. Out of the 10 models developed the best 3D QSAR model having highest cross validated squared correlation coefficient was selected for further study, record as q^2 = 0.8184, Pred_r^2 = 0.7455.

KEY WORDS: *2D QSAR, PLS Regression, DUAL PPAR γ/δ, Diabetes.*

INTRODUCTION

Non-insulin dependent diabetes mellitus (NIDDM) is a major cause of mortality and morbidity in the population of industrialized world. It is a complex, chronic metabolic disorder characterized by insulin resistance in the liver and peripheral tissues and dysfunction of beta -cells[1]. Due to change in dietary habits and sedentary life style [2], it becomes a major concern both in developed as well as developing countries. Insulin resistance is considered to be the underlying mechanism in the pathogenesis of type 2 diabetes, which, if untreated, leads to dyslipidemia, hypertension and obesity, collectively known as metabolic syndrome [3]. Untreated diabetes leads to severe macro and microvascular complications[4].Type 2 diabetes is a metabolic disorder characterized by hyperglycemia and/or insulin resistance. It is often associated with obesity, hypertension and dyslipidemia. Current therapies for reducing plasma glucose include the use of sulfonylureas, metformin, acarbose and thiazolidinones (TZDs). Statins and fibrate drugs act due to the activation of the peroxisome proliferators activated receptors (PPARs) γ and δ respectively. The PPARs constitute a highly conserved set of ligand activated transcription factors in the nuclear hormone receptor subfamily. Three distinct PPAR subtypes have been identified in most mammalian species each forming a functional heterodimer complex with 9 cis retinoic acid receptor (RXR). A series of compounds of 3-{4-[3-(2-aryl-phenoxy) butoxy]-phenyl} propionic acids was selected as novel PPAR γ/δ agonists for QSAR studies[5].The peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor superfamily of ligand-modulated transcription factors. There are three PPAR subtypes, namely PPAR γ, δ and ß. PPAR γ play a central role in regulating the storage and catabolism of dietary fats.[6] PPAR γ agonists, such as fenofibrate, are effective at lowering serum triglycerides and raising high-density lipoprotein

(HDL) cholesterol.[6-7] The role of PPAR γ has been extensively studied and is known to be involved with glucose homeostasis, insulin sensitization, and fat storage. PPAR γ agonists, such as rosiglitazone, 5-{4-[2- (methyl-pyridin-2-yl-amino)-ethoxy]-benzyl}-thiazolidine- 2,4-dione, increase insulin sensitivity and have been approvedfor the treatment of type 2 diabetes.[8] While not as extensively studied as the other subtypes, the role of PPAR ß has become clearer recently with the generation of potent, selective ligands for this PPAR subtype.

As exemplified in studies with GW501516,{2-methyl-4-[4-methyl-2-(4-trifluoromethyl-phenyl)-thiazol ylmethylsulfanyl]- phenoxy}-acetic acid, PPAR **γ/δ** activation appears to increase fatty acid b-oxidation, insulin sensitivity, and HDL cholesterol.[9-10]

MATERIAL AND METHODS

A dataset consisting of a series of 3-{4-[3-(2-aryl-phenoxy) butoxy]-phenyl} propionic acids derivatives acting PPAR **γ/δ** dual activators (Table 1) has been chosen to develop a dual-response QSAR model. The biological activities of the molecules have been expressed as the binding affinities measured as IC50 values in micromolar using recombinant PPAR **γ/δ** by[5]. For QSAR analysis, these have been converted to pIC50 (log _ IC50) values in molar terms (Table 1). Various 2D descriptors (a total of 208) like element counts, molecular weight, molecular refractivity, log P, topological index, electro-topological index, Baumann alignment independent topological descriptors, etc., were calculated using VLifeMDS software [11]. The preprocessing of the independent variables (i.e., descriptors) was done by removing invariable (constant column) and cross-correlated descriptors (with r>0.99) which resulted in total 132 descriptors to be used for QSAR analysis. All the twenty eight compounds were built on workspace of molecular modeling software V-Life MDS 3.5, which is a product VLife Sciences Pvt Ltd., India[11].The compounds were then subjected to conformational analysis and energy minimization using montocarlo conformational search with RMS gradient of 0.001 kcal/mol and iteration limit of 10000 using a MMFF94 force field. Montocarlo conformational search method is similar to the RIPS method that generates a new molecular conformation by randomly perturbing the position of each coordinate of each atom in molecule, followed by energy minimization and optimization is necessary process for proper alignment of molecules around template. Most stable structure for each compound was generated after energy minimization and used for calculating various physico-chemical descriptors like thermodynamic, steric and electronic. The various descriptors selected for 2D QSAR were vdWSurfaceArea (van der Waals surface area of the molecule), –vePotential Surface Area (total van der Waals surface area with negative electrostatic potential of the molecule), +vePotentialSurfaceArea (total van der Waals surface area with positive electrostatic potential of the molecule) dipole moment, YcompDipole (y component of the dipole moment), element count, slogP, path count, cluster, distance based topological indices, connectivity index, hydrophobic and hydrophilic areas like SA Most Hydrophilic (Most hydrophilic value on the vdW surface by Audry Method using Slogp), SAMostHydrophobic Hydrophilic Distance (distance between most hydrophobic and hydrophilic point on the vdW surface by Audry Method using Slogp), SAHydrophilicArea (vdW surface descriptor showing hydrophilic surface area by Audry Method using SlogP) and SKMostHydrophilic (Most hydrophilic value on the vdW surface by Kellog Method using Slogp), radius of gyration, Wiener's index, moment of inertia, semi- empirical descriptors, HOMO (Highest occupied molecular orbital), LUMO (lowest unoccupied molecular orbital), heat of formation and ionization potential. Besides these all alignment independent descriptors were also calculated. The hydrophobic descriptors govern the movement of a drug molecule across the biological membranes in order to interact with the receptor by vander Waals binding forces whereas both electronic and steric descriptors influence the affinity of a drug molecule necessary for proper drug- receptor interaction. The optimal training and test sets were generated by either random selection method or the sphere exclusion algorithm. A commonly used ratio of training to validation objects (test set), which was also adopted in this work, is 70%: 30% [12]. However, rational splitting was accomplished by applying a sphere-exclusion type algorithm [13-17]. In classical sphere-exclusion algorithm the molecules are selected whose similarities with each of the other selected molecules are not higher than a defined threshold. Each selected molecule generates a hyper-sphere around itself, so that any molecule inside the sphere is excluded from the selection in the train set and driven toward the test set. The number of compounds selected and the diversity among them can be determined by adjusting the radius of the sphere (R).

Statistical analysis

Models were generated by using three significant statistical methods, namely, partial least square analysis, multiple regressions, and principle component analysis. The cross-validation analysis was performed using the leave-one-out method. In the selected equations, the cross-correlation limit was set at 0.5, the number of variables at 10, and the term selection criteria at r^2. An F value was specified to evaluate the significance of a variable. The higher the

F value, the more stringent was the significance level: F test ''in'' as 4 and F test ''out'' as 3.99. The variance cutoff was set at 0, and scaling was auto scaling in which the number of random iterations was set at 100.The following statistical parameters were considered for comparison of the generated QSAR models: correlation coefficient (r), squared correlation coefficient (r2), predictive r2 for external test set (pred r2) for external validation, and Fischer's (F).The predicted r2 (pred_r2) value was calculated using Eq. 1, where yi and yˆi are the actual and predicted activities of the i^{th} molecule in the test set, respectively, and ymean is the average activity of all molecules in the training set. Both summations are over all molecules in the test set. The pred_r^2 value indicates the predictive power of the current model for the external test set as follows

$$\text{pred\_}r^2 = 1 - \frac{\sum (y_i - \hat{y}_i)^2}{\sum (y_i - y_{mean})^2} \quad (1)$$

Internal validation was carried out using leave-one-out (q2, LOO) method. For calculating q2, each molecule in the training set was eliminated once and the activity of the eliminated molecule was predicted by using the model developed by the remaining molecules. The q2 was calculated using the equation which describes the internal stability of a model:

$$q^2 = 1 - \frac{\sum (y_i - \hat{y}_i)^2}{\sum (y_i - y_{mean})^2} \quad (2)$$

Where y_i, and $\hat{y}_i$ are the actual and predicted activity of the $_i$th molecule in the training set, respectively, and y_{mean} is the average activity of all molecules in the training set.

RESULT AND DISCUSSION

Biological activity data and various physico-chemical parameters were taken as dependent and independent variables and correlations were established using PLS method. When the compounds were subjected to under goes PLS method to developed QSAR models by using step wise forward-backward variable selection mode, four QSAR models, Model-I and Model-II, Model-III were developed for both the methods respectively as shown below and other good model predicted activity shown abstract. Various 2D descriptors like Polarizability AHC, Polarizability AHP, chi1, 1-pathcount, chi3cluster, kappa3, Hydrogen count, SaaCH count, SdssC count that are responsible for PPAR γ/δ agonistic activity were calculated. The different statistical models were developed using Partial Least Square (PLS) method. The models showed the better correlation between biological activity and physicochemical descriptor values. For model 1 the correlation coefficient (R^2) was 0.9581 and cross validated squared correlation coefficient value (Q^2) was 0.9262. The other data were found to be F test =160.2108, pred_r^2 = 0.3840, pred_r^2se = 0.6413. For model 2 the correlation coefficient (R^2) was 0.9440, cross validated squared correlation coefficient value (Q^2) 0.8987, and the other values were found to be F test = 118.1064, pred_r^2 = 0.7064, pred_r^2se = 0.6559. The equations were generated for assuming the biological activity with the help of physicochemical descriptor values. The equations showed the correlation between biological activity and physicochemical descriptor values.

Model 2

Log10 (IC_50) = -0.0988 T_T_C_7 + 0.3836 T_2_S_3 + 0.2205 T_C_O_2 + 5.2935

Optimum Components = 4, Degrees of Freedom = 16, n = 16, r^2= 0.8361, q2= 0.6416, F test = 54.374 r2 se = 0.361, q2 se = 0.6864, pred_r^2 = 0.7361, SEE = 0.041, SECV= 0.630, SEP=0.170, best_ran_r^2=0.265, best_ran_q^2= 0.236 Zscore_ran_r2 =0.383, Zscore_ran_q2= 0.216, α_ran_r^2 = <0.0001, α _ran_q^2 = <0.001

To improve the external predictivity of the model, PLS analysis with the same data set was performed, which resulted in a coefficient of correlation of 0.5428 and an internal predictive power of 42%, with the good external predictivity of 58%. T_T_C_7 contributes in the same manner as above. T_C_O_2 defines the total number of carbons connected with four single bonds and makes a negative contribution to activity.

Model 3

Log10 (IC_50) = -1.5099 kappa3 + 0.5766 SdssCE-index + 15.4845

Optimum Components = 4, Degrees of Freedom = 16, n = 16, r^2= 0.6951, q2= 0.531, F test = 42.431, r2 se = 0.3725, q2 se = 0.546, pred_r^2 = 0.6541, SEE = 0.175, SECV= 0.218 SEP=0.390, best_ran_r^2 = 0.425 best_ran_q^2 = 0.316, Zscore_ran_r2 =0.431, Zscore_ran_q2= 0.032, α_ran_r^2 =<0.0001, α _ran_q^2 = <0.001

Model –2 shows good squared correlation coefficient (r^2) of 0. 6951, explains 69.51% variance in biological activity. This model also indicates statistical significance >99.9% with F values F = 42.431. Cross validated squared correlation coefficient of this model was 0.8039, which shows the good internal prediction power of this model. The graph of observed vs. predicted biological activities for the training and the test molecules is shown in Figure.

Model 4

Log_{10} (IC_50) = + 0.8838 H-Donor Count + 0.6714 T_2_Cl_6 + 0.6073 chi5chain+0.5364 +0.394 T_N_N_5 - 0.3801 T_2_O_4 + 3.6338

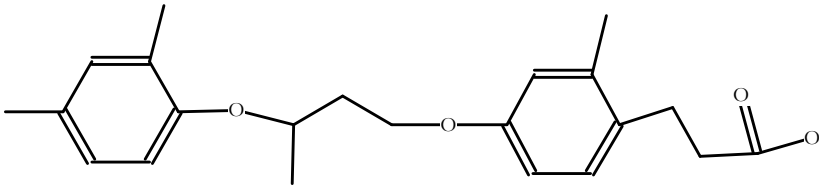
Optimum Components = 3, Degrees of Freedom = 13, n = 16, r^2= 0.7481 q2= 0.5673, F test 59.01 r2 se = 0.6251, q2 se = 0.5481, pred_r^2 = 0.7036, SEE = 0.291, SECV= 0.167, SEP=0.193, best_ran_r^2 = 0.136, best_ran_q^2 = 0.126, Zscore_ran_r2 =0.201, Zscore_ran_q2= 0.048, α_ran_r^2 = <0.00001, α _ran_q^2 = <0.01

Model – 3 shows good squared correlation coefficient (r^2) of 0. 7036 explains 70.36% variance in biological activity. This model also indicates statistical significance >99.9% with F values F = 59.01. Cross validated squared correlation coefficient of this model was 0.5481, which shows the good internal prediction power of this model. The graph of observed vs. predicted biological activities for the training and the test molecules is shown in Figure. In the above equations n is the number of compounds used to derive the model and values in parentheses are the 95% confidence limit of respective coefficient.

CONCLUSION

Models have given significant information to build a strategy to improve the biological activity of the compounds. Substituted methyl and ethyl groups at R^1 are essential for the biological activity. The CF_3 group at R^4 gives compounds with better biological activity than the ethyl substituents. The electrostatic contribution of 2-Pyridyl and 3-Pyridyl groups at R^3 led to compounds with good selectivity over PPARα and potent PPAR γ/δ affinity and functional activity.

Table-1 Substituted 3-{4-[3-(2-aryl-phenoxy) butoxy]-phenyl} propionic acids With IC_{50} and PIC_{50} values



Comp.	Polarizability AHC	Polarizability AHP	chi1	1-path count	chi3cluster	kappa3	Hydrogen count	SaaCH count	SdssC count
1mol2	51.841933	51.936	15.939892	35	2.044998	9.520661	34	11	1
8 mol2	45.062825	48.362	15.456729	34	1.976957	8.628099	31	6	2
9 mol2	45.062825	48.362	15.456729	34	1.976957	8.628099	31	6	2
10 mol2	46.838624	46.897	14.956729	33	1.976957	8.065844	30	9	1
11 mol2	49.105921	49.392	15.456729	34	1.976957	8.628099	31	10	1
12 mol2	49.105921	49.392	15.456729	34	1.976957	8.628099	31	10	1
13 mol2	49.105921	49.392	15.456729	34	1.976957	8.628099	31	10	1
14 mol2	47.684271	48.674	14.956729	33	1.976957	8.065844	29	8	1
15 mol2	47.684271	48.674	14.956729	33	1.976957	8.065844	29	8	1
17 mol2	45.885679	46.248	14.956729	33	1.976957	8.065844	29	8	1
18mol2	47.304559	47.65	14.918724	33	2.061508	8.373087	26	10	1
19 mol2	46.642874	47.284	16.130049	36	1.91717	9.119219	26	10	1
21 mol2	49.315506	49.828	16.668054	37	1.848135	9.356625	29	11	1
7mol2	50.00065	50.101	15.45673	34	1.976957	8.628099	32	11	1
20mol2	50.94605	51.227	15.99473	35	1.907921	8.864266	33	10	1
16mol2	45.88568	46.248	14.95673	33	1.976957	8.065844	29	8	1

Table 2- Calculated descriptors for 2D QSAR

Comp.	Polarizability AHC	Polarizability AHP	chi1	1-path count	chi3cluster	kappa3	Hydrogen count	SaaCH count	SdssC count
1mol2	51.841933	51.936	15.939892	35	2.044998	9.520661	34	11	1
8 mol2	45.062825	48.362	15.456729	34	1.976957	8.628099	31	6	2
9 mol2	45.062825	48.362	15.456729	34	1.976957	8.628099	31	6	2
10 mol2	46.838624	46.897	14.956729	33	1.976957	8.065844	30	9	1
11 mol2	49.105921	49.392	15.456729	34	1.976957	8.628099	31	10	1
12 mol2	49.105921	49.392	15.456729	34	1.976957	8.628099	31	10	1
13 mol2	49.105921	49.392	15.456729	34	1.976957	8.628099	31	10	1
14 mol2	47.684271	48.674	14.956729	33	1.976957	8.065844	29	8	1
15 mol2	47.684271	48.674	14.956729	33	1.976957	8.065844	29	8	1
17 mol2	45.885679	46.248	14.956729	33	1.976957	8.065844	29	8	1
18mol2	47.304559	47.65	14.918724	33	2.061508	8.373087	26	10	1
19 mol2	46.642874	47.284	16.130049	36	1.91717	9.119219	26	10	1
21 mol2	49.315506	49.828	16.668054	37	1.848135	9.356625	29	11	1
7mol2	50.00065	50.101	15.45673	34	1.976957	8.628099	32	11	1
20mol2	50.94605	51.227	15.99473	35	1.907921	8.864266	33	10	1
16mol2	45.88568	46.248	14.95673	33	1.976957	8.065844	29	8	1

SdssCE index	SaaCHE index	SsssCHE index	T_T_T_0	T_T_T_1	T_T_T_7	T_2_O_4	T_2_O_5	T_2_S_3
-0.777802	22.800776	0.016608	33	35	38	5	5	0
0.528201	12.383413	0.028399	32	34	33	6	6	2
0.716561	12.445273	0.038362	32	34	33	6	6	3
-0.784025	17.4986	-0.030439	31	33	30	7	7	0
-0.783101	19.770987	-0.026967	32	34	33	6	6	0
-0.782655	19.741284	-0.019597	32	34	33	6	6	0
-0.782281	19.743587	-0.014814	32	34	33	6	6	0
-0.777394	16.699043	0.011038	31	33	30	6	6	1
-0.776791	16.509719	0.021001	31	33	30	5	5	2
-0.787931	15.041408	-0.058217	31	33	30	7	7	0
-0.781538	19.830979	-0.013401	31	33	30	6	6	0
-0.839849	15.44125	-0.323948	34	36	39	6	6	0
-0.828856	18.009441	-0.296589	35	37	42	6	6	0
-0.77919	22.56846	0.000811	32	34	33	6	8	0
-0.77602	19.89915	-0.02739	33	35	36	6	8	0
-0.78726	14.96514	-0.04716	31	33	30	8	9	0

T_2_T_7	T_2_2_7	T_T_C_7	T_T_N_7	T_T_S_3	T_O_S_4	T_C_O_2	T_C_S_2	T_C_S_3
40	9	67	0	0	0	9	0	0
33	6	55	0	2	0	9	2	2
33	6	57	0	3	1	9	3	3
28	5	51	0	0	0	12	0	0
33	6	57	1	0	0	9	0	0
33	6	55	3	0	0	9	0	0
33	6	55	3	0	0	9	0	0
26	5	48	1	1	0	9	1	1
28	5	50	1	2	1	9	2	2
28	5	50	1	0	0	11	0	0
31	6	48	1	0	0	9	0	0
37	6	60	1	0	0	9	0	0
38	6	65	0	0	0	9	0	0
33	6	58	0	0	0	9	0	0
34	6	61	1	0	0	9	0	0
26	5	48	1	0	0	10	0	0

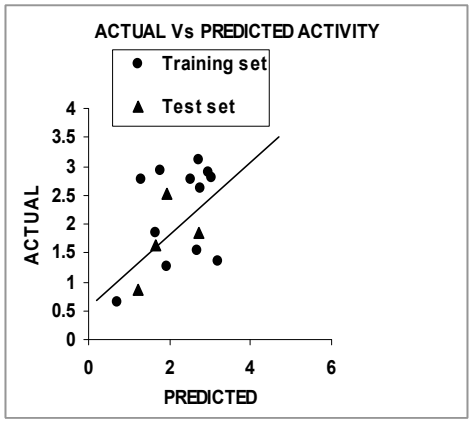
Table -3 – Actual and Predicted values for model-1 and model-2 with Residual values for 2D QSAR analysis

S No.	Molecules	Actual activity	Predicted model-1	Residual value	Predicted model-2	Residual value
1	1mol2	0.69897	0.656516	0.042454	0.660928	0.0038042
2	8mol2	2.75435	2.609566	0.144784	2.761691	-0.007269
3	9mol2	3.03743	2.795467	-0.241963	2.870308	0.167122
4	10mol2	2.97313	2.899258	0.73872	2.853939	0.119191
5	11mol2	1.66276	1.644796	0.017964	2.005534	-0.342774
6	12mol2	1.77815	1.842452	-0.064302	2.005791	-0.227641
7	13mol2	2.71611	1.842452	0.873658	2.006007	0.710103
8	14mol2	2.53908	2.917805	-0.378725	2.857762	-0.318682
9	15mol2	3.18469	3.103706	0.080984	2.85811	0.32658
10	17mol2	2.68395	2.777588	-0.093638	2.851686	-0.167736
11	18mol2	1.94448	2.534248	-0.589768	2.391473	-0.446993
12	19mol2	1.30103	1.348312	-0.047282	1.231278	0.069752

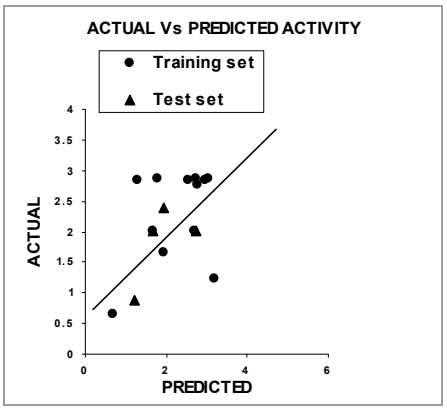
Table 4 - Actual Activity, Predicted Activity and Residual values of test set Compounds

1	21mol2	1.20412	0.854172	0.349948	0.879163	0.324957
2	7mol2	2.332438	1.547600	0.784838	2.007654	0.324784
3	20mol2	1.544068	1.251200	0.292868	1.652892	-0.108824
4	16mol2	2.990783	2.7561	0.234683	2.851948	0.138835

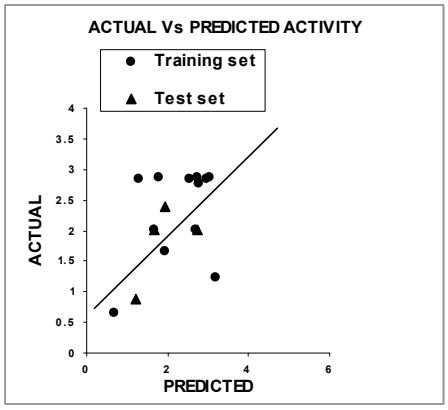
Figure-Actual and Predicted values for model-1 and model-2, model 3, model 4 2D QSAR



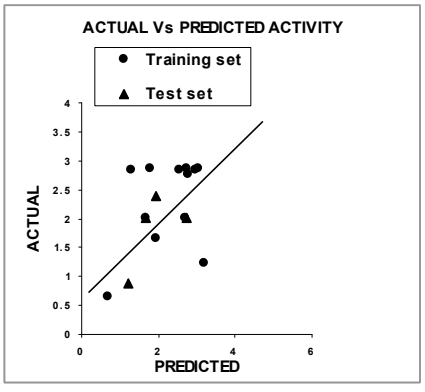
Model 1



Model 2



Model 3



Model 4

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