International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 1, Issue 1, July-Sep. 2009

Research article

A QSAR STUDY ON 5-HT$_7$ RECEPTOR ANTAGONISTS: DERIVATIVES OF (PHENYLPIPERAZINYL-ALKYL) OXINDOLE

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Received- 30 March 09, Revised and Accepted- 14 April 09

ABSTRACT

The 5-HT$_7$ receptor binding affinity of (phenylpiperazinyl-alkyl)oxindole derivatives are quantitatively studied using Fujita-Ban and Hansch type analyses. The Fujita-Ban study resulted in the contributions of different substituents and the parent moiety for the binding affinity. The substituents that have a higher positive contribution to the given activity, relative to substituents of the parent moiety at different positions were then used to obtain a trend for the active analogues. None of the R_1 substituents present at 5-, 6- and 7- positions appears to be advantageous over the substituents of the parent moiety for 5-HT$_7$ binding affinity. Similarly, the 3'-substituents of R_2 and spacer (n = 5) do not contribute positively to the activity. However, the Y-substitution and 4'-R_2 substituents contribute positively to the activity and certainly improve inhibitory actions of the compounds. The appropriate substituents for varying positions, which have highest positive contribution to the parent moiety, may be selected for the future design of more active analogues of the series. The optimal activities seem to be manifested by compounds in which Y and 4'-R_2 are substituted, respectively, by H and Cl or F. The Hansch type analysis, on the other hand, revealed that the R_1-substitutents at 5-position of oxindole benzene ring that exerts a higher positive steric effect and the R_2-substituent that offers higher electronic effects at the *meta* and *para* positions of the phenyl ring attached to the piperazine ring are beneficial in raising the binding affinity of a compound towards 5-HT$_7$ receptor. Similarly, the presence of a F-substitution at *para* position of the phenyl ring is also helpful in improving the activity of a compound.

Keywords: QSAR, 5-HT$_7$ receptor antagonists, Fujita-Ban and Hansch analysis, Binding affinity.

INTRODUCTION

5-hydroxytryptamine$_7$ (5-HT$_7$) receptor, the member of serotonin subfamily of G-protein coupled receptors[1,2], has implications in many CNS functions and disorders, like schizophrenia[3], depression[4-7], epilepsy[8], migraine[9,10] and control of circadian rhythm[11,12]. Thus, renders it as a novel drug target to cure such ailments. Only a few compounds have been reported, in recent years, as antagonists[13-18], selective agonists[15,19],

nonselective agonists[20,21] and partial agonists[15,19,22] at the 5-HT_7 receptor. However, the use of some of the potent compounds was limited due to strong side effects such as blood pressure and heart rate changes[20] or poor experimental metabolic stability. The representative chemical species showing metabolic stability and selectivity at 5-HT_7 receptor include tetrahydrobenzindoles[23], (2-methoxyphenyl) piperazinyls[24], DR4365[25], DR4446[26] and DR4485[27]. A synthetic study, with the aim to obtain compounds having strong 5-HT_7 receptor affinity and good selectivity over other receptors, was performed by Volk *et al.*[28]. The structure-activity relationship (SAR) studies on the aforesaid series of 1,3-dihydro-2*H*-indol-2-one (oxindole) were mainly concerned with the limited alteration of substituents at oxindole benzene ring, nitrogen atom, spacer and at different positions of the phenyl ring attached to the piperazine and provided no rationale to reduce the trial-and-error factors. Hence, a quantitative SAR (QSAR) study on aforesaid analogues is carried out here so as to provide the rationale for drug design and explore the possible mechanism of their action.

MATERIALS AND METHODS

The general structure of reported (phenylpiperazinyl-alkyl)oxindole analogues is shown in Figure 1.

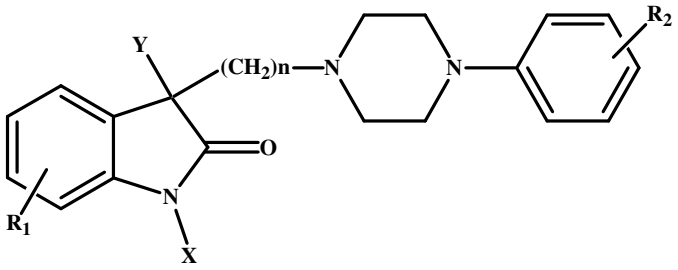

Fig. 1 : Structures of (phenylpiperazinyl-alkyl)oxindole analogues.

The biological effects and the appropriate quantifying parameters of substituents, present at different positions of the parent structure, are compiled in Table 1. The biological effects, reported as K_i, represents the radioligand binding of [^{3}H]LSD (NEN) on cloned human serotonin serotonin receptor subtype 7 (h5-HT_7) produced in CHO cells. In present study, for a given compound, it is expressed as pK_i on a molar basis. Both the Fujita-Ban and the Hansch types of analyses were carried out on these compounds to derive a QSAR employing the method of multiple regression analysis (MRA). The Fujita-Ban analysis[29] based on an additivity principle is a nonparametric

approach and requires, relatively, a larger data-set. In addition, the approach also requires certain group to occur two or more times at a given varying position in a molecule.

Table 1 : Structures, QSAR parameters and 5-HT$_7$ binding affinities of (Phenylpiperazinyl-alkyl) oxindole analogues.

S. No.	R_1	X	Y	n	R_2	Es_5	$\sigma_{3'}$	$\sigma_{4'}$	$I_{4'}$	pK_i(M) Obs[a]	F.B.	Eq 2)	LOO
1	H	H	Et	4	3´-Cl	0.00	0.37	0.00	0	9.39	9.20	9.01	8.97
2	H	H	Et	5	3´-Cl	0.00	0.37	0.00	0	8.84	8.86	9.01	9.03
3	H	H	Et	4	4´-Cl	0.00	0.00	0.23	0	9.42	9.23	8.91	8.94
4	H	H	Et	5	4´-Cl	0.00	0.00	0.23	0	8.91	8.89	9.02	9.03
5	H	H	Et	4	H	0.00	0.00	0.00	0	8.68	9.04	8.35	8.31
6	H	Me	Et	4	H	0.00	0.00	0.00	0	7.48	-[b]	-[c]	-[c]
7	H	H	Et	4	2´-OMe	0.00	0.00	0.00	0	8.27	-[b]	8.35	8.36
8	H	Bn	Et	4	2´-OMe	0.00	0.00	0.00	0	8.18	-[b]	8.35	8.38
9	5-F	H	Et	4	3´-Cl	-0.46	0.37	0.00	0	8.68	8.60	8.55	8.54
10	5-F	H	H	4	3´-Cl	-0.46	0.37	0.00	0	8.71	8.62	8.55	8.54
11	5-F	H	Et	4	4´-F	-0.46	0.00	0.06	1	8.82	8.63	8.73	8.69
12	5-F	H	H	4	4´-F	-0.46	0.00	0.06	1	8.47	8.65	8.73	8.87
13	H	H	i-Bu	4	3´-Cl	0.00	0.37	0.00	0	8.75	-[b]	9.01	9.05
14	H	H	H	4	3´-Cl	0.00	0.37	0.00	0	9.31	9.22	9.01	8.98
15	H	H	H	4	4´-Cl	0.00	0.00	0.23	0	8.16	-[c]	-[c]	-[c]
16	5-F	H	H	4	H	-0.46	0.00	0.00	0	7.36	-[c]	7.89	7.97
17	H	H	Et	4	3´-OMe	0.00	0.12	0.00	0	8.59	-[b]	8.57	8.57
18	H	H	Et	4	4´-OMe	0.00	0.00	-0.27	0	7.60	-[b]	7.58	7.56
19	H	H	Et	4	2´-Cl	0.00	0.00	0.00	0	8.29	-[b]	8.35	8.36
20	H	H	Et	4	4´-F	0.00	0.00	0.06	1	9.37	9.22	9.19	9.10
21	H	H	Et	4	3´,4´-di-Cl	0.00	0.37	0.23	0	9.20	9.39	9.67	9.86
22	H	H	Et	4	3´-Cl,4´-F	0.00	0.37	0.06	1	9.22	9.39	-[c]	-[c]
23	H	H	Et	4	3´-Cl,4´-Me	0.00	0.37	-0.17	0	9.18	-[b]	-[c]	-[c]
24	5-Cl	H	Et	4	3´-Cl	-0.97	0.37	0.00	0	8.08	8.25	8.04	8.03
25	6-F	H	Et	4	3´-Cl	0.00	0.37	0.00	0	9.17	9.08	9.01	8.99
26	5-Cl,6-F	H	Et	4	3´-Cl	-0.97	0.37	0.00	0	8.04	8.13	8.04	8.04
27	5-F,7-Cl	H	Et	4	3´-Cl	-0.46	0.37	0.00	0	8.32	8.31	8.55	8.57
28	5,7-di-Cl	H	Et	4	3´-Cl	-0.97	0.37	0.00	0	8.02	7.95	8.04	8.05
29	5-F	H	Et	4	4´-Cl	-0.46	0.00	0.23	0	8.55	8.63	8.55	8.56
30	5-Cl	H	Et	4	4´-Cl	-0.97	0.00	0.23	0	8.43	8.27	8.04	7.95
31	6-F	H	Et	4	4´-Cl	0.00	0.00	0.23	0	9.10	9.11	9.02	9.00
32	5-Cl,6-F	H	Et	4	4´-Cl	-0.97	0.00	0.23	0	8.16	8.16	8.04	8.02
33	5-F,7-Cl	H	Et	4	4´-Cl	-0.46	0.00	0.23	0	8.22	8.33	8.55	8.60
34	5,7-di-Cl	H	Et	4	4´-Cl	-0.97	0.00	0.23	0	7.99	7.98	8.04	8.06

[a]Taken from Ref. [28]; [b]Compound not included in Fujita-Ban study; [c]Outlier(s) of present study.

This may in turn give a better insight into the substitutional requirements for those analogues which have yet to be synthesized. The Hansch approach, on the other hand, is a parametric approach in which physicochemical or structural parameters are most commonly used as the correlative parameters. This method is generally used to increase the understanding of the mechanisms of action of a set of congeners and to direct drug design in a congeneric series as well as to attempt to predict biological activities quantitatively. In general, the approach is to set up the equations involving different combinations of the substituents constants, then to allow the correlative methods to aid in the selection of the 'best equation' justifying it statistically and avoiding chance correlations. For the present study, the most suitable quantifying parameters were found to be the Taft's steric parameter, *Es*, electronic, σ (*meta* and *para*). For this purpose, the physicochemical parameters were taken directly from the literature[30]. In addition to these parameters the indicator variables, representing the presence or absence of certain structural characteristics, was also used in the series.

All the models identified have further been put to a randomization test[31] by repeated randomization of the activity to discover the chance correlations, if any, associated with them. For this every model has been subjected to 100 simulation runs with scrambled activity. The scrambled activity models with regression statistics better than or equal to that of the original activity model have been counted to express the percent chance correlation of the model under scrutiny.

For each model, derived in n data points, a number of statistical parameters were obtained to access its overall statistical significance. These are: the multiple correlation coefficient (r), the standard deviation (s), the F-ratio between the variances of calculated and observed activities (F), the cross-validated indices, Q^2_{LOO}[32] and Q^2_{L4O} respectively from leave-one-out and leave-four-out procedures. In leave-five-out procedure a group of five compounds is randomly kept outside the analysis each time in such a way that all compounds, for once, become the part of the predictive groups. To be a reasonable QSAR model, q2 should be greater than 0.6, and a value of this index greater than 0.9 indicates an excellent model. Another parameter, r_{randY}(s.d.), is the mean random correlation coefficient of the regressions in the activity (Y) randomization study with its standard deviation from 100 simulations.

Additional statistical parameters, such as the Akaike's information criterion, AIC[33,34], the Kubinyi function, FIT[35,36] and the Friedman's lack of fit, LOF[37], have also been calculated to further validate the derived models. The AIC takes into account the statistical goodness of fit and the number of parameters that have to be estimated to achieve that degree of fit. The FIT, closely related to the F-value, proved to be a useful parameter for evaluating the quality of the models. A model which is derived in k independent descriptors, its F-value will be more sensitive if k is small while it becomes less sensitive if k is large. The FIT, on the other hand, will be less sensitive if k is small whereas it becomes more sensitive if k is large. The model that produces the lowest AIC value and highest FIT value is considered potentially the most useful and the best. The LOF factor takes into account the number of terms used in the equation and is not biased, as are other indicators, toward large number of parameters.

RESULTS AND DISCUSSION

In construction of the Fujita-Ban matrix, twenty six compounds of Table 1 were initially retained and compound 1 was considered as the reference or parent congener. Eight compounds (**6-8**, **13, 17-19**, **23**) from this Table were, however, not included in the above training set as the frequency of occurrence of certain groups in these compounds was only once. To be concise, the matrix comprising of 26 compounds (rows) and 10 substituents (including parent compound) pertaining to varying positions of the parent moiety (columns) is not documented here. The rows and columns of this matrix representing respectively the data-points and the independent variables while the activity values (pK_is) being considered as the dependent variable were subjected to MRA. The resulting statistical parameters of the study were:

$n = 26, r = 0.881, s = 0.332, F[10,15] = 5.217.$

where n, r, s and F are respectively the number of data-points in the training set, multiple regression coefficient, standard error of estimate and F-ratio between the variances of calculated and observed activities. Except the r-value, which accounts for 78 % of variance (r^2 = 77.6), the remaining statistical parameters of the analysis are slightly too poor to account for significant results. Possibly certain outlier compounds, present in the original training-set, are responsible for the inferiority in these parameters. The congener **15** and **16,** having 4'-Cl and 5-F substitutions respectively, are the compounds whose calculated pK_i values

were found to be much higher than the observed values. The data-points were, therefore, ignored further. In doing so, the corresponding row was removed from the Fujita-Ban matrix and the MRA of the new matrix lead to the results summarized in Table 2. The data given within the parentheses therein are the 90% confidence intervals. The improved statistical parameters of the study are:

$n = 24, r = 0.956, s = 0.189, F[10,13] = 13.761$

The r^2-value now accounts for 91% of the variance and the F-value stands significant at 99% level. The calculated values of pK_i for all the compounds in Table 1 are also in close agreement with the observed ones. The contributions of different substituents and that of the parent moiety obtained for the 5-HT_7 receptor binding affinity of oxindole analogues are given in are given in Table 2.

Table 2: Fujita-Ban contributions of substituents and parent moiety to the 5-HT_7 receptor binding affinities of titled compounds

Position	Substitution	Contribution to pK_i
$R_1(5)$	Cl	-0.953(±0.19)
	F	-0.595(±0.19)
$R_1(6)$	F	-0.118(±0.21)
$R_1(7)$	Cl	-0.298(±0.22)
Y	H	0.018(±0.23)
n	5	-0.340(±0.27)
$R_2(3')$	H	-0.163(±0.21)
$R_2(4')$	Cl	0.191(±0.23)
	F	0.186(±0.25)
Contribution of parent compound, μ		9.199(±0.15)

From this Table, the substituents that have a higher positive contribution to activity, relative to substituents of the parent moiety at different positions may easily be obtained. None of the R_1 substituents present at 5-, 6- and 7-positions appears to be advantageous over the substituents of the parent moiety for 5-HT_7 binding affinity. Similarly, the 3'- substituents of R_2 and spacer, n = 5, do not contribute positively to the activity. However, the Y-substitution and 4'-R_2 substituents contribute positively to the activity and certainly improve inhibitory actions of the compounds. The appropriate

substituents for varying positions, which have highest positive contribution to the parent moiety, may be selected for the future design of more active analogues of the series. The optimal activities seem to be manifested by compounds in which Y and 4'-R_2 are substituted, respectively, by H and Cl or F.

It is important to note that the Fujita-Ban approach cannot extrapolate beyond the substituents of the training set whereas the Hansch approach, discussed below for the entire data-set, can do so. A number of physicochemical parameters for the R_1-substituents of the oxindole benzene ring and R_2-substituents of phenyl ring attached to piperazine ring were selected in a systematic manner. A data-set consisting of substituent constants such as hydrophobicity, π, hydrogen-bond donor, HD, hydrogen-bond acceptor, HA, electronic (*meta* and *para*), σ, field, F, resonance, R, dipole moment, μ, Taft's steric, *Es*, molar refraction, MR, molecular weight, MW and van der Waals volume, V_w for each of the positions was considered for the compounds in Table 1. MR, MW, V_w was considered for the spacer $(CH_2)_n$. In this way, independent variables were then permuted appropriately for the varying positions and subjected to MRA. This leads to a large number of QSAR equations which were then subjected to various statistical tests. The correlation equation, which returned the highest r- and F-values and lowest s-value was finally retained for further consideration. From the generated data set for the analogues in Table 1, the Taft's steric parameter, *Es*, accounting for the steric hindrance exerted by R_1-substituents at 5-position, and electronic (*meta* and *para*), σ, accounting for the electronic effects of R_2-substituents at 3'- and 4', emerged as the most appropriate quantifying parameter and none for X- and Y-substituents and spacer. In addition, the indicator variables reflecting certain structural variations played an important role in developing significant correlations. The stepwise development of the most significant equation is shown in Table 3 and represented as Equation (1) for further discussion.

Table 3: Stepwise development of Equation (1); $pK_i = a_0 + a_1 Es_5 + a_2\sigma_{3'} + a_3\sigma_{4'} + a_4 I_{4'}$

a_0	a_1	a_2	a_3	a_4	r	s	F	Q^2_{LOO}	step
8.746	0.673(±0.40)				0.452	0.508	8.219	0.126	(i)
8.579	0.698(±0.37)	1.111(±0.76)			0.579	0.471	7.830	0.222	(ii)
8.396	0.882(±0.32)	1.713(±0.70)	2.280(±1.05)		0.736	0.398	11.853	0.430	(iii)
8.299	0.868(±0.29)	1.860(±0.68)	2.358(±0.94)	0.558(±0.33)	0.804	0.356	13.239	0.507	(iv)

pK_i = 8.299 + 0.868(±0.29)Es_5 + 1.860 (±0.68)$\sigma_{3'}$ + 2.358(±0.94)$\sigma_{4'}$ + 0.558 (±0.33)$I_{4'}$, n = 34, r = 0.804, s = 0.356, F [4,29] = 13.239, Q^2_{LOO} = 0.507, Q^2_{L4O} = 0.522,r_{randY}(sd) = 0.330 (0.115), FIT = 1.059, LOF = 0.185, AIC = 0.170 …(1)

where the indicator variable $I_{4'}$ highlights the presence of an F-substituent at the R_2-position in the phenyl ring. A value 1 or 0 for this variable indicates the presence or absence of an F-substituent in the 4'-position of the phenyl ring, bonded to the piperazine ring. In the randomization study (100 simulations runs), the identified models has not shown any chance correlation. The derived F-value for above Equation remained significant at 99% level and the q^2 index accounted for a significant model but the r^2-value has explained only for 65% of variance in observed activity values. To improve the significance of above equation the compounds having high residual activity were considered as the outliers. An outlier to a QSAR is identified normally by having a large standard residual activity and can indicate the limits of applicability of QSAR models[38]. There are many reasons for their occurrence in QSAR studies; for example, chemicals might be acting by a mechanism different from that of the majority of the data points. It is also likely that outlier might be a result of a random experimental error that could be significant when analyzing a large data set. Equation (1) is further improved by eliminating compounds **6, 15, 22** and **23** from the data set as their calculated activity value showed a large deviation from the observed one. No appropriate reason seems to be immediately apparent for their 'outlier' behavior. Me-substitution at oxindole nitrogen in compound **6**, Cl-substitution at *para*-position in compound **15** and for compounds **22** and **23** Cl-substitution at *meta*-position and a F- and Me-substitution, respectively, at *para*-position of the phenyl ring attached to the piperazine ring possibly entail an error in the reported experimental activity data. The resulting correlation, by ignoring the outlier congeners, is shown in Equation (2)

pK_i = 8.354 + 1.002(±0.22)Es_5 + 1.779 (±0.51)$\sigma_{3'}$ + 2.876(±0.75)$\sigma_{4'}$ + 0.667 (±0.28) $I_{4'}$ n = 30, r = 0.892, s = 0.259, F [4,25] = 24.3740 Q^2_{LOO} = 0.702, Q^2_{L4O} = 0.677, r_{randY}(sd) = 0.327(0.110), FIT = 2.119, LOF = 0.104, AIC = 0.094 …(2)

The statistical parameters of Equation (2) have now improved over to that of Equation (1). The r^2-value has explained for 80% of variance in observed activity values and q^2 index has accounted comparatively for a better robust model. The decreased values of parameters AIC and LOF and increased value of FIT

have further shown the superiority of this model over to that of the model in Equation (1). Equation (2) was also subjected to randomization process, where 100 simulations were carried out but none of the identified models has shown any chance correlation.

Further, the The variables used in deriving Equation (2), possess poor inter-correlations (Table 4) amongst themselves and thus satisfy an important criterion of statistical significance, the mutual orthogonal condition or independency. The equation was, therefore, used to calculate the activity of compounds of the test data set. These values, listed in Table 1, were found to be in close agreement with the observed values.

Table 4 : Intercorrelation matrix[a] amongst the predictor variables of Eq. (2)

	Es_5	$\sigma_{3'}$	$\sigma_{4'}$	$I_{4'}$
Es_5	1.000	0.070	0.227	0.009
$\sigma_{3'}$		1.000	0.336	0.283
$\sigma_{4'}$			1.000	0.017
$I_{4'}$				1.000

[a]Matrix elements are the r-values.

The predicted values of all the analogues, obtained through the LOO approach, were also given in this Table for the sake of comparison. The plot showing the variation of observed versus calculated through Equation (2) and predicted activities obtained through LOO method for the compounds in Tables 1 is shown in Figure 2.

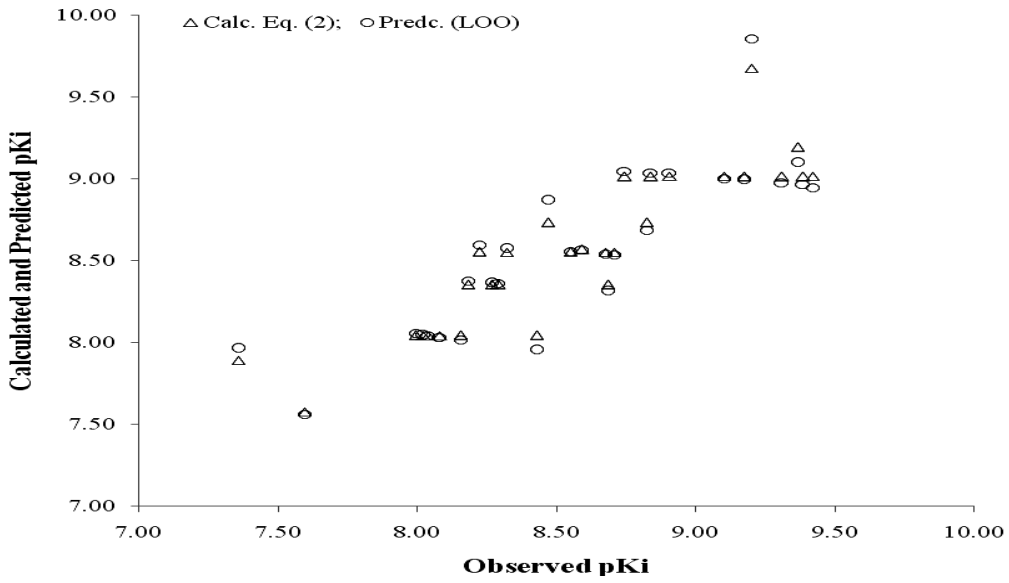

Fig 2. Plot between observed versus calculated and predicted pK_i values.

Such a demonstration may help to understand the goodness of fit and to identify systematic variation of observed versus calculated and predicted activities for the compounds under present study. The derived regression coefficients of

various descriptors in Equation (2) are all positive. It therefore follows that the R_1-substitutents at 5-position of oxindole benzene ring that exerts a higher positive steric effect and the R_2-substituent that offers higher electronic effects at the *meta* and *para* positions of the phenyl ring attached to the piperazine ring are beneficial in raising the binding affinity of a compound towards 5-HT_7 receptor. Similarly, the presence of a F-substitution at *para* position of the phenyl ring is also helpful in improving the activity of a compound.

CONCLUSION

This strategy may, therefore, be followed for designing higher potency compounds for future synthesis. These guidelines may, therefore, provide a basis for rationalizing substituent selection in the future designing of selective 5-HT_7 receptor ligands based on oxindole moiety. The study may also help in proposing the possible mode of action of oxindole analogues at the molecular level.

Acknowledgements

Authors are thankful to the Department and the Institution for providing the necessary facilities to complete this work.

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