Supplementary Information for

Designing and screening of fluoroquinolone substitutes using combined in-silico approaches: biological metabolism-bioconcentration bilateral selection and their mechanism analyses

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2. Materials and methods

2.4. Construction of the 3D-QSA^{2}R model for the bidirectional selective effect of the biological metabolism and concentration of FQs using the SYBYL-X2.0

In this study, the comparative molecular similarity index analysis (CoMSIA) was used to construct the model; according to the three-dimensional quantitative structure-activity relationship between molecular structure and BSEV of FQs, the least square method was used for the analysis.[1] The leave-one-out method was used to cross-validate the training set compounds, and the cross-validation coefficient q^2 and the best principal component N were obtained. Regression analysis by non-cross validation was performed, and the non-cross validation coefficient r^2, standard deviation SEE, and test value F were evaluated. The Q^2_{ext} and cSDEP values were obtained by the perturbation stability test. Finally, r^2_{pred} was obtained by cross-validation of test sets (Cross-Validate). After the above parameters met the requirements, the CoMSIA model was constructed.[2] According to the "View QSAR" module in the constructed 3D-QSA^{2}R model, the three-dimensional contour maps of the CoMSIA model were analyzed to determine the substitution groups and substitution sites of the target molecule.

2.6. Drug effect, human health, and ecological risk evaluation of the substitutes of FQs

2.6.1. Evaluation of the effect of the substitutes of FQs using the ADMET model

The ADMET model in the Discovery Studio 2020 (DS) software can provide an efficacy evaluation model (water solubility at 25 °C, permeability of blood-brain barrier, intestinal absorption, human cytochrome P450 2D6 enzyme binding, and plasma protein binding), which can be used to predict the absorption, distribution, metabolism, excretion, and hepatotoxicity of substitute molecules.[3]

2.6.2. Human health and ecological risk evaluation of the substitutes of FQs using the TOPKAT model

Based on the DS software ADMET module, the hepatotoxicity of the substitute molecules was predicted, and the TOPKAT module was used to predict the toxicity of the substitute molecule.[4] The TOPKAT module contains 14 toxicity prediction models. In the study, Rodent Carcinogenicity (NTP

and FDA), Mutagenicity (Ames test), Developmental Toxicity Potential (DTP), Skin Sensitization, Skin Irritancy, Ocular Irritancy, Rat Chronic Oral LOAEL, Rat Oral LD_{50}, and Inhalational LC_{50} were selected as human health risk evaluation parameters. Fathead Minnow LC_{50}, and Daphnia Magna EC_{50}, and Aerobic Biodegradability were used as the ecological risk evaluation parameters.

3. Results and discussion

3.5. Drug characteristics, human health and ecological risk evaluation of NOR substitute molecules based on ADMET and TOPKAT

3.5.1. Evaluation of molecular drug properties of NOR substitutes based on the ADMET model

The ADMET model was used to predict and evaluate the absorption, distribution, metabolism, excretion, and toxicity of 30 NOR substitutes. The results of the prediction and evaluation are shown in Table S4 and Fig. S1.

Table S1 The ΔG_b of FQs of metabolic and concentration enzymes and their BSEV in fish

Compounds	FQs	Binding energy (kJ/mol)		Dimensionless values		BSEV
		Dock to 4R1Z	Dock to 4HGM	BMI	BEI	
1[b, c, f]	Amifloxacin	-24.027	-47.049	0.000	0.799	0.336
2[a, c, e]	Balofloxacin	-84.667	-150.620	0.913	0.000	0.529
3[a, d]	Besifloxacin	-74.005	-39.721	0.752	0.856	0.796
4[a, c, e]	Cinoxacin	-44.157	-40.624	0.303	0.849	0.532
5[a, c]	Ciprofloxacin	-40.829	-65.347	0.253	0.658	0.423
6[e]	Clinafloxacin	-60.209	-106.563	0.545	0.340	0.459
7[a, f]	Danofloxacin	-57.717	-23.322	0.507	0.982	0.707
8[c]	Difloxacin	-61.187	-101.864	0.559	0.376	0.482
9[a]	Enoxacin	-55.361	-60.869	0.472	0.693	0.565
10[a, d, e]	Enrofloxacin	-50.994	-46.514	0.406	0.803	0.573
11[a, c, e]	Fleroxacin	-46.226	-40.395	0.334	0.851	0.551
12[a, d, e]	Gatifloxacin	-57.319	-109.039	0.501	0.321	0.425
13[a]	Grepafloxacin	-52.775	-63.598	0.433	0.671	0.533
14[e]	Levofloxacin	-90.464	-33.756	1.000	0.902	0.959
15[c, e]	Lomefloxacin	-85.240	-47.657	0.921	0.795	0.868

16[c, e]	Marbofloxacin	-51.146	-21.026	0.408	1.000	0.657
17[a, c, e]	Moxifloxacin	-90.298	-95.608	0.998	0.424	0.756
18[a, c, e]	Nadifloxacin	-45.464	-60.359	0.323	0.696	0.480
19[a, c, d, e]	Norfloxacin	-28.525	-63.290	0.068	0.674	0.323
20	Orbifloxacin	-59.639	-23.299	0.536	0.982	0.723
21[b, c, f]	Pefloxacin	-44.545	-42.147	0.309	0.837	0.531
22[a, b, d, e, f]	Pipemidic Acid	-40.489	-33.154	0.248	0.906	0.524
23[b, e]	Rufloxacin	-68.873	-24.040	0.675	0.977	0.802
24[b]	Sitafloxacin	-63.058	-63.333	0.587	0.674	0.624
25[a, c]	Sparfloxacin	-68.076	-62.747	0.663	0.678	0.669
26[b, c, e]	Temafloxacin	-74.066	-29.166	0.753	0.937	0.830

a: The bidirectional selectivity effect 3D-QSA2R model training set and b: Test set; c: Bio-metabolic model training set and d: Test set;

e: Bio-concentration model training set and f: Test set.

Table S2 Predicting the NOR substitutes using the 3D-QSA^{2}R model and screening of the 3D-QSAR model based on bio-metabolism effect and bio-concentration effect.

Compounds	Substituent groups	BMI	Change rate (%)	BEI	Change rate (%)	BSEV	Change rate (%)	Compounds	Substituent groups	BMI	Change rate (%)	BEI	Change rate (%)	BSEV	Change rate (%)
NOR		0.066		0.674		0.314				0.066		0.674		0.314	
N-001	1-A	0.153	131.82	0.732	8.61	0.363	15.61	N-062	1-A, 4-I	0.287	334.85	0.660	-2.08	0.422	34.39
N-002	1-B	0.221	234.85	0.621	-7.86	0.338	7.64	N-063	1-A,4-S	0.185	180.30	0.842	24.93	0.429	36.62
N-003	1-C	0.201	204.55	0.628	-6.82	0.338	7.64	N-064	1-A, 4-J	0.240	263.64	0.864	28.19	0.456	45.22
N-004	1-D	0.545	725.76	0.435	-35.46	0.482	53.50	N-065	1-G, 4-O	0.324	390.91	0.767	13.80	0.475	51.27
N-005	1-E	0.395	498.48	0.593	-12.02	0.403	28.34	N-066	1-G, 4-H	0.323	389.39	0.817	21.22	0.476	51.59
N-006	1-F	0.217	228.79	0.603	-10.53	0.339	7.96	N-067	1-G, 4-A	0.265	301.52	0.704	4.45	0.442	40.76
N-007	1-G	0.208	215.15	0.684	1.48	0.360	14.65	N-068	1-G, 4-G	0.323	389.39	0.768	13.95	0.446	42.04
N-008	1-H	0.056	-15.15	0.789	17.06	0.352	12.10	N-069	1-G, 4-E	0.313	374.24	0.854	26.71	0.474	50.96
N-009	1-I	0.312	372.73	0.672	-0.30	0.411	30.89	N-070	1-G, 4-D	0.332	403.03	0.845	25.37	0.478	52.23
N-010	1-J	0.404	512.12	0.536	-20.47	0.432	37.58	N-071	1-G, 4-P	0.338	412.12	0.798	18.40	0.487	55.10
N-011	1-K	0.628	851.52	0.490	-27.30	0.499	58.92	N-072	1-G, 4-C	0.316	378.79	0.848	25.82	0.464	47.77
N-012	1-L	0.616	833.33	0.558	-17.21	0.500	59.24	N-073	1-G, 4-I	0.331	401.52	0.627	-6.97	0.432	37.58
N-013	1-M	0.349	428.79	0.680	0.89	0.406	29.30	N-074	1-G, 4-S	0.265	301.52	0.820	21.66	0.448	42.68
N-014	1-N	0.143	116.67	0.660	-2.08	0.316	0.64	N-075	1-G, 4-J	0.340	415.15	0.844	25.22	0.484	54.14
N-015	2-A	0.230	248.48	0.811	20.33	0.389	23.89	N-076	2-A, 3-Q	0.239	262.12	0.803	19.14	0.391	24.52
N-016	2-G	0.104	57.58	0.679	0.74	0.312	-0.64	N-077	2-C, 3-Q	0.171	159.09	0.672	-0.30	0.327	4.14

N-017	2-H	0.022	-66.67	0.609	-9.64	0.288	-8.28	N-078	2-D, 3-Q	0.266	303.03	0.807	19.73	0.397	26.43
N-018	2-C	0.157	137.88	0.681	1.04	0.314	0.00	N-079	2-A, 4-O	0.266	303.03	0.848	25.82	0.477	51.91
N-019	2-E	0.149	125.76	0.664	-1.48	0.353	12.42	N-080	2-A, 4-A	0.236	257.58	0.908	34.72	0.464	47.77
N-020	2-D	0.181	174.24	0.740	9.79	0.328	4.46	N-081	2-A, 4-H	0.262	296.97	0.872	29.38	0.487	55.10
N-021	2-F	0.176	166.67	0.653	-3.12	0.320	1.91	N-082	2-A, 4-G	0.271	310.61	0.947	40.50	0.468	49.04
N-022	2-M	0.109	65.15	0.672	-0.30	0.347	10.51	N-083	2-A, 4-E	0.252	281.82	0.891	32.20	0.487	55.10
N-023	3-Q	0.090	36.36	0.679	0.74	0.324	3.18	N-084	2-A, 4-D	0.260	293.94	0.851	26.26	0.481	53.18
N-024	3-R	0.102	54.55	0.672	-0.30	0.326	3.82	N-085	2-A, 4-P	0.270	309.09	0.853	26.56	0.484	54.14
N-025	3-O	0.125	89.39	0.661	-1.93	0.330	5.10	N-086	2-A, 4-C	0.261	295.45	0.894	32.64	0.476	51.59
N-026	3-P	0.107	62.12	0.653	-3.12	0.315	0.32	N-087	2-A, 4-I	0.118	78.79	0.938	39.17	0.402	28.03
N-027	3-N	0.088	33.33	0.659	-2.23	0.325	3.50	N-088	2-A, 4-S	0.214	224.24	0.883	31.01	0.469	49.36
N-028	3-A	-0.084	-227.27	0.337	-50.00	0.223	-28.98	N-089	2-A, 4-J	0.288	336.36	0.874	29.67	0.493	57.01
N-029	3-C	0.115	74.24	0.646	-4.15	0.323	2.87	N-090	2-C, 4-P	0.329	398.48	0.865	28.34	0.469	49.36
N-030	3-J	0.120	81.82	0.634	-5.93	0.315	0.32	N-091	2-C, 4-C	0.309	368.18	0.925	37.24	0.446	42.04
N-031	4-O	0.253	283.33	0.859	27.45	0.475	51.27	N-092	2-C, 4-I	0.349	428.79	0.677	0.45	0.440	40.13
N-032	4-H	0.262	296.97	0.895	32.79	0.477	51.91	N-093	2-C, 4-S	0.262	296.97	0.888	31.75	0.430	36.94
N-033	4-A	0.230	248.48	0.687	1.93	0.427	35.99	N-094	2-C, 4-J	0.328	396.97	0.898	33.23	0.469	49.36
N-034	4-G	0.231	250.00	0.914	35.61	0.461	46.82	N-095	2-C, 4-O	0.309	368.18	0.841	24.78	0.455	44.90
N-035	4-E	0.216	227.27	0.903	33.98	0.452	43.95	N-096	2-C, 4-A	0.279	322.73	0.769	14.09	0.440	40.13
N-036	4-D	0.264	300.00	0.871	29.23	0.473	50.64	N-097	2-C, 4-H	0.316	378.79	0.882	30.86	0.457	45.54
N-037	4-P	0.271	310.61	0.876	29.97	0.485	54.46	N-098	2-C, 4-G	0.312	372.73	0.847	25.67	0.434	38.22

N-038	4-C	0.256	287.88	0.939	39.32	0.465	48.09	N-099	2-C, 4-E	0.304	360.61	0.930	37.98	0.460	46.50
N-039	4-I	0.221	234.85	1.087	61.28	0.438	39.49	N-100	2-C, 4-D	0.310	369.70	0.853	26.56	0.448	42.68
N-040	4-S	0.216	227.27	0.906	34.42	0.454	44.59	N-101	2-D, 4-H	0.331	401.52	0.891	32.20	0.468	49.04
N-041	4-J	0.291	340.91	0.924	37.09	0.496	57.96	N-102	2-D, 4-A	0.267	304.55	0.817	21.22	0.433	37.90
N-042	1-A, 2-A	-0.091	-237.88	0.638	-5.34	0.250	-20.38	N-103	2-D, 4-O	0.332	403.03	0.844	25.22	0.459	46.18
N-043	1-A, 2-C	0.205	210.61	0.724	7.42	0.350	11.46	N-104	2-D, 4-G	0.320	384.85	0.934	38.58	0.439	39.81
N-044	1-A, 2-D	0.216	227.27	0.720	6.82	0.340	8.28	N-105	2-D, 4-E	0.314	375.76	0.928	37.69	0.481	53.18
N-045	1-H, 2-A	-0.138	-309.09	0.652	-3.26	0.247	-21.34	N-106	2-D, 4-D	0.322	387.88	0.890	32.05	0.463	47.45
N-046	1-H, 2-C	0.108	63.64	0.781	15.88	0.342	8.92	N-107	2-D, 4-P	0.356	439.39	0.876	29.97	0.478	52.23
N-047	1-H, 2-D	0.041	-37.88	0.749	11.13	0.281	-10.51	N-108	2-D, 4-C	0.330	400.00	0.935	38.72	0.462	47.13
N-048	1-G, 2-A	0.143	116.67	0.641	-4.90	0.345	9.87	N-109	2-D, 4-I	0.344	421.21	0.728	8.01	0.438	39.49
N-049	1-G, 2-C	0.279	322.73	0.693	2.82	0.365	16.24	N-110	2-D, 4-S	0.296	348.48	0.925	37.24	0.444	41.40
N-050	1-G, 2-D	0.209	216.67	0.605	-10.24	0.334	6.37	N-111	2-D, 4-J	0.361	446.97	0.918	36.20	0.484	54.14
N-051	1-A, 3-Q	0.173	162.12	0.723	7.27	0.360	14.65	N-112	3-Q, 4-O	0.278	321.21	0.858	27.30	0.484	54.14
N-052	1-H, 3-Q	0.073	10.61	0.779	15.58	0.349	11.15	N-113	3-Q, 4-H	0.281	325.76	0.887	31.60	0.491	56.37
N-053	1-G, 3-Q	0.224	239.39	0.674	0.00	0.363	15.61	N-114	3-Q, 4-A	0.259	292.42	0.907	34.57	0.450	43.31
N-054	1-A, 4-O	0.238	260.61	0.799	18.55	0.459	46.18	N-115	3-Q, 4-G	0.280	324.24	0.894	32.64	0.464	47.77
N-055	1-A, 4-H	0.232	251.52	0.846	25.52	0.456	45.22	N-116	3-Q, 4-E	0.262	296.97	0.915	35.76	0.500	59.24
N-056	1-A, 4-A	0.158	139.39	0.724	7.42	0.408	29.94	N-117	3-Q, 4-D	0.289	337.88	0.879	30.42	0.487	55.10
N-057	1-A, 4-G	0.234	254.55	0.774	14.84	0.416	32.48	N-118	3-Q, 4-C	0.286	333.33	0.939	39.32	0.479	52.55
N-058	1-A, 4-E	0.190	187.88	0.849	25.96	0.449	42.99	N-119	3-Q, 4-P	0.301	356.06	0.875	29.82	0.497	58.28

N-059	1-A, 4-D	0.248	275.76	0.856	27.00	0.460	46.50	N-120	3-Q, 4-I	0.216	227.27	0.745	10.53	0.397	26.43
N-060	1-A, 4-P	0.246	272.73	0.828	22.85	0.466	48.41	N-121	3-Q, 4-S	0.238	260.61	0.849	25.96	0.444	41.40
N-061	1-A, 4-C	0.225	240.91	0.857	27.15	0.439	39.81	N-122	3-Q, 4-J	0.294	345.45	0.897	33.09	0.496	57.96

Table S3 Prediction and evaluation of functional characteristics and environmental safety of NOR substitute molecules

Compounds	Genotoxicity		Biodegradation		Compounds	Genotoxicity		Biodegradation	
	Predicted	Change rate (%)	Predicted	Change rate (%)		Predicted	Change rate (%)	Predicted	Change rate (%)
NOR	7.875		0.800		NOR	7.875		0.800	
N-031	8.388	6.51	1.326	65.75	N-085	8.460	7.43	0.525	-34.38
N-032	8.830	12.13	1.435	79.38	N-086	8.725	10.79	0.971	21.38
N-034	8.290	5.27	1.332	66.50	N-088	8.794	11.67	0.879	9.87
N-035	8.564	8.75	1.685	110.63	N-089	9.575	21.59	0.775	-3.13
N-036	8.718	10.70	1.662	107.75	N-090	8.038	2.07	0.693	-13.38
N-037	7.986	1.41	1.430	78.75	N-091	8.207	4.22	1.097	37.13
N-038	8.091	2.74	1.733	116.63	N-093	8.340	5.90	1.076	34.50
N-039	9.019	14.53	1.978	147.25	N-094	8.989	14.15	0.941	17.63
N-040	8.306	5.47	1.691	111.38	N-095	8.453	7.34	0.595	-25.63
N-041	9.038	14.77	1.688	111.00	N-096	10.155	28.95	0.364	-54.50
N-054	8.650	9.84	2.538	217.25	N-097	8.908	13.12	0.702	-12.25
N-055	9.092	15.45	2.707	238.38	N-098	8.491	7.82	0.580	-27.50
N-057	8.551	8.58	2.794	249.25	N-099	8.634	9.64	1.064	33.00
N-058	8.825	12.06	3.192	299.00	N-100	8.747	11.07	0.985	23.13
N-059	8.980	14.03	2.792	249.00	N-101	8.591	9.09	0.582	-27.25
N-060	8.248	4.74	2.643	230.38	N-102	9.833	24.86	0.324	-59.50
N-061	8.353	6.07	3.033	279.13	N-103	8.127	3.20	0.425	-46.88
N-063	8.567	8.79	3.281	310.13	N-104	8.090	2.73	0.509	-36.38
N-064	9.300	18.10	2.822	252.75	N-105	8.292	5.30	1.045	30.63
N-065	8.668	10.07	1.846	130.75	N-106	8.417	6.88	0.881	10.13
N-066	9.109	15.67	2.008	151.00	N-107	7.770	-1.33	0.465	-41.88
N-068	8.569	8.81	1.985	148.13	N-108	7.858	-0.22	0.995	24.38
N-069	8.843	12.29	2.455	206.88	N-110	8.024	1.89	0.810	1.25
N-070	8.998	14.26	2.217	177.13	N-111	8.702	10.50	0.769	-3.88
N-071	8.266	4.97	1.973	146.63	N-112	8.299	5.38	1.464	83.00
N-072	8.371	6.30	2.431	203.88	N-113	9.000	14.29	1.559	94.88

N-074	8.585	9.02	2.530	216.25	N-114	10.347	31.39	1.542	92.75
N-075	9.317	18.31	2.215	176.88	N-115	8.626	9.54	1.293	61.63
N-078	7.857	-0.23	0.671	-16.13	N-116	8.339	5.89	2.141	167.63
N-079	8.900	13.02	0.462	-42.25	N-117	8.616	9.41	1.845	130.63
N-080	10.697	35.83	0.423	-47.13	N-118	8.374	6.34	1.877	134.63
N-081	9.411	19.50	0.617	-22.88	N-119	8.029	1.96	1.529	91.13
N-082	8.911	13.16	0.703	-12.13	N-120	8.872	12.66	1.010	26.25
N-083	9.087	15.39	0.819	2.37	N-121	8.222	4.41	1.669	108.63
N-084	9.166	16.39	0.778	-2.75	N-122	9.413	19.53	1.722	115.25

Table S4 Prediction and evaluation of NOR and its substitutes, based on the ADMET model

Compounds	Human intestinal absorption	Aqueous solubility (25℃)		Blood-brain barrier penetration		Cytochrome P450 2D6 (CYP2D6) enzyme inhibition		Plasma protein binding	
	Prediction/Level	Log (S_w)/Level	Change rate (%)	logBB/Level	Change rate (%)	Bayesian score	Change rate (%)	AlogP98	Change rate (%)
NOR	0/Good	-2.832/Good		-0.940/Low		-3.860/Non*		1.294/Non	
N-031	0/Good	-3.046/Good	-7.56	-0.598/Low	36.38	-5.241/Non	-35.77	1.914/Non	47.91
N-032	0/Good	-3.188/Good	-12.57	-1.264/Low	-34.47	-6.431/Non	-66.60	1.119/Non	-13.52
N-035	0/Good	-2.990/Good	-5.58	-1.069/Low	-13.72	-9.337/Non	-141.89	1.733/Non	33.93
N-036	0/Good	-2.454/Good	13.35	-1.127/Low	-19.89	-11.938/Non	-209.26	1.090/Non	-15.77
N-039	0/Good	-2.672/Good	5.65	-1.612/Low	-71.49	-12.019/Non	-211.36	0.880/Non	-31.99
N-041	0/Good	-3.261/Good	-15.15	-0.650/Low	30.85	-5.523/Non	-43.08	1.919/Non	48.30
N-054	0/Good	-3.136/Good	-10.73	-1.078/Low	-14.68	-4.490/Non	-16.32	2.083/Non	60.97
N-055	0/Good	-3.798/Good	-34.11	Undefined	Unknown	-5.039/Non	-30.55	1.288/Non	-0.46
N-057	0/Good	-3.198/Good	-12.92	Undefined	Unknown	-5.039/Non	-30.55	1.626/Non	25.66
N-058	0/Good	-3.393/Good	-19.81	Undefined	Unknown	-8.576/Non	-122.18	1.902/Non	46.99
N-059	0/Good	-2.701/Good	4.63	Undefined	Unknown	-12.287/Non	-218.31	1.259/Non	-2.70
N-061	0/Good	-3.424/Good	-20.90	-1.297/Low	-37.98	-4.868/Non	-26.11	1.832/Non	41.58
N-063	0/Good	-2.603/Good	8.09	Undefined	Unknown	-9.220/Non	-138.86	1.180/Non	-8.81
N-064	0/Good	-3.677/Good	-29.84	-1.130/Low	-20.21	-5.144/Non	-33.25	2.088/Non	61.36
N-065	0/Good	-3.223/Good	-13.81	-0.792/Low	15.74	-4.198/Non	-8.75	2.354/Non	81.92

N-066	0/Good	-3.710/Good	31.00	Undefined	Unknown	-5.318/Non	37.78	1.558/Non	20.40
N-068	0/Good	-3.112/Good	-9.89	-1.263/Low	-34.36	-4.220/Non	-9.31	1.896/Non	46.52
N-069	0/Good	-3.323/Good	-17.34	Undefined	Unknown	-8.903/Non	-130.64	2.172/Non	67.85
N-070	0/Good	-2.709/Good	4.34	-1.320/Low	-40.43	-10.936/Non	-183.31	1.529/Non	18.16
N-072	0/Good	-3.342/Good	-18.01	-1.011/Low	-7.55	-4.451/Non	-15.31	2.102/Non	62.44
N-074	0/Good	-2.606/Good	7.98	-1.345/Low	-43.09	-9.410/Non	-143.76	1.450/Non	12.06
N-075	0/Good	-3.600/Good	-27.12	-0.843/Low	10.32	-3.879/Non	-0.50	2.359/Non	82.30
N-099	0/Good	-3.045/Good	-7.52	-1.216/Low	-29.36	-9.651/Non	-150.02	1.716/Non	32.61
N-113	0/Good	-3.579/Good	-26.38	-1.147/Low	-22.02	-5.726/Non	-48.34	1.497/Non	15.69
N-114	0/Good	-3.429/Good	-21.08	-1.187/Low	-26.28	-5.750/Non	-48.95	1.731/Non	33.77
N-115	0/Good	-3.052/Good	-7.77	-0.952/Low	-1.28	-4.628/Non	-19.90	1.834/Non	41.73
N-117	0/Good	-2.841/Good	-0.32	-1.010/Low	-7.45	-10.440/Non	-170.46	1.467/Non	13.37
N-118	0/Good	-3.385/Good	-19.53	-0.701/Low	25.43	-3.986/Non	-3.26	2.04/Non	57.65
N-120	0/Good	-3.058/Good	-7.98	-1.495/Low	-59.04	-11.024/Non	-185.59	1.257/Non	-2.86
N-122	0/Good	-3.648/Good	-28.81	-0.533/Low	43.30	-4.056/Non	-5.06	2.297/Non	77.51

*Non: None.

Table S5–1 The prediction and evaluation of the animal carcinogenicity and mutagenicity of NOR and its substitutes, based on the TOPKAT model

Compounds	Probability value/Level								
	Ames mutagenicity	NTP rodent carcinogenicity				FDA rodent carcinogenicity			
		Female mouse	Male mouse	Female rat	Male rat	Female mouse Non vs. Carc	Male mouse Single vs. Mult	Female rat Non vs. Carc	Male rat Non vs. Carc
NOR	0.937/Mut*	0.378/Non*	0.674/Carc*	0.500/Non	0.671/Carc	0.217/Non	0.125/Non	0.137/Non	0.091/Non
N-031	0.920/Mut	0.454/Non	0.738/Carc	0.515/Carc	0.671/Carc	0.231/Non	0.102/Non	0.152/Non	0.125/Non
N-032	0.949/Mut	0.366/Non	0.684/Carc	0.534/Carc	0.706/Carc	0.231/Non	0.112/Non	0.157/Non	0.141/Non
N-035	0.799/Mut	0.388/Non	0.607/Carc	0.548/Non	0.669/Carc	0.207/Non	0.131/Non	0.139/Non	0.144/Non
N-036	0.758/Mut	0.317/Non	0.593/Carc	0.495/Non	0.685/Carc	0.208/Non	0.113/Non	0.142/Non	0.136/Non
N-039	0.803/Mut	0.332/Non	0.456/Non	0.494/Non	0.696/Carc	0.221/Non	0.137/Non	0.151/Non	0.138/Non
N-041	0.926/Mut	0.310/Non	0.631/Carc	0.515/Non	0.642/Carc	0.225/Non	0.133/Non	0.164/Non	0.156/Non
N-054	0.733/Mut	0.469/Non	0.631/Carc	0.505/Non	0.534/Non	0.232/Non	0.137/Non	0.178/Non	0.176/Non
N-061	0.713/Non	0.383/Non	0.614/Carc	0.506/Non	0.535/Non	0.217/Non	0.151/Non	0.176/Non	0.168/Non
N-064	0.732/Mut	0.327/Non	0.608/Carc	0.508/Non	0.521/Non	0.222/Non	0.145/Non	0.176/Non	0.173/Non
N-065	0.757/Mut	0.483/Non	0.612/Carc	0.480/Non	0.553/Non	0.228/Non	0.136/Non	0.180/Non	0.176/Non
N-068	0.750/Mut	0.354/Non	0.609/Carc	0.504/Non	0.555/Non	0.227/Non	0.141/Non	0.181/Non	0.181/Non
N-070	0.663/Non	0.355/Non	0.510/Non	0.448/Non	0.570/Non	0.206/Non	0.117/Non	0.171/Non	0.178/Non
N-072	0.750/Mut	0.386/Non	0.614/Carc	0.498/Non	0.549/Non	0.224/Non	0.135/Non	0.179/Non	0.181/Non

N-074	0.766/Mut	0.433/Non	0.535/Non	0.472/Non	0.580/Non	0.232/Non	0.136/Non	0.186/Non	0.183/Non
N-075	0.768/Mut	0.331/Non	0.592/Carc	0.483/Non	0.498/Non	0.222/Non	0.139/Non	0.183/Non	0.187/Non
N-099	0.543/Non	0.388/Non	0.609/Carc	0.578/Carc	0.742/Carc	0.219/Non	0.139/Non	0.189/Non	0.200/Non
N-113	0.801/Mut	0.389/Non	0.628/Carc	0.525/Non	0.709/Carc	0.233/Non	0.100/Non	0.170/Non	0.147/Non
N-114	0.791/Mut	0.329/Non	0.675/Carc	0.535/Carc	0.650/Carc	0.233/Non	0.123/Non	0.169/Non	0.146/Non
N-115	0.783/Mut	0.385/Non	0.620/Carc	0.535/Carc	0.702/Carc	0.232/Non	0.114/Non	0.169/Non	0.150/Non
N-117	0.776/Mut	0.345/Non	0.599/Carc	0.501/Non	0.688/Carc	0.223/Non	0.115/Non	0.172/Non	0.136/Non
N-118	0.778/Mut	0.394/Non	0.680/Carc	0.541/Carc	0.681/Carc	0.228/Non	0.118/Non	0.171/Non	0.155/Non
N-120	0.793/Mut	0.356/Non	0.476/Non	0.496/Non	0.699/Carc	0.228/Non	0.132/Non	0.172/Non	0.139/Non
N-122	0.793/Mut	0.338/Non	0.613/Carc	0.523/Non	0.644/Carc	0.229/Non	0.122/Non	0.171/Non	0.154/Non

*Non: None, Mut: Mutagen, Carc: Carcinogen.

Table S5–2 Prediction and evaluation of skin sensitization, skin irritation, and eye irritation of NOR and its substitutes based on the TOPKAT model

Compounds	**Probability value**					
	Skin irritancy	**Skin sensitization NON vs. SENS**	**Skin sensitization (Weak vs. Stro)**	**Ocular irritancy (NON vs Irr)**	**Ocular irritancy Mild vs. SEV**	**Ocular irritancy Mod vs. SEV**
NOR	0.957/Non*	0.800/SENS*	0.897/Stro*	0.999/Irr*	0.861/Mod~Sev*	0.585/Sin*
N-035	0.965/Non	0.795/SENS	0.971/Stro	0.999/Irr	0.829/Mod~Sev	0.337/Sin
N-036	0.914/Non	0.690/Non	——	0.999/Irr	0.828/Mod~Sev	0.245/Sin
N-039	0.942/Non	0.782/SENS	0.926/Stro	0.999/Irr	0.833/Mod~Sev	0.238/Sin

N-041	0.965/Non	0.848/SENS	0.910/Stro	0.999/Irr	0.842/Mod~Sev	0.551/Sin
N-054	0.949/Non	0.813/SENS	0.921/Stro	0.999/Irr	0.831/Mod~Sev	0.572/Sin
N-061	0.951/Non	0.857/SENS	0.905/Stro	0.999/Irr	0.837/Mod~Sev	0.509/Sin
N-064	0.945/Non	0.857/SENS	0.904/Stro	0.999/Irr	0.839/Mod~Sev	0.533/Sin
N-065	0.957/Non	0.802/SENS	0.918/Stro	0.999/Irr	0.835/Mod~Sev	0.586/Sin
N-068	0.954/Non	0.849/SENS	0.911/Stro	0.999/Irr	0.841/Mod~Sev	0.525/Sin
N-070	0.882/Non	0.685/Non	——	0.999/Irr	0.828/Mod~Sev	0.262/Sin
N-072	0.960/Non	0.849/SENS	0.913/Stro	0.999/Irr	0.840/Mod~Sev	0.528/Sin
N-074	0.952/Non	0.675/Non	——	0.999/Irr	0.827/Mod~Sev	0.331/Sin
N-075	0.957/Non	0.843/SENS	0.912/Stro	0.999/Irr	0.846/Mod~Sev	0.545/Sin
N-112	0.965/Non	0.784/SENS	0.914/Stro	0.999/Irr	0.837/Mod~Sev	0.600/Sin
N-113	0.953/Non	0.852/SENS	0.910/Stro	0.999/Irr	0.840/Mod~Sev	0.536/Sin
N-117	0.918/Non	0.710/Non	——	0.999/Irr	0.837/Mod~Sev	0.273/Sin
N-120	0.947/Non	0.773/SENS	0.915/Stro	0.999/Irr	0.834/Mod~Sev	0.249/Sin
N-122	0.966/Non	0.848/SENS	0.915/Stro	0.999/Irr	0.850/Mod~Sev	0.576/Sin

*Non: None, SENS: Sensitization, Carc: Carcinogen, Stro: Strong, Irr: Irritant, Mod~Sev: Moderate-Severe, Sin: Single

Table S5–3 Human health risk and ecological risk evaluation of NOR and its substitutes based on the TOPKAT model

Compounds	Human health risk assessment					Ecological risk assessment		
	Rat chronic oral LOAEL*	Rat oral LD_{50*} g/kg	Rat inhalational LC_{50*}	Developmental toxicity	Hepatotoxicity	Aerobic biodegradability	Fathead minnow LC_{50*} (g/L)	Daphnia EC_{50*} (mg/L)
	Concentration /Level			Probability value/Level	Value/Level	Probability value/Level	Concentration/Level	
NOR	0.024/High	1.955/Low	22609.500/Mild	0.707/Toxic	1.861/Toxic	0.163/Non	3.586/Low	249.773/Low
N-036	0.065/High	1.995/Low	26321.100/Mild	0.717/Toxic	-0.475/Toxic	0.233/Non	0.041/Mild	19.575/Mod
N-039	0.018/High	1.239/Low	9023.380/Mild	0.701/Toxic	1.510/Toxic	0.150/Non	0.216/Low	19.300/Mod
N-041	0.027/High	0.301/Low	18066.100/Mild	0.683/Toxic	1.377/Toxic	0.135/Non	0.288/Low	46.872/Mod
N-054	0.031/High	0.327/Low	17100.900/Mild	0.639/Toxic	0.241/Toxic	0.132/Non	0.054/Mild	24.270/Mod
N-061	0.022/High	0.227/Mod	13692.300/Mild	0.661/Toxic	1.583/Toxic	0.141/Non	0.812/Low	31.208/Mod
N-064	0.033/High	0.121/Mod	8150.460/Mild	0.668/Toxic	0.907/Toxic	0.127/Non	1.235/Low	34.954/Mod
N-065	0.042/High	1.105/Low	23891.900/Mild	0.629/Toxic	0.585/Toxic	0.150/Non	0.011/Mild	14.478/Mod
N-068	0.034/High	2.012/Low	14998.300/Mild	0.618/Toxic	1.172/Toxic	0.129/Non	0.538/Low	33.413/Mod
N-070	0.070/High	1.178/Low	16622.100/Mild	0.716/Toxic	-0.772/Toxic	0.211/Non	0.035/Mild	12.820/Mod
N-072	0.029/High	0.560/Low	19138.000/Mild	0.648/Toxic	1.038/Toxic	0.149/Non	0.163/Low	9.947/High
N-074	0.042/High	1.523/Low	13538.700/Mild	0.677/Toxic	0.685/Toxic	0.152/Non	0.074/Mild	21.116/Mod
N-075	0.050/High	0.266/Mod	11407.600/Mild	0.641/Toxic	0.768/Toxic	0.134/Non	0.248/Low	19.840/Mod
N-113	0.014/High	0.702/Low	14793.100/Mild	0.702/Toxic	1.242/Toxic	0.117/Non	0.450/Low	80.014/Mod

N-117	0.029/High	2.400/Low	28540.200/Mild	0.659/Toxic	0.358/Toxic	0.206/Non	0.024/Mild	24.968/Mod
N-120	0.010/High	1.284/Low	9783.150/Mild	0.707/Toxic	1.325/Toxic	0.146/Non	0.125/Low	20.162/Mod
N-122	0.014/High	0.328/Low	19587.200/Mild	0.699/Toxic	0.661/Toxic	0.135/Non	0.166/Low	56.555/Mod

* The evaluation was performed based on the "Acute Oral, Inhalation, and Percutaneous Toxicity Classification for Rats" (Table 2) and the "Ecotoxicological Hazard Classification" (Table 16), cited from the Guidelines for the Hazard Evaluation of New Chemical Substances (HJ/T154–2004), Ministry of Ecology and Environment of the People's Republic of China, April 13, 2004. Non: None, Mod: Moderate.

Table S6 Calculations of the energy gap, energy, and positive frequency of six NOR substitutes

Compounds	**Energy gap (eV)**	**Change rate (%)**	**energy (a. u.)**	**Change rate (%)**	**Positive frequency (cm^{-1})**
NOR	4.288		-1109.90		28.31
N-039	4.463	4.06	-1279.02	-15.24	9.09
N-041	3.999	-6.74	-1165.62	-5.02	23.61
N-068	4.476	4.38	-1260.70	-13.59	23.37
N-113	4.290	0.05	-1204.94	-8.56	23.98
N-120	4.450	3.76	-1318.33	-18.78	16.64
N-122	4.225	-1.48	-1283.55	-15.65	20.06

Table S7 The binding energy of NOR and its four substitute molecules with 4R1Z and 4HGM

Compounds	Metabolic enzyme (4R1Z)		concentration enzyme (4HGM)	
	Binding energy (kJ/mol)	Change rate (%)	Binding energy (kJ/mol)	Change rate (%)
NOR	-28.525		-63.289	
N-039	-46.131	61.72	-21.455	-66.10
N-068	-47.044	64.92	-47.571	-24.83
N-113	-16.324	-42.77	-88.152	39.28
N-120	-60.179	110.97	-49.316	-22.08

Table S8 Interaction type and the average distance between NOR substitutes and the receptor (4R1Z)

Compounds	Electrostatic total		Halogen total		Hydrogen bond total		Hydrophobic total	
	Amount	Average distance (Å)	Amount	Average distance (Å)	Amount	Average distance (Å)	Amount	Average distance (Å)
NOR	0	0.000	0	0.000	7	2.780	4	4.785
N-031	2	3.825	1	3.170	10	2.887	8	4.790
N-032	2	3.555	1	3.390	7	2.557	4	5.098
N-035	0	0.000	0	0.000	8	2.529	7	4.547
N-036	0	0.000	0	0.000	5	2.660	4	4.405
N-039	0	0.000	3	3.053	9	2.601	8	4.604
N-041	2	3.930	1	3.260	7	2.590	4	4.680
N-054	0	0.000	2	3.170	3	2.670	6	3.870
N-055	1	3.470	0	0.000	6	2.527	2	4.325
N-057	1	4.470	0	0.000	5	2.480	3	4.713
N-058	0	0.000	2	3.240	6	2.390	6	4.040
N-059	2	3.335	0	0.000	9	2.327	5	5.090
N-061	0	0.000	2	3.360	7	2.733	4	4.258
N-063	2	3.410	1	3.350	10	2.758	6	4.948
N-064	0	0.000	1	3.470	5	2.226	4	4.300
N-065	0	0.000	0	0.000	4	2.678	6	4.458
N-066	1	4.110	4	3.320	7	2.550	0	0.000
N-068	1	4.150	3	3.213	5	2.470	1	5.360

N-069	0	0.000	2	3.285	4	2.510	6	4.463
N-070	2	4.130	0	0.000	5	2.396	4	4.390
N-072	2	3.455	1	3.270	10	2.774	7	5.053
N-074	1	4.990	2	2.885	6	2.145	4	4.015
N-075	1	4.210	3	3.200	6	2.615	4	5.000
N-099	1	4.460	0	0.000	8	2.480	7	4.876
N-113	1	4.880	2	2.905	8	2.433	6	4.608
N-114	0	0.000	0	0.000	7	2.561	3	4.507
N-115	1	4.880	2	3.410	3	2.730	3	3.460
N-117	0	0.000	0	0.000	4	2.330	7	4.758
N-118	2	3.645	1	3.070	7	2.794	7	4.703
N-120	0	0.000	2	3.190	5	2.592	6	4.547
N-122	0	0.000	1	3.510	7	2.444	8	4.571

Table S9 Risk evaluation of NOR and its substitutes by determining nutrition amplification in the food chain

Compounds	**Algae**		**Shrimp**		**Fish**		**Human**	
	ΔG_b (kJ/mol)	**Change rate (%)**	**ΔG_b (kJ/mol)**	**Change rate (%)**	**ΔG_b (kJ/mol)**	**Change Rate (%)**	**ΔG_b (kJ/mol)**	**Change Rate (%)**
NOR	-85.384		-33.283		-28.525		-68.504	
N-039	-91.437	7.09	-34.098	2.45	-46.131	61.72	-88.325	28.93
N-068	-95.233	11.53	-36.300	9.06	-47.044	64.92	-105.72	54.33
N-120	-109.733	28.52	-58.345	75.30	-60.179	110.97	-99.032	44.56

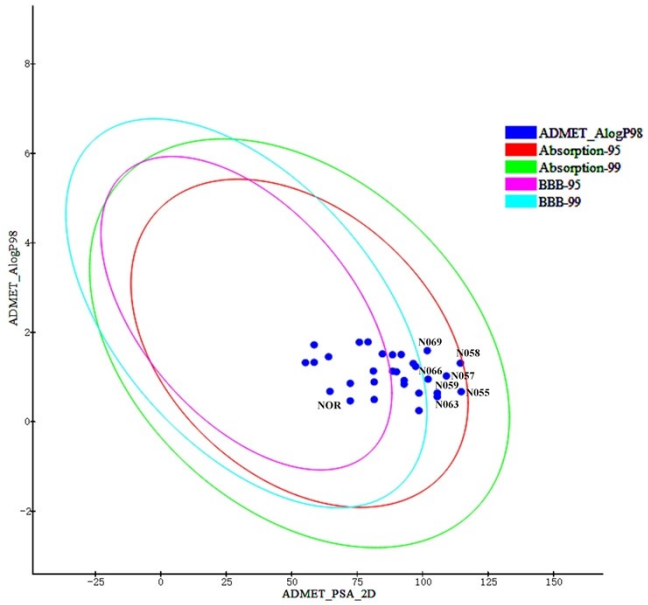

Fig. S1 The distribution of evaluation indices of the blood-brain barrier penetration (BBB) and the human intestinal absorption (HIA) model of NOR and its substitutes

*ADMET_PSA_2D, or rapid polar surface area, is a descriptive index for evaluating the transportability of drugs in cells; AlogP98 is the distribution coefficient of lipid water.

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