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QSAR Study on Some Dihydrofolate Reductase Inhibitors[#]

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Abstract

Dihydrofolate reductase (DHFR) inhibitors have proved to be of value as antibacterial, antimalarial, and antitumor agents. Some 2,4–diamino–5–methyl–6–[(substituted anilino)methyl]pyrrido[2,3–*d*]pyrimidines were reported earlier as DHFR inhibitors. Using non–parabolic Hansch models, a QSAR study was performed in an attempt to find out the required physicochemical and structural features of these compounds for DHFR inhibition. This study revealed the importance of resonance effect at R2 and R3 positions and sum of molar refractivity (ΣMR) at R2, R3, R4, and R5 positions of the ring C. Lipophilicity of the whole molecule (log *P*) also played an important role. The presence of OCH_3 group at R4 of the phenyl C ring and CH_3 at R1 of anilino N might be advantageous to DHFR inhibition. This QSAR study is beneficial for future studies to carry out further tailoring of this type of compounds with an objective to increase DHFR inhibitory activity.

Keywords. QSAR; pyrimidines; dihydrofolate reductase; DHFR; inhibitors; Hansch analysis.

1 INTRODUCTION

Inhibition of dihydrofolate reductase (DHFR) is of particular interest since DHFR inhibitors have already proved to be having value as antibacterial [1–2], antimalarial [3–4], and antitumor agents [5–6]. DHFR catalyzes the NADPH–dependent formation of 5,6,7,8– tetrahydrofolate (THF) from 7,8–dihydrofolate (DHF). The most significance consequence of DHFR inhibition by antifolate is a decrease of thymidylate biosynthesis by means of depletion of the N5, N10–methylene–THF pool resulting in inhibition of DNA synthesis and cell death [7]. Several series of antifolates were reported as inhibitors of rat liver (rl), *Toxoplasma gondii* (tg), and *Pneumocystis carinii* (pc) DHFR [8–11].

Quantitative structure–activity relationships (QSAR) models are highly effective in explaining the structural basis of biological activity, and a literature survey shows a lot of work going on this subject. Some recent QSAR applications to various classes of compounds and their biological

[#] Dedicated to Professor Haruo Hosoya on the occasion of the 65th birthday.

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targets are evidence of that. Hirashima *et. al*. reported inhibition of sex–pheromone production in *Plodia interpunctella* [12], Hadjipavlou–Litina and Pontiki worked on lipoxygenase inhibitors [13], Garcia–Domenech and coworkers [14] showed prediction of antifungal activity could be done with topological descriptors, Debnath and his group performed QSAR study on some antitumor agents [15] while Vračko and Gasteiger studied flavonoid derivatives [16]. QSAR studies were performed for different dihydrofolate reductase inhibition by various compounds earlier. Selassie *et. al*. performed QSAR study on inhibition of chicken liver dihydrofolate reductase by a series of benzyl pyrimidines [17], Booth and his coworkers worked with QSAR study on inhibition of Leishmania DHFR by triazines [18], Selassie *et. al*. reported QSAR result of inhibition of DHFR from *Lactobacillus casei* and chicken liver by diaminopyrimidines [19] and that of Escherichia coli by benzyl pyrimidines [20], Selassie and coworkers also reported QSAR study on inhibition of Leukemia 1210 (L1210) DHFR by triazines [21].

In this article, we report the QSAR study on some 2,4–diamino–5–methyl–6–[(substituted anilino)methyl]pyrrido[2,3–*d*]pyrimidines [11] as DHFR inhibitors using Hansch analysis in order to identify the physicochemical and structural features required or responsible for DHFR inhibition as a part of our composite program of rational drug design [22–26]. Figure 1 shows the general structure of 2,4–diamino–5–methyl–6–[(substituted anilino)methyl]pyrrido[2,3–*d*]pyrimidines.

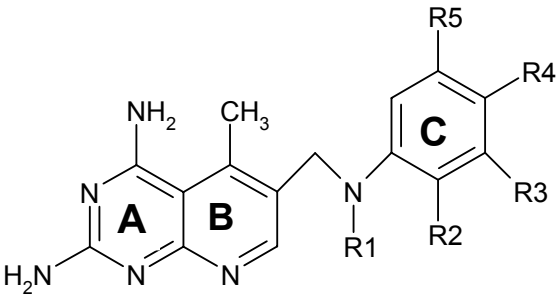

Figure 1. General structure of 2,4–diamino–5–methyl–6–[(substituted anilino)methyl]pyrrido[2,3–*d*]pyrimidines.

2 MATERIALS AND METHODS

The QSAR study was performed using the non–parabolic Hansch model where DHFR inhibitory activities in logarithmic scale were considered as dependent variable while physicochemical and structural parameters as independent variables. Regression analysis was done using the software Statistica. The DHFR inhibition data, reported by Gangjee *et.al*. [11], of 2,4–diamino–5–methyl–6–[(substituted anilino)methyl]pyrrido[2,3–*d*]pyrimidines on *Pneumocystis carinii* dihydrofolate reductase (pcDHFR), rat liver dihydrofolate reductase (rlDHFR) and *Toxoplasma gondii* dihydrofolate reductase (tgDHFR) are listed in Table 1. IC_{50} in nM were transformed to pIC_{50} (negative logarithm of IC_{50}) to consider under the non–parabolic Hansch model (pcDHFR to pC_1; rlDHFR to pC_2; tgDHFR to pC_3). The physicochemical parameters used which are listed in Table 2, namely the hydrophobic constant π, resonance effect R, molar refractivity MR, which were

compiled from the literature [27–29]. The hydrophobic parameter for the whole molecule, log *P* was calculated using ChemOffice software. Also, several indicator parameters were also used to describe the effects of some substituents at specific positions.

Table 1. Biological activity data and log *P* values of the 2,4–diamino–5–methyl–6–[(substituted anilino)methyl] pyrrido[2,3–*d*]pyrimidines

Cd [a]	R1	R2	R3	R4	R5	IC_{50}, nM [b]			log *P* [c]
						pcDHFR	rlDHFR	tgDHFR	
1	H	H	OCH_3	OCH_3	OCH_3	86	2.1	7.4	2.35
2	CH_3	H	OCH_3	OCH_3	OCH_3	13	7.6	0.85	3.14
3	H	H	Cl	Cl	Cl	63	33	12	4.40
4	CH_3	H	Cl	Cl	Cl	104.5	36.3	38.1	5.19
5	H	H	OCH_3	OCH_3	H	44	7.6	8.8	2.48
6	CH_3	H	OCH_3	OCH_3	H	320	44	29	3.26
7	CH_3	OCH_3	H	H	OCH_3	216	407	30.1	3.26
8	H	H	Cl	Cl	H	320	53	28	3.84
9	CH_3	H	Cl	Cl	H	100	42	27	4.63
10	H	H	H	H	H	80	170	17	2.73
11	H	OCH_3	H	H	H	117	169	23	2.60
12	H	H	OCH_3	H	H	68.9	80.1	7.4	2.60
13	H	H	H	OCH_3	H	95.4	55.6	12	2.60
14	H	Cl	H	H	H	47	88	7.1	3.29
15	H	H	Cl	H	H	23	37	11	3.29
16	H	H	H	Cl	H	55.4	51	19	3.29
17	H	H	H	Br	H	80.8	34.9	9.5	3.56
18	CH_3	H	OCH_3	H	H	30	18	6.3	3.39
19	CH_3	H	H	OCH_3	H	35	13	7.3	3.39
20	CH_3	Cl	H	H	H	84	100	18	4.07
21	CH_3	H	H	Cl	H	29	26	5.4	4.07
22	CH_3	H	H	Br	H	37	36	30	4.35

[a] Cd = compound number; [b] taken from Ref. [11]; [c] calculated using ChemOffice

Table 2. Physicochemical parameters used for the aromatic substituents compiled from Refs. [27–29]

Substituents	R	π	MR
OCH_3	–0.51	–0.02	0.79
Cl	–0.15	0.71	0.60
Br	–	–	0.89

3 RESULTS AND DISCUSSION

When *Pneumocystis carinii* dihydrofolate reductase (pcDHFR) inhibition data [11] were correlated with physicochemical parameters, the statistical parameters do not show a significant QSAR model for Model 1 from Eq. (1):

$$pC_1 = -1.738(\pm0.103) + 0.867(\pm0.516)R\_R2 - 0.279(\pm0.258)\pi R3 + 0.014\ (\pm0.224)I_1 \qquad (1)$$

where R_R2 is the resonance effect of the substituents at position R2, πR3 is the hydrophobic parameter for the substituents at R3 position and I_1 is the indicator variable for simultaneous presence of OCH_3 group at R4 of ring C respectively and CH_3 at R1 position linked with anilino N joining pyrrido–pyrimidine. The statistical parameters of Eq. (1) are shown in Table 3. The values

within the parenthesis are the confidence intervals for the parameters at a certain probability and shown in Table 4.

Table 3. Statistical parameters of Eqs. (1) to (6)

Model	Eq.	n [a]	DC [b]	*r* [c]	%EV [d]	F [e]	P [f]	S.E.E. [g]
1	(1)	22	–	0.410	16.81	1.21	0.334	0.343
	(2)	18	3, 6, 15, 21	0.813	66.13	9.11	0.001	0.205
2	(3)	22	–	0.836	69.92	13.94	0.000	0.298
	(4)	18	6, 13, 18, 21	0.943	88.90	37.21	0.000	0.202
3	(5)	22	–	0.538	28.97	2.45	0.097	0.337
	(6)	18	6, 14, 17, 21	0.918	84.28	25.01	0.000	0.169

[a] n, [b] DC, [c] *r*, [d] %EV, [e] F, [f] p, [g] S.E.E. are number of data points, deleted compound, correlation coefficient, percentage of explained variance, ratio between the variances of observed and calculated activities, probability factor and standard error of estimate, respectively

Table 4. p values for the equations of different QSAR equations of Model 1 to 3

Model	Eq.	Parameters	*p*–value	Confidence intervals (%)	Eq.	Parameters	*p*–value	Confidence intervals (%)
1	(1)	Intercept	0.000	100.0	(2)	Intercept	0.000	100.0
		R_R2	0.110	89.0		R_R2	0.110	89.0
		πR_3	0.295	70.5		πR_3	0.008	99.2
		I_1	0.952	4.8		I_1	0.017	98.3
2	(3)	Intercept	0.000	100.0	(4)	Intercept	0.000	100.0
		R_R2	0.001	99.9		R_R2	0.000	100.0
		ΣMR	0.019	98.1		ΣMR	0.178	82.2
		I_2	0.083	91.7		I_2	0.000	100.0
3	(5)	Intercept	0.059	94.1	(6)	Intercept	0.000	100.0
		R_R3	0.300	70.0		R_R3	0.000	100.0
		Log P	0.243	75.7		Log P	0.054	94.6
		I_1	0.202	79.8		I_1	0.000	100.0

After deleting the outliers (**3**, **6**, **15**, **21**), which may be acting indirectly, we obtain:

$$pC_1 = -1.767(\pm0.064) + 0.800(\pm0.311)R\_R2 - 0.578(\pm0.188)\pi R3 + 0.433\ (\pm0.159)I_1 \quad (2)$$

The statistical parameters of Eq. (2) are shown in Table 3. Eq. (2) indicated that the resonance effect at R2 position of the phenyl C ring and simultaneous presence of methyl at R1 of anilino N and methoxy at R4 position of ring C are contributing to the activity. Hydrophobic substituents at R3 position of phenyl C ring are detrimental to the activity. The correlation matrix, observed and calculated values of activity data for Eqs. (1) and (2) of Model 1 are shown in Table 5 and 6, respectively.

In case of rat liver dihydrofolate reductase (rlDHFR) inhibition [11] in Model 2, biological activity is better modeled using physicochemical and indicator parameter as found in Eq. (3):

$$pC_2 = -2.071(\pm0.186) + 1.710(\pm0.444)R\_R2 + 0.368(\pm0.143)\Sigma MR + 0.316(\pm0.172)I_2 \quad (3)$$

where ΣMR is the sum of the molar refractivity for the positions R2, R3, R4, and R5, and I_2 is the indicator variable for the methoxy (OCH_3) group at position R4 of the phenyl C ring. The value of I_2 is 1 for the presence of OCH_3 at position R4, otherwise zero. The positive coefficient of R_R2 indicates that resonance effect at position R2 might be favorable to the biological activity. Positive coefficient of ΣMR revealed that steric effect at R2, R3, R4, R5 of ring C is advantageous to the activity. The statistical parameters of Eq. (3) are shown in Table 3.

Table 5. Correlation matrices for Eqs. (1) and (2) of Model 1

Eq. (1)	R_R2	πR3	I_1	pC_1	Eq. (2)	R_R2	πR3	I_1	pC_1
R_R2	1.00	0.21	0.16	0.33	R_R2	1.00	0.19	0.16	0.39
πR3	0.21	1.00	–0.22	–0.17	πR3	0.19	1.00	–0.16	–0.49
I_1	0.16	–0.22	1.00	0.13	I_1	0.16	–0.16	1.00	0.59
pC_1	0.33	–0.17	0.13	1.00	pC_1	0.39	–0.49	0.59	1.00

Table 6. Observed, calculated, residual and LOO–calculated values for Eqs. (1) and (2) of Model 1

Cd [a]	Obs [b]	Eq. (1)			Eq. (2)		
		Calc [c]	Res [d]	L–calc [e]	Calc [c]	Res [d]	L–calc [e]
1	–1.935	–1.733	–0.202	–1.718	–1.756	–0.179	–1.735
2	–1.114	–1.719	0.605	–2.022	–1.323	0.209	–1.533
3	–1.799	–1.936	0.137	–1.97	–	–	–
4	–2.019	–1.936	–0.083	–1.916	–2.178	0.159	–2.257
5	–1.644	–1.733	0.089	–1.742	–1.756	0.112	–1.769
6	–2.505	–1.719	–0.786	–1.326	–	–	–
7	–2.335	–2.181	–0.154	–2.048	–2.175	–0.160	–2.037
8	–2.505	–1.936	–0.569	–1.794	–2.178	–0.327	–2.014
9	–2.000	–1.936	–0.064	–1.920	–2.178	0.178	–2.267
10	–1.903	–1.739	–0.165	–1.722	–1.767	–0.136	–1.753
11	–2.068	–2.181	0.113	–2.278	–2.175	0.107	–2.268
12	–1.838	–1.733	–0.105	–1.722	–1.756	–0.082	–1.746
13	–1.980	–1.738	–0.242	–1.715	–1.767	–0.213	–1.744
14	–1.672	–1.868	0.197	–1.885	–1.887	0.215	–1.906
15	–1.362	–1.936	0.574	–2.08	–	–	–
16	–1.744	–1.738	–0.006	–1.738	–1.767	0.023	–1.77
17	–1.907	–1.738	–0.169	–1.722	–1.767	–0.139	–1.752
18	–1.477	–1.733	0.256	–1.760	–1.756	0.279	–1.788
19	–1.544	–1.725	0.181	–1.815	–1.335	–0.209	–1.125
20	–1.924	–1.868	–0.055	–1.864	–1.887	–0.037	–1.884
21	–1.462	–1.738	0.276	–1.766	–	–	–
22	–1.568	–1.738	0.170	–1.755	–1.767	0.199	–1.789

[a] Cd. = compound number; [b] Obs = observed value; [c] Calc = calculated value; [d] Res = residual; [e] L–calc = LOO calculated value

When compounds **6**, **13**, **18**, **21** were deleted on the same basis as in Eq. (1), much better relationship was obtained as shown in Eq. (4):

$$pC_2 = -2.039(\pm0.140) + 1.527(\pm0.306)\ R\_R2 + 0.284(\pm0.106)\Sigma MR + 0.683(\pm0.143)I_2 \quad (4)$$

The statistical parameters of Eq. (4) are shown in Table 3. These compounds (**6**, **13**, **18**, **21**) did not fit with the model, possibly due to some different type(s) of inhibitory action. The Eq. (4)

explains the activity data up to 88.90% and also indicates that the presence of methoxy group at R4 position is favorable to the activity. The correlation matrix, observed and calculated values of the activity data for the Eqs. (3) and (4) of Model 2 are shown in the Table 7 and 8, respectively.

Table 7. Correlation matrices for Eqs. (3) and (4) of Model 2

Eq. (3)	R_R2	ΣMR	I_2	pC_2	Eqn. (4)	R_R2	ΣMR	I_2	pC_2
R_R2	1.00	0.02	0.25	0.60	R_R2	1.00	0.05	0.24	0.61
ΣMR	0.02	1.00	0.51	0.55	ΣMR	0.05	1.00	0.56	0.61
I_2	0.25	0.51	1.00	0.61	I_2	0.24	0.56	1.00	0.81
pC_2	0.60	0.55	0.61	1.00	pC_2	0.61	0.61	0.81	1.00

Table 8. Observed, calculated, residual and LOO–calculated values for Eqs. (3) and (4) of Model 2

Cd [a]	Obs [b]	Eq. (3)			Eq. (4)		
		Calc [c]	Res [d]	L–calc [e]	Calc [c]	Res [d]	L–calc [e]
1	–0.322	–0.845	0.523	–1.045	–0.653	0.331	–0.811
2	–0.881	–0.845	–0.036	–0.832	–0.653	–0.228	–0.545
3	–1.519	–1.371	–0.148	–1.333	–1.498	–0.020	–1.492
4	–1.560	–1.371	–0.189	–1.323	–1.498	–0.062	–1.480
5	–0.881	–1.098	0.217	–1.141	–0.848	–0.033	–0.837
6	–1.644	–1.098	–0.546	–0.989	–	–	–
7	–2.610	–2.286	–0.323	–1.954	–2.310	–0.299	–1.991
8	–1.724	–1.554	–0.169	–1.537	–1.639	–0.085	–1.630
9	–1.623	–1.554	–0.069	–1.547	–1.639	0.016	–1.641
10	–2.230	–1.920	–0.310	–1.839	–1.922	–0.309	–1.815
11	–2.228	–2.539	0.311	–2.824	–2.505	0.277	–2.762
12	–1.904	–1.667	–0.237	–1.647	–1.727	–0.178	–1.708
13	–1.745	–1.351	–0.394	–1.200	–	–	–
14	–1.945	–1.993	0.048	–1.999	–2.010	0.065	–2.018
15	–1.568	–1.737	0.169	–1.754	–1.781	0.212	–1.808
16	–1.708	–1.737	0.029	–1.740	–1.781	0.073	–1.790
17	–1.543	–1.630	0.087	–1.638	–1.698	0.155	–1.714
18	–1.255	–1.667	0.412	–1.702	–	–	–
19	–1.114	–1.351	0.236	–1.441	–1.043	–0.070	–0.985
20	–2.000	–1.993	–0.006	–1.993	–2.010	0.010	–2.011
21	–1.415	–1.737	0.322	–1.770	–	–	–
22	–1.556	–1.630	0.074	–1.636	–1.698	0.142	–1.713

[a] Cd. = compound number; [b] Obs = observed value; [c] Calc = calculated value;
[d] Res = residual; [e] L–calc = LOO calculated value

When *Toxoplasma gondii* dihydrofolate reductase (tgDHFR) inhibition data [11] were investigated for correlation with physicochemical parameters in Model 3, it was found that the statistical indices of Eq. (5) are not high enough:

$$pC_3 = -0.776(\pm0.384) - 0.398(\pm0.371)R\_R3 + 0.124(\pm0.102)\log P + 0.292(\pm0.220)I_1 \quad (5)$$

where, R_R3 is the resonance effect of the substituents at R3 position of the phenyl C ring and $\log P$ is the hydrophobic constant for the whole molecule. The negative coefficient of R_R3 indicated that resonance effect at R3 position of ring C might be detrimental to the activity. The lipophilicity of the compounds might help to the activity. Lipophilic property of these compounds might be required for penetrating the cell membrane barrier to exhibit DHFR inhibitory activity. The statistical parameters of the Eq. (5) are shown in Table 3. When outliers (**6**, **14**, **17**, **21**) were

deleted using the same basis as for Eqs. (1) and (3), significant improved relationship was obtained as found in Eq. (6):

$$pC_3 = -0.981(\pm0.198) - 0.908(\pm0.199)R\_R3 + 0.110(\pm0.052)\log P + 0.712(\pm0.128)I_1 \quad (6)$$

The statistical parameters for Eq. (6) are shown in Table 3. Eq. (6) explains the variances of the activity data up to 84.28 %. Eq. (6) also indicates that simultaneous presence of OCH_3 group at R4 of the phenyl C ring and CH_3 at R1 position of anilino N are required for better activity. The correlation matrix, observed and calculated activities of Eqs. (5) and (6) of model 3 are shown in Table 9 and 10 respectively. The predictive powers of the Eqs. (1) to (6) were confirmed by leave–one–out (LOO) method [25]. The LOO calculated values for Models 1–3 are shown in Tables 6, 8 and 10, respectively.

Table 9. Correlation matrices for Eqs. (5) and (6) of Model 3

Eq. (5)	R_R3	log *P*	I_1	pC_3	Eqn. (6)	R_R3	log *P*	I_1	pC_3
R_R3	1.00	0.33	–0.31	–0.40	R_R3	1.00	0.32	–0.12	–0.66
log *P*	0.33	1.00	–0.10	–0.36	log *P*	0.32	1.00	–0.07	–0.44
I_1	–0.31	–0.10	1.00	0.37	I_1	–0.12	–0.07	1.00	0.67
pC_3	–0.40	–0.36	0.37	1.00	pC_3	–0.66	–0.44	0.67	1.00

Table 10. Observed, calculated, residual and LOO values for Eqs. (5) and (6) of Model 3

Cd [a]	Obs [b]	Eq. (5)			Eq. (6)		
		Calc [c]	Res [d]	L–calc [e]	Calc [c]	Res [d]	L–calc [e]
1	–0.869	–0.863	–0.006	–0.861	–0.777	–0.093	–0.744
2	0.071	–0.668	0.739	–1.095	–0.151	0.222	–0.462
3	–1.079	–1.259	0.180	–1.287	–1.329	0.250	–1.371
4	–1.581	–1.357	–0.224	–1.250	–1.416	–0.165	–1.327
5	–0.945	–0.879	–0.066	–0.860	–0.791	–0.154	–0.742
6	–1.462	–0.683	–0.779	–0.229	–	–	–
7	–1.479	–1.179	–0.301	–1.149	–1.340	–0.139	–1.322
8	–1.447	–1.90	–0.257	–1.172	–1.267	–0.180	–1.252
9	–1.431	–1.288	–0.143	–1.257	–1.354	–0.077	–1.335
10	–1.230	–1.112	–0.117	–1.092	–1.281	0.051	–1.293
11	–1.362	–1.097	–0.265	–1.040	–1.267	–0.095	–1.243
12	–0.869	–0.894	0.025	–0.901	–0.804	–0.065	–0.783
13	–1.079	–1.097	0.018	–1.101	–1.267	0.188	–1.264
14	–0.851	–1.182	0.331	–1.214	–	–	–
15	–1.041	–1.122	0.081	–1.127	–1.207	0.165	–1.218
16	–1.279	–1.182	–0.097	–1.173	–1.343	0.064	–1.351
17	–0.978	–1.215	0.237	–1.235	–	–	–
18	–0.799	–0.995	0.196	–1.045	–0.891	0.092	–0.916
19	–0.863	–0.902	0.039	–0.937	–0.642	–0.222	–0.330
20	–1.255	–1.278	0.023	–1.281	–1.429	0.174	–1.452
21	–0.732	–1.278	0.546	–1.335	–	–	–
22	–1.477	–1.313	–0.164	–1.290	–1.460	–0.017	–1.456

[a] Cd. = compound number; [b] Obs = observed value; [c] Calc = calculated value;
[d] Res = residual; [e] L–calc = LOO calculated value

4 CONCLUSIONS

This QSAR study throws some light first time on substitutional requirements for *Pneumocystis carinii*, rat liver, and *Toxoplasma gondii* DHFR inhibitory action of 2,4–diamino–5–methyl–6–[(substituted anilino) methyl]pyrrido[2,3–*d*]pyrimidines. Resonance effects on R2 and R3, sum of molar refractivity at R2, R3, R4, and R5 of the phenyl ring C, lipophilic property of the molecule, and presence of OCH_3 at R4 of ring C and CH_3 at R1 position of anilino N play major roles in pcDHFR, rlDHFR and tgDHFR inhibition as evidenced by this work and were well supported by X–ray data as well as conformational changes of DHFR from different species [30–31]. This work may be useful for tailoring of this type of compounds keeping the anilino moiety with phenyl C ring, which will be beneficial for future drug design of antifolates.

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