SUPPORTING INFORMATION

Naomi Scarano [1], Elena Abbotto [2], Francesca Musumeci[1], Annalisa Salis[2], Chiara Brullo[1], Paola Fossa[1], Silvia Schenone[1], Santina Bruzzone[2,3], Elena Cichero[1*]

[1] Department of Pharmacy, Section of Medicinal Chemistry, School of Medical and Pharmaceutical Sciences, University of Genoa, Viale Benedetto XV, 3, 16132 Genoa, Italy; naomi.scarano@edu.unige.it (N.S.); francesca.musumeci@unige.it (F.M.); chiara.brullo@unige.it (C.B.); paola.fossa@unige.it (P.F.); silvia.schenone@unige.it (S.S.)

[2] Department of Experimental Medicine, Section of Biochemistry, University of Genoa, Viale Benedetto XV 1, 16132 Genoa, Italy; elena.abbotto@unige.it (E.A.); annalisa.salis@unige.it (A.S.); santina.bruzzone@unige.it (S.B.)

[3] IRCCS Ospedale Policlinico San Martino, Genova, Italy

* Correspondence: elena.cichero@unige.it (E.C.); Tel.: +39-010-353-8350 (E.C.)
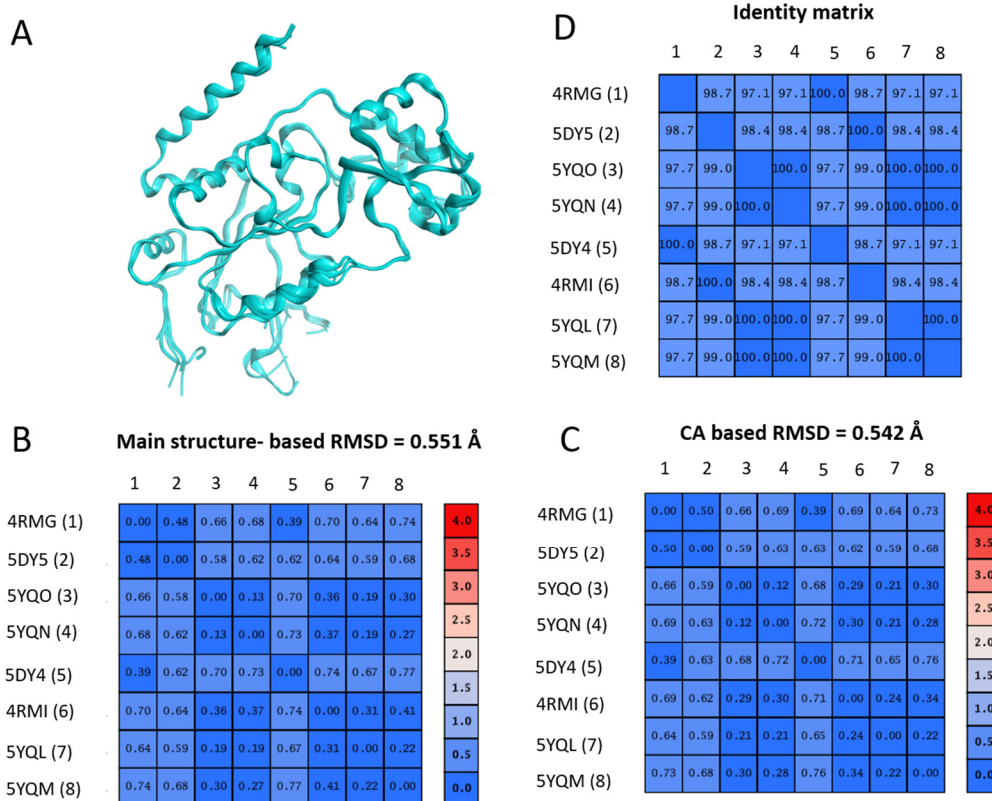
Figure S1. Superimposition of the SIRT2 X-ray complexes 4RMG, 5DY5, 5YQO, 5YQN, 5DY4, 4RMI, 5YQL, 5YQM, featuring SirReal2 and related analogues as enzyme inhibitors (A). Scheme of the derived RMSD matrix based on distances as estimated between each couple of proteins with respect to the main protein structure (B) or to the alpha-carbon atom CA (C) comparison. The related identity matrix is also shown (D).
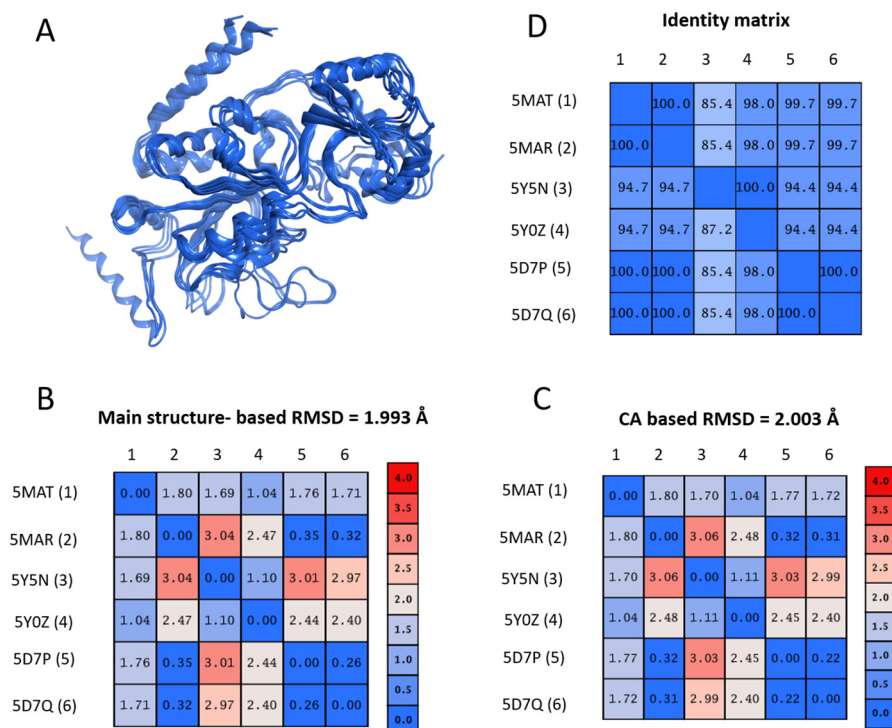
A

D

Identity matrix

	1	2	3	4	5	6
5MAT (1)		100.0	85.4	98.0	99.7	99.7
5MAR (2)	100.0		85.4	98.0	99.7	99.7
5Y5N (3)	94.7	94.7		100.0	94.4	94.4
5Y0Z (4)	94.7	94.7	87.2		94.4	94.4
5D7P (5)	100.0	100.0	85.4	98.0		100.0
5D7Q (6)	100.0	100.0	85.4	98.0	100.0	

B

Main structure- based RMSD = 1.993 Å

	1	2	3	4	5	6
5MAT (1)	0.00	1.80	1.69	1.04	1.76	1.71
5MAR (2)	1.80	0.00	3.04	2.47	0.35	0.32
5Y5N (3)	1.69	3.04	0.00	1.10	3.01	2.97
5Y0Z (4)	1.04	2.47	1.10	0.00	2.44	2.40
5D7P (5)	1.76	0.35	3.01	2.44	0.00	0.26
5D7Q (6)	1.71	0.32	2.97	2.40	0.26	0.00

C

CA based RMSD = 2.003 Å

	1	2	3	4	5	6
5MAT (1)	0.00	1.80	1.70	1.04	1.77	1.72
5MAR (2)	1.80	0.00	3.06	2.48	0.32	0.31
5Y5N (3)	1.70	3.06	0.00	1.11	3.03	2.99
5Y0Z (4)	1.04	2.48	1.11	0.00	2.45	2.40
5D7P (5)	1.77	0.32	3.03	2.45	0.00	0.22
5D7Q (6)	1.72	0.31	2.99	2.40	0.22	0.00

Figure S2. Superimposition of the SIRT2 X-ray complexes 5MAT, 5MAR, 5Y5N, 5Y0Z, 5D7P, 5D7Q, featuring non-SirReal2 chemical structure (A). Scheme of the derived RMSD matrix based on distances as estimated between each couple of proteins with respect to the main protein structure (B) or to the alpha-carbon atom CA (C) comparison. The related identity matrix is also shown (D).
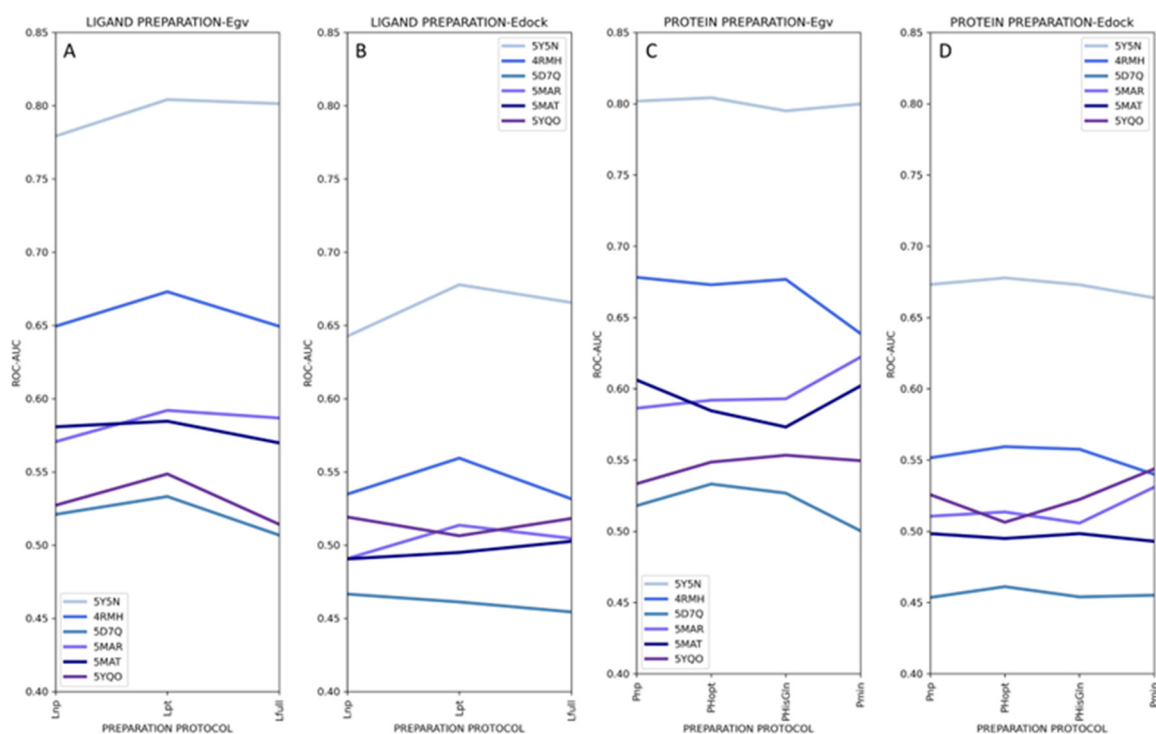
Figure S3. Preparation protocol effect on screening performances for a subset of conformationally diverse X-Rays (5Y5N, 4RMH, 5DYQ, 5MAR, 5MAT, 5YQO). Both the ligand (A-B) and the protein (C-D) preparation was investigated, according to the two selected scoring functions E-gv and E-dock.
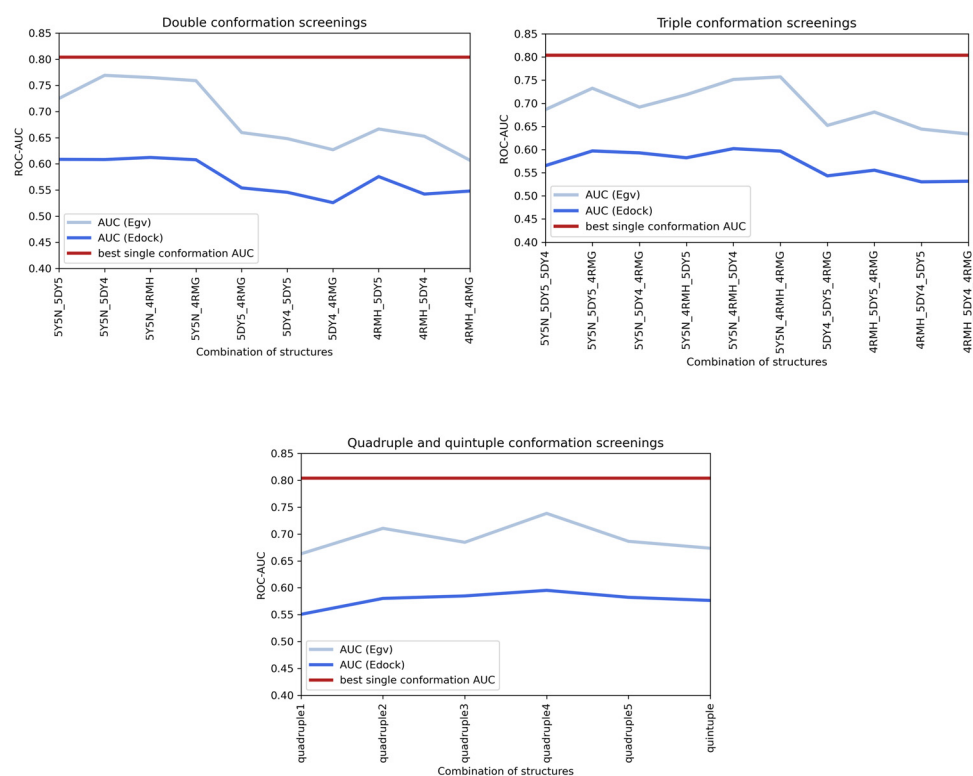
Figure S4. Multi-conformation screening performances. The AUC values of the screenings are reported on the y-axis, for each ensemble of conformation (x-axis). No multi-conformation screening outperformed the best single conformation AUC (shown as red line).
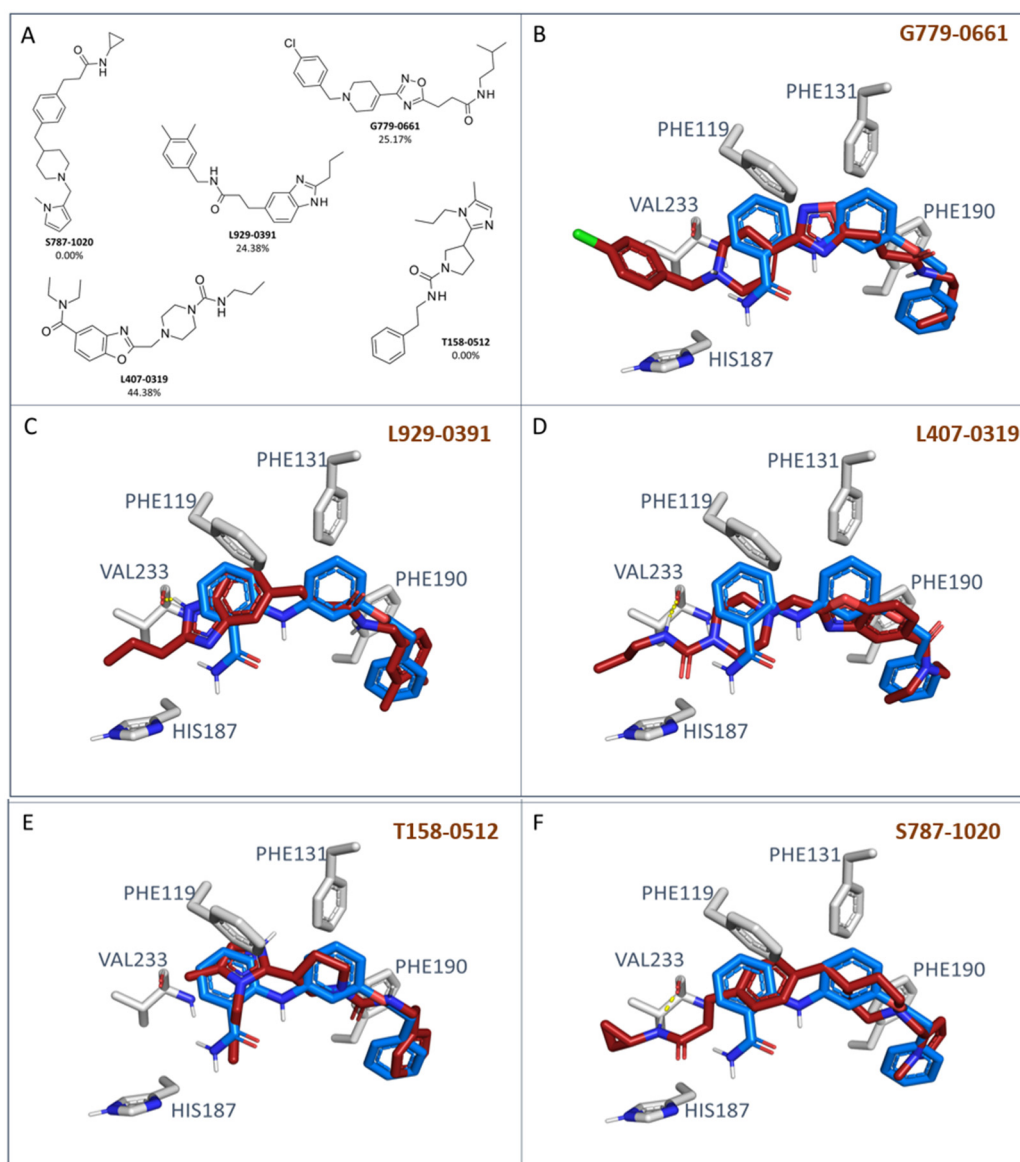
Figure S5. Tested compounds structures and their percentage of inhibition (@150microM) (A). Putative binding mode (docking pose) as obtained via preliminary ICM-Pro docking studies about: the (weakly) active compounds (dark red), in comparison with the crystallographic ligand of 5Y5N (blue) (B-D); inactive compounds (E-F). H-bonds are shown as yellow dotted lines.

Figure S6. Molecular docking poses of the selected ChemDiv compounds T158-0512, L929-0391, S787-1020 and G779-0661 in dark red, as derived via Induced Fit method, are shown. The X-ray crystallographic positioning of the 5Y5N inhibitor is also reported (blue). H-bonds are shown as yellow dotted lines.

Figure S7. Molecular docking poses of **AGK2** in dark red, as derived via Induced Fit method. The X-ray crystallographic positioning of the 5Y5N inhibitor is also reported (blue).

Figure S8. Molecular docking poses of compound **3** in dark red, as derived via Induced Fit method. The X-ray crystallographic positioning of the 5Y5N inhibitor is also reported (blue). H-bonds are shown as yellow dotted lines.

Figure S9. Prediction of putative target preferences displayed by SirReal2 and compounds **1**-**2** via SwissTarget [58].
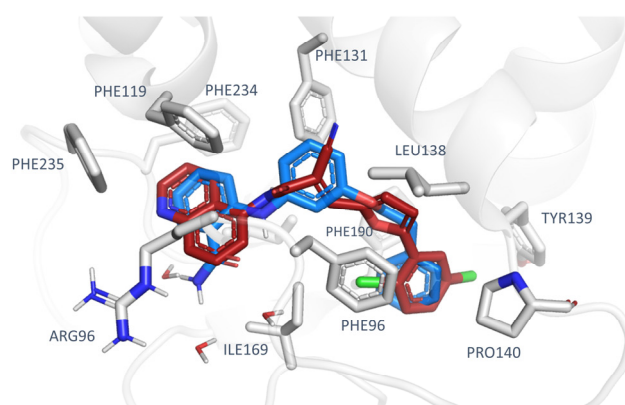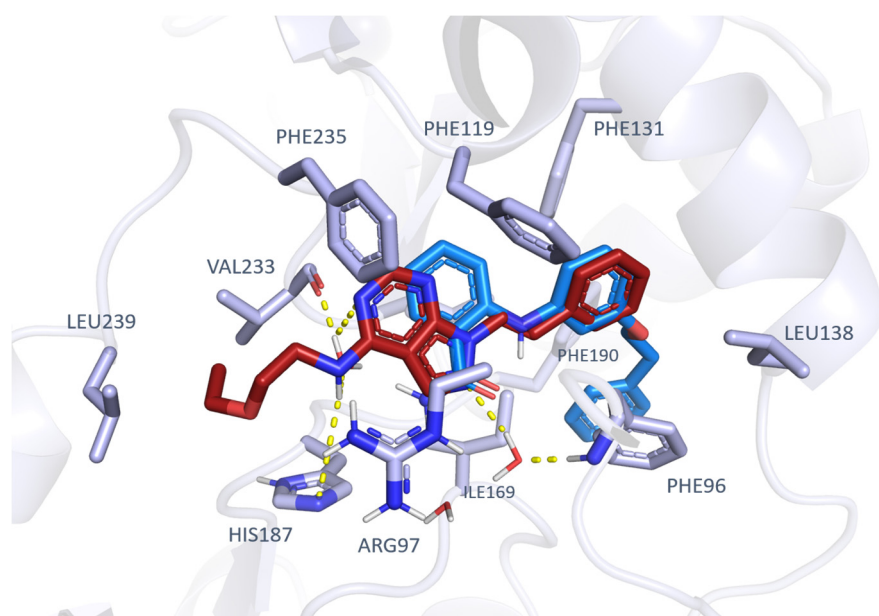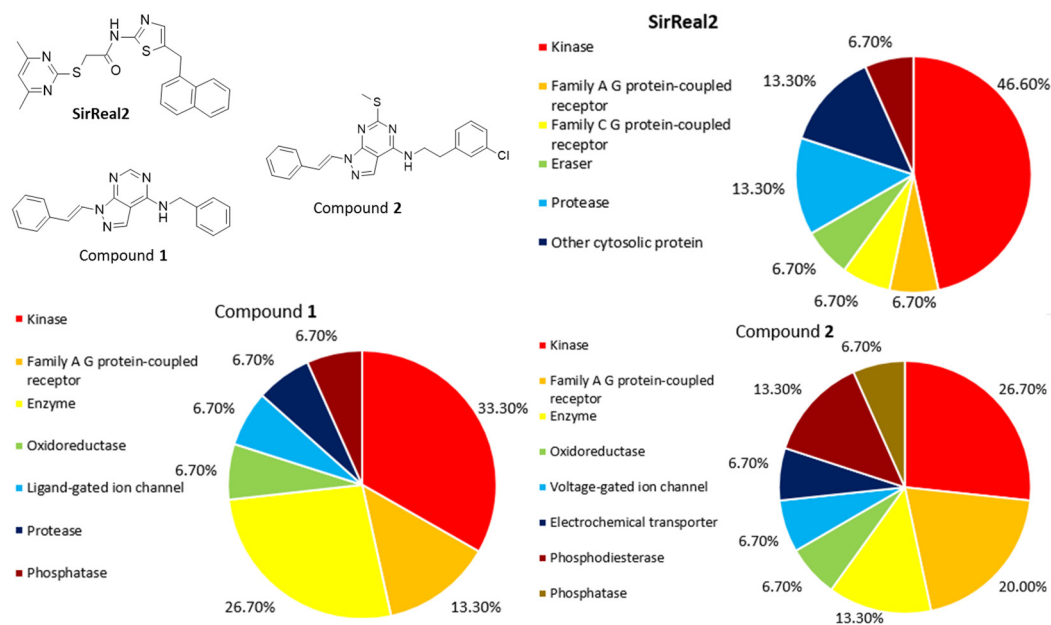
Table S1. Screening performances according to all the scores available in ICM Pro are shown. A color gradient from yellow (low performances) to green (high performance) was used to highlight the best structures for each scoring function.

Structure	AUC (Egv)	AUC (Edock)	AUC (Egb)	AUC (Ege)	AUC (Egs)
5Y5N	0.804	0.678	0.294	0.315	0.601
4RMH	0.673	0.559	0.409	0.409	0.520
5DY4	0.643	0.535	0.423	0.524	0.532
5DY5	0.642	0.564	0.373	0.641	0.469
4RMG	0.634	0.548	0.520	0.555	0.515
5MAR_	0.592	0.514	0.526	0.639	0.362
5YQL	0.591	0.517	0.507	0.618	0.446
5MAT	0.585	0.495	0.420	0.537	0.462
5YQM	0.576	0.502	0.467	0.558	0.481
4RMJ	0.557	0.484	0.543	0.505	0.344
1J8F	0.551	0.486	0.601	0.608	0.456
5YQO	0.549	0.506	0.579	0.574	0.435
4RMI	0.547	0.476	0.451	0.623	0.519
5D7Q	0.533	0.461	0.549	0.530	0.547
5YQN	0.525	0.494	0.601	0.608	0.506
5D7P	0.518	0.472	0.594	0.591	0.463
5D7O	0.516	0.492	0.675	0.617	0.455
3ZGO	0.516	0.466	0.592	0.604	0.400
3ZGV	0.511	0.469	0.610	0.641	0.386
6QCN	0.511	0.466	0.478	0.536	0.447
5Y0Z	0.497	0.443	0.392	0.608	0.352

Table S2. Prospectical VS hitlist derived by the shared best ranked ChemDiv compounds based on the Edock and Egv lists. The Egv and Edock overall values spanning from -100 to -27 and from -79 to +154, respectively. The best ranked compounds herein reported as **1**,**2**,**3**, **30** and **32** correspond to G779-0661, L929-0391, T158-0512, S787-1020 and L407-0319, respectively.

Compound	Edock	Egb	Ege	Egs	Egv

1	-74.1010	-0.0015	-3.1336	-3.9952	-98.1924
2	-73.1753	-2.3449	-2.1623	-5.7277	-97.6812
3	-73.1105	-0.0372	-1.4511	-4.0467	-98.3821
4	-73.0800	-0.0017	-3.7219	-4.2774	-93.4413
5	-72.9835	-0.0135	-0.7816	-5.5705	-95.7549
6	-71.7816	-0.3475	-0.1681	-4.7204	-98.9049
7	-71.5094	-0.0248	-1.3601	-4.0547	-93.7375
8	-69.9059	-2.7525	-4.1420	-3.8886	-94.5518
9	-69.0341	-1.9179	-1.8940	-4.8107	-93.4752
10	-66.9884	-0.0094	-1.6075	-4.1240	-99.7078
11	-65.0870	0.0000	0.3325	-5.6524	-95.6800
12	-64.6306	-0.0009	-0.6915	-6.4698	-97.4875
13	-64.5609	0.0000	-1.3661	-4.6546	-94.0085
14	-64.4532	-1.9053	-2.3282	-5.1569	-104.8000
15	-64.2745	-0.5489	-2.8789	-3.9479	-94.9102
16	-64.2445	-2.5014	-2.7627	-3.3587	-94.7383
17	-63.9475	0.0000	-2.4020	-2.7798	-93.3818
18	-63.6607	-2.5639	-3.3902	-4.5794	-99.5819
19	-63.5323	-0.3831	-1.8376	-3.8880	-101.4200
20	-63.3577	-2.2523	-4.1377	-5.0910	-102.2760
21	-63.1814	-0.8960	-3.2990	-4.5044	-105.1260
22	-62.9697	-2.5862	-3.3027	-4.9437	-103.6320
23	-62.7408	-0.0002	-1.3012	-5.0084	-96.5661
24	-62.5634	-0.7837	-2.8341	-5.2580	-101.2800
25	-62.4619	-0.0389	-1.9369	-4.3631	-97.5730
26	-62.4229	-0.1062	-0.5760	-5.2396	-98.4668
27	-62.3099	-0.0044	-2.2245	-5.1281	-97.9797
28	-62.2316	-0.0005	-4.2195	-4.4760	-94.0403
29	-62.0531	-0.0001	-1.6504	-4.3139	-96.7160
30	-61.9968	-2.5799	-3.5404	-4.4009	-98.1765
31	-61.8770	-0.0637	-0.2563	-4.5305	-95.2331
32	-61.7207	-2.8779	-1.5110	-6.1593	-95.9497
33	-61.5949	-0.8718	-4.6105	-4.6819	-104.8680
34	-61.4346	-0.1157	-0.8091	-5.5982	-97.5079
35	-61.3821	-0.8690	-3.2354	-4.0503	-105.1150
36	-61.3133	-2.5714	-3.8932	-3.9555	-100.8300
37	-61.2720	-2.6280	-3.5496	-4.7674	-102.0800
38	-61.1623	-0.0006	-2.2522	-4.0464	-93.9030
39	-60.6692	-0.6984	-2.3879	-4.4881	-94.3983
40	-60.6456	-0.7940	-3.2206	-3.8874	-101.5290
41	-60.6189	-0.0877	-1.8410	-4.3481	-93.7782
42	-60.4993	-0.0001	-4.2309	-3.7123	-94.5240
43	-60.4117	-1.1882	-2.0536	-4.2117	-98.9525
44	-60.3808	-0.5576	-2.0215	-4.4631	-93.9850
45	-60.2867	-1.4237	-3.9941	-3.8708	-98.7257

46	-60.2196	-2.8228	-2.9219	-6.2219	-95.0871
47	-60.0609	0.0000	-2.5361	-3.5206	-93.2596
48	-59.8568	-1.5263	-2.2573	-6.2298	-95.2045
49	-59.7578	-2.0971	-1.2352	-3.6144	-98.7815
50	-59.5333	-0.0016	-0.9065	-5.3786	-108.2460
51	-59.4172	-0.6711	-4.6271	-3.9765	-101.1850
52	-59.3197	-0.0006	-1.7961	-4.4074	-93.5351
53	-59.3128	-0.0028	-0.2272	-4.5865	-94.5272
54	-59.2622	-0.6176	-3.0479	-4.6180	-108.2000
55	-59.1593	-0.6183	-3.2467	-4.1109	-103.0490
56	-59.1300	-0.1482	-0.9154	-4.6027	-94.3369
57	-59.0794	-0.9283	-3.2935	-4.3358	-105.0790
58	-59.0727	-0.0009	-1.2124	-6.0624	-93.6329
59	-58.9055	-0.0415	-2.6879	-4.6307	-103.3830
60	-58.8999	-1.1390	-2.5991	-5.5194	-100.0400
61	-58.8606	-0.6398	-4.5510	-4.0584	-96.8498
62	-58.2566	-0.9527	-4.0778	-4.2850	-96.8092
63	-58.1412	-1.4077	-2.2307	-4.9205	-94.1710
64	-58.0369	-0.0003	-2.2669	-4.8472	-97.2731
65	-57.8591	-1.5607	-2.9456	-6.1299	-95.9226
66	-57.6873	-0.2497	-1.6056	-4.1594	-95.0826
67	-57.3347	-0.0661	-1.2544	-4.5302	-98.0901
68	-57.2832	-2.7845	-2.1933	-6.4634	-99.0789
69	-57.2566	-0.0091	-2.4627	-4.3892	-93.8245
70	-57.1401	-0.0017	-3.1232	-3.6074	-94.7271
71	-57.1012	-0.0006	-3.6977	-3.6625	-95.7957
72	-56.7250	-2.3513	-3.1346	-4.4361	-97.6273
73	-56.7175	-0.1313	-1.1070	-5.1882	-107.7800
74	-56.7174	-0.0090	-2.4388	-4.7531	-93.7740
75	-56.6428	-2.1925	-2.0195	-3.3692	-98.1029
76	-56.4770	-0.0618	-2.8450	-4.5229	-95.4513
77	-56.4366	0.0000	-3.4258	-3.9035	-96.2544
78	-56.3899	-1.4357	-5.4366	-4.8446	-97.4825
79	-56.3272	-2.0205	-6.1612	-4.0414	-96.3520
80	-56.2961	-0.0024	-1.5220	-5.1081	-102.2000
81	-56.0894	-0.9316	-3.2638	-4.2895	-93.1568
82	-55.9252	-0.0674	-1.6011	-4.7996	-96.9458
83	-55.7163	-0.0137	-0.7777	-3.5116	-94.5758
84	-55.7091	-0.0049	-2.3269	-4.6630	-93.0870
85	-55.6702	-1.5347	-2.6694	-6.4437	-95.6793
86	-55.6015	-0.3624	-0.6558	-4.2062	-95.3834
87	-55.5642	-2.1875	-2.9041	-5.0136	-94.8598
88	-55.3460	-0.0081	-3.1021	-2.8365	-94.6978
89	-55.2981	-0.2023	-3.2514	-5.2092	-97.1420
90	-55.2835	-2.2083	-3.1451	-5.2238	-96.3453
91	-55.2227	-1.4475	-0.6717	-4.1146	-100.5660

92	-55.1365	-0.0292	-2.6166	-4.0825	-100.4020
93	-55.0291	-0.0119	-2.3920	-3.9357	-95.0055
94	-54.9067	-0.7622	-4.3134	-3.9374	-102.6300
95	-54.7922	-2.8035	-5.6293	-5.1247	-98.3778
96	-54.7778	-0.2550	-1.9831	-4.4438	-97.0222
97	-54.6720	-0.0073	-1.4701	-4.6710	-96.5072
98	-54.6015	-0.1328	-1.0676	-4.8723	-96.4729
99	-54.4797	-0.0001	-4.6508	-4.3936	-93.9290
100	-54.4442	-0.6699	-2.3302	-4.7279	-100.2480
101	-54.2429	-1.4547	-2.7816	-4.4133	-94.2846
102	-54.2413	-0.0849	-3.6666	-5.0271	-102.0420
103	-54.1346	-2.6935	-0.1961	-4.5565	-100.3350
104	-54.1119	-0.0563	-4.1697	-3.1698	-103.1620
105	-53.8940	-0.0755	-1.2928	-4.1627	-94.9840
106	-53.8502	-0.0095	-1.3343	-4.1158	-95.3158
107	-53.6962	0.0000	-2.9883	-4.2593	-95.4560
108	-53.6106	-0.0084	-2.5255	-4.2487	-104.4230
109	-53.0678	-0.1815	-1.9526	-5.7253	-99.8734
110	-52.9804	-0.0099	-3.0822	-3.1097	-93.3653
111	-52.9516	-0.0047	-3.4885	-4.6460	-95.7773
112	-52.7471	0.0000	0.6760	-6.2456	-95.8534
113	-52.7269	-0.1925	-2.9846	-5.4825	-97.4698
114	-52.5380	-0.0298	-2.5814	-4.2068	-96.6420
115	-52.5095	-1.1778	-0.5474	-4.6805	-97.6060
116	-52.4309	-2.9244	-2.0999	-4.7908	-94.4248
117	-52.3048	-0.0006	-2.4517	-4.4511	-96.1636
118	-52.2837	0.0000	-1.8656	-4.8776	-94.2555
119	-52.0834	-0.1454	-2.6446	-5.0120	-101.6250
120	-52.0529	-0.1993	-1.5629	-5.4449	-98.0737
121	-52.0324	-0.0202	-2.6802	-3.7517	-97.8603
122	-51.9735	-2.3193	-2.1231	-4.7037	-93.0883
123	-51.9201	-0.0264	-2.5331	-4.0478	-99.0031
124	-51.8823	0.0000	-3.4827	-5.1025	-95.7570
125	-51.8631	-1.9610	-7.1271	-3.7703	-96.7123
126	-51.8626	-2.5053	-4.5323	-4.2030	-96.5582
127	-51.7673	-0.6655	-2.1999	-4.4758	-96.0942
128	-51.5975	-0.1582	-1.2830	-4.4468	-99.0986
129	-51.4326	0.0000	-0.8297	-4.3733	-96.9515
130	-51.3839	-0.3403	-1.3218	-3.9589	-95.6809
131	-51.2375	-0.0378	-3.1637	-3.0342	-94.6419
132	-51.2301	-0.0038	-1.2090	-4.5928	-99.4314
133	-51.2019	-0.0160	-5.1322	-3.8772	-99.6118
134	-51.1852	-0.0858	1.2446	-4.4477	-96.7682
135	-51.1296	-0.0003	-5.1402	-3.4187	-93.6378
136	-51.0505	-0.9464	-2.0487	-3.6821	-95.2863
137	-50.9949	-3.9262	-4.5268	-4.9822	-100.8360

138	-50.9850	-2.0310	-4.5483	-2.8759	-93.3405
139	-50.9300	-2.1170	-2.7666	-5.1867	-94.6087
140	-50.8604	-0.0442	-1.7295	-5.0401	-97.4395
141	-50.7745	-1.8347	-3.3052	-2.9077	-99.4844
142	-50.6867	-0.0159	-5.2116	-3.8444	-102.4420
143	-50.5105	-0.1448	-3.5850	-3.3833	-95.5918
144	-50.5047	-0.0770	-3.8328	-4.2863	-98.2482

Table S3. List of the docked positioning at the PDB code 5Y5N as obtained for the ChemDiv compounds L407-0319, T158-0512, L929-0391, S787-1020 and G779-0661 by the MOE software, via Induced Fit Method. The related scoring functions are reported (see Materials and Methods for details). Each compound best ranked positioning is numbered in brackets.

Compound	S	E_conf	E_place	E_score1	E_refine	E_score2
S787-1020 (1)	-12.9629	-56.3911	-28.1496	-6.9868	-6.0236	-12.9629
S787-1020 (2)	-12.8906	-58.1482	-29.4084	-10.9565	-3.8008	-12.8906
S787-1020 (3)	-12.7075	-52.3098	-28.3601	-10.6552	-0.3903	-12.7075
S787-1020 (4)	-12.5217	-49.5478	-33.5206	-8.4583	3.3984	-12.5217
S787-1020 (5)	-12.3914	-47.3594	-27.6094	-6.4112	21.2888	-12.3914
S787-1020 (6)	-11.3572	-38.6717	-30.9583	-7.0214	38.3946	-11.3572
S787-1020 (7)	-11.2510	-42.8353	-33.3823	-7.8193	24.5549	-11.2510
S787-1020 (8)	-11.1606	-19.9268	-30.0418	-5.2849	43.0576	-11.1606
S787-1020 (9)	-11.0530	-7.2810	-31.1057	-6.3583	39.6091	-11.0530
S787-1020 (10)	-10.7224	-43.2943	-15.7638	-5.5554	-0.0271	-10.7224
G779-0661 (1)	-11.0609	-93.0590	-29.4996	-6.5924	-3.2255	-11.0609
G779-0661 (2)	-10.8909	-100.1790	-27.5835	-6.2791	-1.4830	-10.8909
G779-0661 (3)	-10.8036	-91.2914	-22.1520	-8.4753	-7.3028	-10.8036
G779-0661 (4)	-10.7843	-92.5569	-19.2095	-6.9647	-4.3070	-10.7843
G779-0661 (5)	-10.7174	-91.0160	-19.5722	-6.2984	-3.9622	-10.7174
G779-0661 (6)	-10.5314	-94.8143	-22.9798	-6.8813	-8.0070	-10.5314
G779-0661 (7)	-10.1919	-92.2446	-25.3268	-6.3812	-6.4496	-10.1919
G779-0661 (8)	-10.1027	-97.9148	-23.9967	-8.3370	-3.0042	-10.1027
G779-0661 (9)	-9.6690	-71.9892	-18.8026	-7.9855	15.0713	-9.6690
G779-0661 (10)	-9.4174	-99.1317	-19.8047	-7.2923	-2.0284	-9.4174
L929-0391 (1)	-11.4816	-13.6089	-25.7211	-6.6640	-9.8897	-11.4816
L929-0391 (2)	-11.3849	-6.9678	-22.6603	-7.5868	9.4724	-11.3849
L929-0391 (3)	-11.2309	-8.1932	-38.7532	-11.0587	-6.0178	-11.2309
L929-0391 (4)	-10.9817	4.4021	-28.3159	-7.2305	3.0133	-10.9817
L929-0391 (5)	-10.9782	3.5696	-26.7625	-7.3105	13.8290	-10.9782
L929-0391 (6)	-10.9284	4.6133	-23.8885	-7.7201	8.9119	-10.9284
L929-0391 (7)	-10.7332	-12.4066	-24.5360	-6.9871	-3.3393	-10.7332

Comp.	S	E_conf	E_place	E_score1	E_refine	E_score2
L929-0391 (8)	-10.6277	-2.8772	-32.5934	-8.3447	9.5693	-10.6277
L929-0391 (9)	-10.4772	-4.1015	-25.9803	-6.6437	3.8881	-10.4772
L929-0391 (10)	-10.0302	7.1742	-31.5802	-7.6948	2.0055	-10.0302
L407-0319 (1)	-10.9991	22.4051	-28.9169	-8.6297	0.2398	-10.9991
L407-0319 (2)	-10.6003	19.9485	-32.1959	-6.8201	17.6211	-10.6003
L407-0319 (3)	-10.5481	16.9632	-23.9989	-7.1032	8.9507	-10.5481
L407-0319 (4)	-10.4156	0.8449	-26.3849	-6.9491	3.5253	-10.4156
L407-0319 (5)	-10.4034	24.4647	-29.2555	-8.8818	6.7530	-10.4034
L407-0319 (6)	-10.3856	11.8233	-24.8855	-7.3133	14.6330	-10.3856
L407-0319 (7)	-10.3253	27.9439	-28.9309	-7.9709	29.5893	-10.3253
L407-0319 (8)	-10.3247	36.7086	-32.1794	-8.6008	25.6048	-10.3247
L407-0319 (9)	-10.2434	20.5182	-28.6654	-7.2897	14.9559	-10.2434
L407-0319 (10)	-10.0754	11.5451	-25.3714	-7.2537	21.5696	-10.0754
T158-0512 (1)	-11.2678	-84.1918	-38.2289	-10.4326	-17.1116	-11.2678
T158-0512 (2)	-11.1999	-75.5950	-22.8768	-7.0069	-0.6193	-11.1999
T158-0512 (3)	-10.9673	-58.5861	-18.6111	-7.7297	10.1871	-10.9673
T158-0512 (4)	-10.9055	-69.7515	-24.2180	-7.4382	0.6890	-10.9055
T158-0512 (5)	-10.8869	-66.3196	-24.5560	-7.5278	-7.1103	-10.8869
T158-0512 (6)	-10.6946	-72.8045	-30.8092	-7.1964	-4.7896	-10.6946
T158-0512 (7)	-10.6148	-72.1135	-22.0245	-7.6108	1.8093	-10.6148
T158-0512 (8)	-10.6148	-75.5431	-25.8115	-7.2905	8.2533	-10.6148
T158-0512 (9)	-10.5834	-62.1496	-33.4858	-9.3133	5.5085	-10.5834
T158-0512 (10)	-10.5123	-60.4017	-25.0967	-11.0992	37.9273	-10.5123

Table S4. List of the docked positioning at the PDB code 5Y5N as obtained for the in-house pyrazolopyrimidine derivatives **1-5** and for **AGK2** by the MOE software, via Induced Fit Method. The related scoring functions are reported (see Materials and Methods for details). Each compound (Comp.) best ranked positioning is numbered in brackets.

Comp.	SIRT2 activity, % inhibition (at 150µM)	S	E_conf	E_place	E_score1	E_refine	E_score2
AGK2 (1)		-13.3422	42.0134	-24.8074	-4.9204	-8.0481	-13.3422
AGK2 (2)		-13.1782	32.6195	-24.9241	-5.6795	-12.1494	-13.1782
AGK2 (3)		-13.0759	44.7573	-24.4907	-7.1738	7.9173	-13.0759
AGK2 (4)		-12.7270	56.8438	-22.2558	-5.2599	11.4438	-12.7270
AGK2 (5)		-12.6897	34.5196	-19.6450	-5.5092	4.5140	-12.6897
AGK2 (6)		-12.5827	49.8805	-25.7144	-4.7816	5.9234	-12.5827
AGK2 (7)		-12.3439	84.1997	-28.3850	-6.9059	20.8000	-12.3439
AGK2 (8)	97±10	-12.0308	91.3584	-21.4676	-4.7684	73.4215	-12.0308
AGK2 (9)		-11.3564	30.9502	-17.1473	-5.7395	-11.3315	-11.3564
AGK2 (10)		-11.0339	34.0729	-15.3933	-5.6749	-8.0045	-11.0339
Comp. **1** (1)		-10.1235	32.7543	-20.9708	-8.0659	-9.2104	-10.1235
Comp. **1** (2)		-9.4463	25.1215	-20.3997	-7.8768	-11.5007	-9.4463
Comp. **1** (3)		-9.1678	23.2598	-17.0396	-8.9267	-9.1595	-9.1678
Comp. **1** (4)		-8.8686	18.7720	-22.2926	-8.3693	-12.4879	-8.8686

Comp. 1 (5)	81.2±7.3	-8.5445	21.2742	-18.5652	-9.3955	-5.7665	-8.5445
Comp. 1 (6)		-8.4330	21.0701	-17.6653	-8.0572	-14.1552	-8.4330
Comp. 1 (7)		-8.3396	32.8019	-27.8831	-8.8722	12.4342	-8.3396
Comp. 1 (8)		-7.6088	22.3001	-17.8534	-8.0659	-9.3113	-7.6088
Comp. 1 (9)		-7.5627	23.6958	-13.9132	-10.3548	-10.1522	-7.5627
Comp. 1 (10)		-7.2794	22.3064	-15.0052	-8.4840	-9.2212	-7.2794
Comp. 2 (1)	79.9±5.7	-10.6011	78.4224	-23.4699	-5.1424	46.1146	-10.6011
Comp. 2 (2)		-10.4132	45.4204	-10.2007	-5.1029	11.4587	-10.4132
Comp. 2 (3)		-10.1726	29.4811	-18.7644	-7.7152	4.3011	-10.1726
Comp. 2 (4)		-10.1081	29.1231	-10.5693	-5.2501	6.0333	-10.1081
Comp. 2 (5)		-10.0823	27.7032	-13.7296	-6.6773	7.4934	-10.0823
Comp. 2 (6)		-10.0200	43.8283	-15.6982	-7.8617	15.4735	-10.0200
Comp. 2 (7)		-10.0159	27.7095	-16.6155	-5.5088	8.2891	-10.0159
Comp. 2 (8)		-9.6562	15.5165	-17.0470	-9.1955	4.8109	-9.6562
Comp. 2 (9)		-9.6085	35.7236	-11.2675	-6.6172	18.2979	-9.6085
Comp. 2 (10)		-9.4280	18.1683	-22.3381	-9.3905	21.9861	-9.4280
Comp. 3 (1)	51.1±15.2	-8.9098	32.3784	-18.8613	-10.0137	-4.1164	-8.9098
Comp. 3 (2)		-8.8555	34.7034	-19.6096	-9.3624	-8.7683	-8.8555
Comp. 3 (3)		-8.5589	32.5533	-17.0152	-9.1667	-9.1626	-8.5589
Comp. 3 (4)		-8.4621	40.5428	-22.9374	-8.8308	14.8686	-8.4621
Comp. 3 (5)		-8.1030	25.4036	-18.6497	-8.5754	-13.4334	-8.1030
Comp. 3 (6)		-8.0371	37.0049	-17.9247	-9.1068	-7.0459	-8.0371
Comp. 3 (7)		-7.9924	54.3745	-17.6777	-8.9269	11.6464	-7.9924
Comp. 3 (8)		-7.8812	42.2742	-22.0742	-8.5052	10.1671	-7.8812
Comp. 3 (9)		-7.8392	44.4200	-22.1329	-9.4621	13.3254	-7.8392
Comp. 3 (10)		-7.4396	57.1753	-19.1514	-9.5890	-4.0853	-7.4396
Comp. 4 (1)	35.7±2.5	-10.2455	50.4786	-28.9652	-9.0331	0.6912	-10.2455
Comp. 4 (2)		-9.8068	50.9603	-22.3115	-7.5869	23.6100	-9.8068
Comp. 4 (3)		-9.7042	64.1719	-30.9991	-8.2975	10.7053	-9.7042
Comp. 4 (4)		-9.5734	41.1254	-23.5727	-8.0481	24.9652	-9.5734
Comp. 4 (5)		-9.3790	54.8781	-22.5588	-7.7471	15.8612	-9.3790
Comp. 4 (6)		-9.0861	45.4645	-23.9362	-8.5249	32.7465	-9.0861
Comp. 4 (7)		-8.4322	29.3551	-20.6842	-8.6190	-5.9255	-8.4322
Comp. 4 (8)		-8.1193	32.5835	-20.8686	-9.4813	8.3224	-8.1193
Comp. 4 (9)		-8.0110	65.7885	-19.2858	-8.1719	31.8408	-8.0110
Comp. 4 (10)		-7.7538	33.4744	-20.7952	-8.2916	17.8685	-7.7538
Comp. 5 (1)	31.4	-8.3733	14.1298	-21.5673	-10.2491	-5.6128	-8.3733
Comp. 5 (2)		-8.0763	11.6770	-22.4232	-9.7738	-10.3065	-8.0763
Comp. 5 (3)		-8.0670	21.7656	-23.8852	-9.3472	20.0158	-8.0670
Comp. 5 (4)		-7.9753	11.7914	-23.5345	-9.7690	-9.6618	-7.9753
Comp. 5 (5)		-7.9118	16.5507	-21.9831	-10.4226	-9.4711	-7.9118
Comp. 5 (6)		-7.7647	29.9488	-26.6388	-9.4354	-5.7638	-7.7647
Comp. 5 (7)		-7.7461	24.2195	-21.1790	-9.5222	-2.2892	-7.7461
Comp. 5 (8)		-7.5208	25.7669	-22.3904	-10.0996	2.3178	-7.5208
Comp. 5 (9)		-7.3605	31.3796	-22.4805	-10.5198	14.2673	-7.3605
Comp. 5 (10)		-7.1799	21.2991	-26.7483	-9.4834	-5.3364	-7.1799

Table S5. Calculated features as suggested by the Lipinski's and Veber's rules and by other pharmacokinetic (PK) properties, referred to the novel compounds (Comp.) **1–5**. The same prediction for the reference inhibitor SirReal2 has been listed. The predicted values for the effective pyrazolo-pyrimidine **1** as ameliorated druglike compound, with respect to SirReal2 (in grey), are highlighted in light cyan. Reliability index values for a number of descriptors are shown as R.I. (values higher than 0.30 are ranked as reliable by the software).

Comp.	MW[a] (g/mol)	HBA[b]	HBD[c]	N. rot. bonds[d]	LogP[e]	TPSA[f]	HIA (%)[g]	Vd (l/kg)[h]	%PPB[i] (R.I. ≥ 0.60)	LogKa[l HSA] (R.I. ≥ 0.40)	%F (oral)[m] 50mg
1	327.38	3	1	5	2.88	55.63	100	2.7	98.57	4.77	96.6
2	421.95	3	1	7	4.21	80.93	100	4.3	99.97	5.04	38.0
3	309.37	4	1	7	2.00	64.86	100	1.9	95.55	4.09	99.3
4	319.40	3	0	3	2.87	46.84	100	3.5	96.54	4.30	91.6
5	277.32	3	1	4	2.16	55.63	100	2.1	96.24	3.79	98.4
SirReal2	420.55	4	1	7	2.65	121.31	100	2.5	98.71	4.69	21.9

[a] Molecular weight; [b] Number of H-bond acceptors; [c] Number of H-bond donors; [d] Number of rotatable bonds; [e] Logarithmic ratio of the octanol–water partitioning coefficient; [f] Topological polar surface area; [g] HIA represents the human intestinal absorption, expressed as percentage of the molecule able to pass through the intestinal membrane; [h] Prediction of Volume of Distribution (Vd) of the compound in the body; [i] Ligand affinity toward human serum albumin (HSA); [m] Percentage oral bioavailability

Table S6. Calculated ADMET descriptors related to metabolism, excretion and toxicity properties, referred to the novel compounds (Comp.) **1–5** and to SirReal2. The pyrazolo-pyrimidine **1** and SirReal2 are highlighted in light cyan and grey, respectively.

Comp.	Endocrine System Disruption[a] (R.I. ≥ 0.30)		CYP3A4[b] (R.I. ≥ 0.30)		LD$_{50}$ (mg/kg)[c] Mouse oral (R.I. ≥ 0.30)	hERG inhibitor [d] (R.I = 0.20)	PAINS (Pan Assay Interference structures)
	LogRBA > -3	LogRBA > 0	Inhibitor (IC$_{50}$ < 10μM)	Substrate			
1	**0.58**	**0.03**	0.76	0.87	1000	0.15	0 ALERT
2	0.52	0.02	0.79	0.95	1000	0.24	0 ALERT
3	0.15	0.00	0.47	0.63	930	0.17	0 ALERT
4	0.41	0.05	0.36	0.74	650	0.30	0 ALERT

5	0.09	0.00	0.44	0.58	540	0.09	0 ALERT
SirReal2	0.34	0.09	0.38	0.97	1100	0.15	0 ALERT

[a] RBA represents the relative binding affinity with respect to that of estradiol. Compounds showing LogRBA > 0 are classified as strong estrogen binders, while those showing LogRBA < -3 are considered as non-binders; [b] Prediction of the ligand inhibitor/substrate behavior towards cytochrome CYP3A4 [c] Acute toxicity (LD$_{50}$) for mice after oral administration; [d] Probability of human ether-á-go-go-related gene (hERG) channel inhibition at clinically relevant concentrations (K_i<10 μM).