SUPPORTING INFORMATION

Protein Flexibility in Virtual Screening: The BACE-1 Case Study.

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Table S1. Root-mean square deviations (RMSD) of all the considered BACE-1 structures superimposed on the structure with PDB code 1XS7.[1]

PDB	RMSD (Cα)	PDB	RMSD (Cα)	PDB	RMSD (Cα)	PDB	RMSD (Cα)	PDB	RMSD (Cα)
1FKN	0.685	2PH8	2.104	2ZHS	2.047	3IVI	1.862	3L5B	2.441
1M4H	0.691	2Q11	0.828	2ZHT	2.036	3IXJ	1.267	3L5C	2.419
1SGZ	0.465	2Q15	1.534	2ZHU	2.031	3IXK	1.278	3L5D	2.309
1TQF	1.868	2QK5	2.766	2ZHV	2.077	3K5C	1.380	3L5E	2.615
1W50	1.690	2QMD	2.612	2ZJH	2.632	3K5D	1.633	3L5F	2.609
1W51	1.885	2QMF	2.619	2ZJI	2.229	3K5F	1.752	3LHG	1.163
1XN2	0.370	2QMG	2.594	2ZJJ	2.168	3K5G	1.410	3LNK	2.620
1XN3	0.462	2QP8	2.617	2ZJK	2.105	3KMX	2.798	3LPI	2.601
1YM2	1.373	2QU2	1.412	2ZJL	2.477	3KMY	2.730	3LPJ	2.620
1YM4	1.085	2QU3	1.279	2ZJM	2.009	3KN0	2.445	3LPK	2.569
2B8L	0.792	2QZK	1.398	2ZJN	2.272	3KYR	2.040	3MSJ	1.714
2B8V	1.621	2QZL	2.039	3BRA	2.157	3L38	1.052	3MSK	1.688
2F3E	1.368	2VA5	1.638	3BUF	2.059	3L3A	1.257	3MSL	1.795
2F3F	1.655	2VA6	1.664	3BUG	2.223	3L58	2.763	3N4L	1.077
2FDP	1.692	2VA7	1.959	3BUH	2.138	3L59	2.594	3NSH	1.329
2G94	0.448	2VIE	1.704	3CIB	2.623	3L5B	2.441		
2HIZ	1.879	2VIJ	1.691	3CIC	2.621	3L5C	2.419		
2HM1	1.916	2VIY	1.992	3CID	2.608	3L5D	2.309		
2IQG	1.912	2VIZ	2.409	3CKP	1.130	3L5E	2.615		
2IRZ	0.777	2VJ6	2.424	3CKR	1.153	3L5F	2.609		
2IS0	0.791	2VJ7	2.504	3DM6	1.567	3LHG	1.163		
2NTR	0.782	2VJ9	2.400	3DUY	1.890	3LNK	2.620		
2OAH	0.919	2VKM	0.523	3DV1	1.367	3LPI	2.601		
2OF0	1.700	2VNM	1.396	3DV5	1.409	3LPJ	2.620		
2OHK	1.662	2VNN	1.392	3EXO	2.026	3LPK	2.569		
2OHL	1.683	2WEZ	1.999	3FKT	1.310	3MSJ	1.714		
2OHM	1.631	2WF0	2.002	3H0B	2.092	3MSK	1.688		
2OHN	1.664	2WF1	1.386	3HVG	0.909	3MSL	1.795		
2OHP	1.718	2WF2	1.390	3HW1	1.943	3N4L	1.077		
2OHQ	1.674	2WF3	1.379	3I25	1.757	3NSH	1.329		
2OHR	1.781	2WF4	2.007	3IGB	1.035	3KMX	2.798		
2OHS	2.014	2WJO	1.705	3IN3	1.385	3KMY	2.730		
2OHT	1.678	2XFI	2.007	3IN4	1.360	3KN0	2.445		
2OHU	1.645	2XFJ	1.656	3IND	1.513	3KYR	2.040		
2P4J	0.508	2XFK	2.287	3INE	0.870	3L38	1.052		
2P83	1.872	2ZDZ	1.407	3INF	1.214	3L3A	1.257		
2P8H	0.797	2ZE1	0.728	3INH	0.972	3L58	2.763		
2PH6	1.847	2ZHR	0.466	3IVH	2.031	3L59	2.594		

Table S2. PDB codes of the analysed X-Ray BACE-1 structures clustered according to their conformational behaviour

Motion Description	Reference Structure	Structures in the Cluster
Flap open conformation	1XN3	1W50, 2OF0, 2OHK, 2OHL, 2OHM, 2OHN, 2OHP, 2OHQ, 2OHR, 2OHS, 2OHT, 2OHU, 2Q11, 2Q15, 2QU2, 2QU3, 2VA5, 2VA6, 2VA7, 2WJO, 2ZDZ, 2ZE1, 2ZHS, 2ZHT, 2ZHU, 2ZHV, 2ZJH, 2ZJI, 2ZJK, 2ZJL, 2ZJN, 2BRA, 3BUF, 3BUG, 3BUH, 3EXO, 3FKT, 3H0B, 3HW1, 3IGB, 3IN3, 3IN4, 3IND, 3INE, 3INF, 3KMY, 3KN0, 3L38, 3L3A, 3L59, 3L5B, 3L5C, 3L5D, 3L5E, 3L5F, 3LHG, 3LNK, 3MSJ, 3MSK, 3MSL
10s loop closed conformation	1FKN	1M4H, 1SGZ, 1XN2, 1XN3, 1XS7, 1YM2, 3F3F, 2G94, 2NTR, 2OAH, 2OHS, 2PH8 , 2Q11, 2Q15, 2QK5, 2QMD, 2QMF, 2QMG, 2QP8, 2VA6, 2VA7, 2VIZ, 2VJ6, 2VJ9, 2XFK, 2ZE1, 2ZHR, 3CIB, 3CIC, 3CID, 3K5D, 3KMX, 3KMY, 3KN0, 3L58, 3L59, 3L5B, 3L5C, 3L5D, 3L5E, 3L5F, 3LNK, 3LPI, 3LPJ, 3LPK, 3MSJ, 3MSK, 3MSL, 3N4L, 3NSH
10s loop open conformation	1W51	1W50, 1YM4, 2B8L, 2B8V, 2F3E, 2FDP, 2HIZ, 2HM1, 2IQG, 2IRZ, 2IS0, 2OF0, 2OHK, 2OHL, 2OHM, 2OHN, 2OHP, 2OHQ, 2OHR, 2OHT, 2OHU, 2P83, 2P8H, 2QU2, 2QU3, 2QZK, 2QZL, 2VA5, 2VIE, 2VIJ, 2VIY, 2VJ7, 2VNM, 2VNN, 2WEZ, 2WF0, 2WF1, 2WF2, 2WF3, 2WF4, 2WJO, 2XFI, 2XFJ, 2ZDZ, 2ZE1, 2ZHS, 2ZHT, 2ZHU, 2ZHV, 2ZJH, 2ZJI, 2ZJJ, 2ZJK, 2ZJL, 2ZJM, 2ZJN, 3BRA, 3BUF,

		3BUG, 3BUH, 3CKP, 3CKR, 3DM6, 3DUY, 3DV1, 3DV5, 3EXO, 3FKT, 3H0B, 3HVG, 3HW1, 3I25, 3IGB, 3IN3, 3IN4, 3IND, 3INE, 3INF, 3INH, 3IVH, 3IVI, 3IXJ, 3IXK, 3K5C, 3K5F, 3K5G, 3KYR, 3L38, 3L3A
10s loop outlier conformation	1TQF	2P4J, 2PH6, 2VKM,

Table S3. Stuctures of the 45 known active inhibitors included in the docking simulations.

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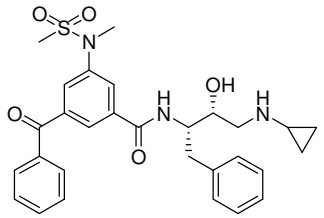
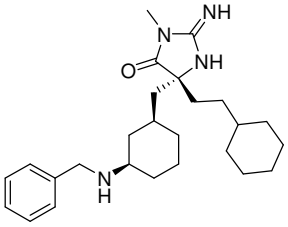
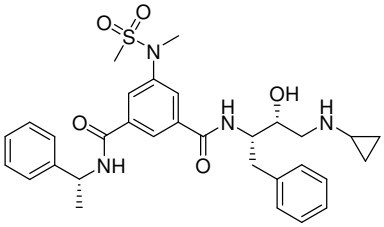
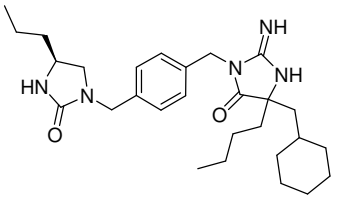
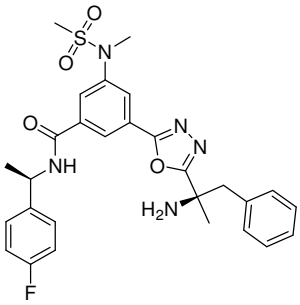
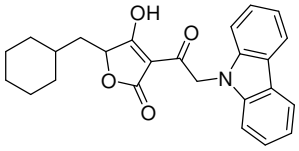
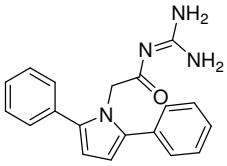
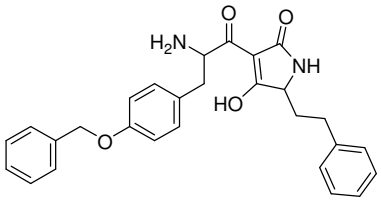
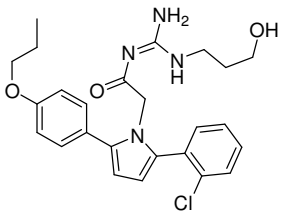
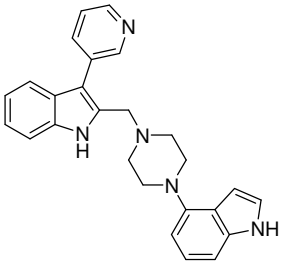
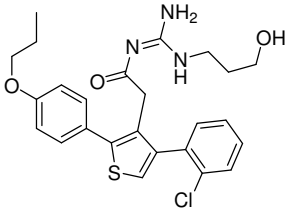
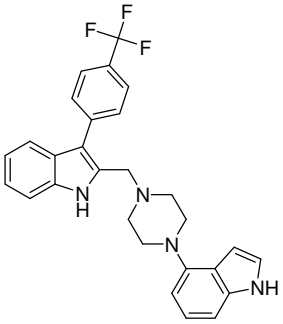
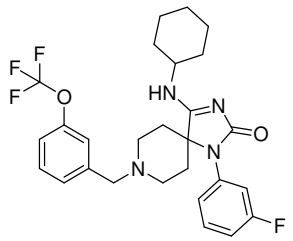
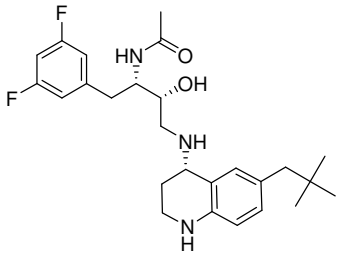
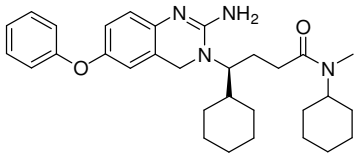
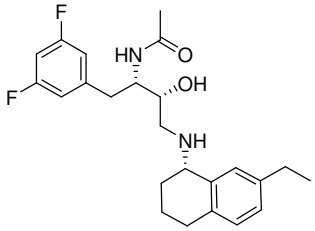
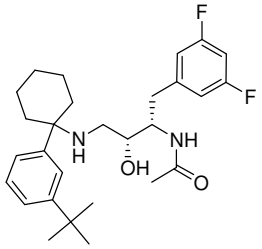
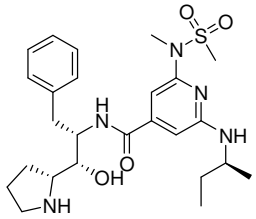
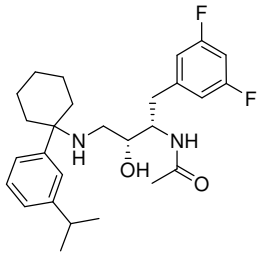
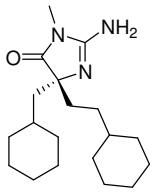
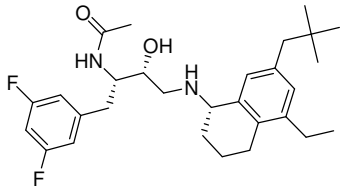
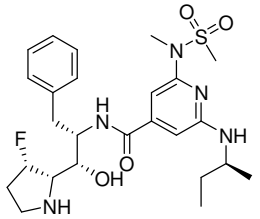
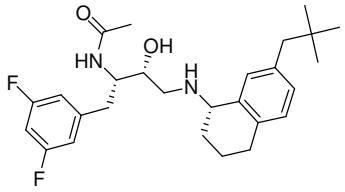
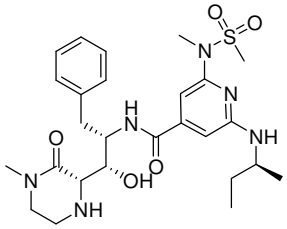
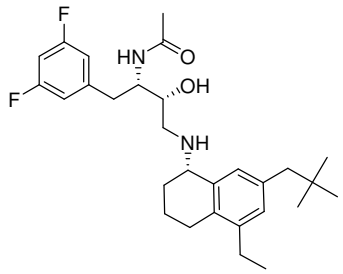
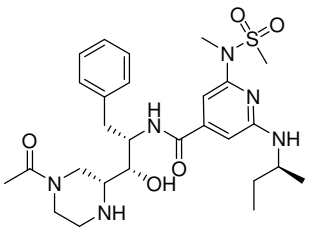
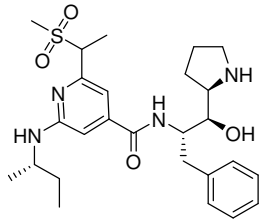
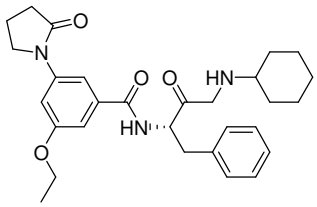
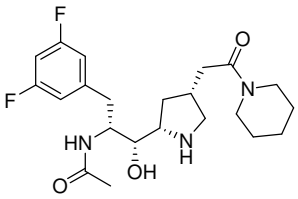
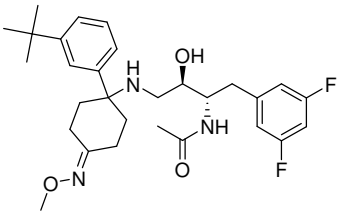
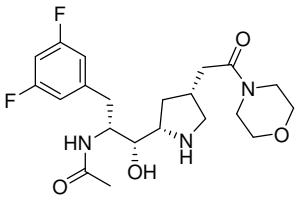
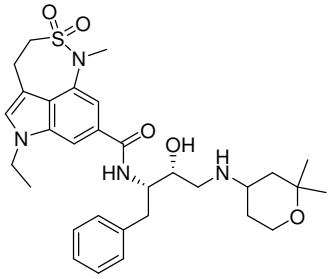
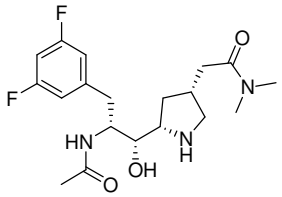
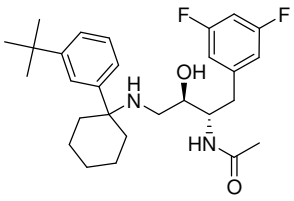
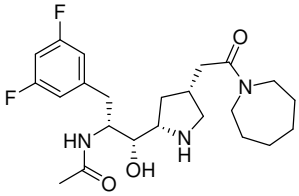
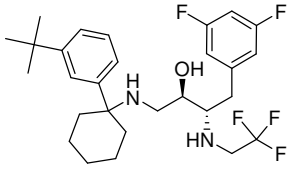
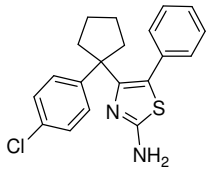
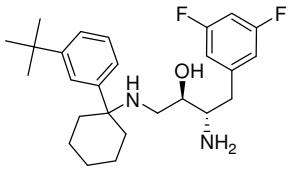
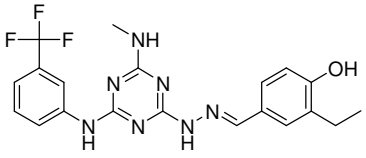
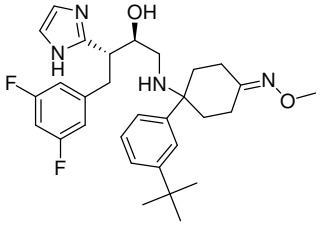
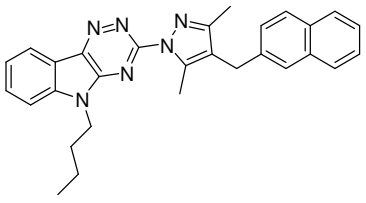
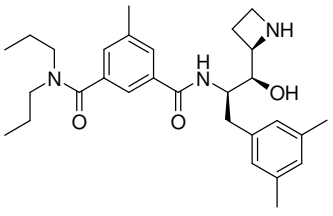
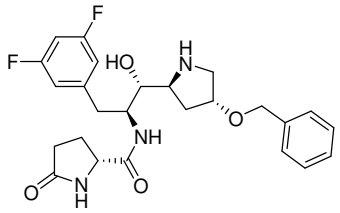
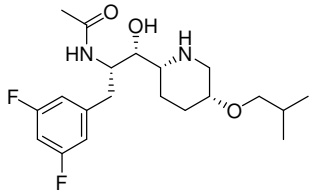
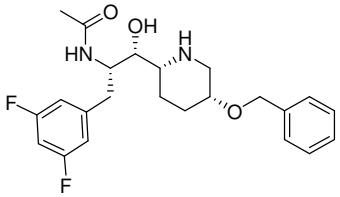
	0.098	2		<1	2
	0.015	2		<1	3
	0.012	4		11	4
	3.7	6		16	5
	0.12	6		2.2	7
	0.15	6		4.3	7

	<100	8		0.19	10
	0.021	9		0.21	10
	0.047	10		0.0018	11
	n.a.	10		0.1	11
	0.012	10		0.0039	11
	0.009	10		0.001	11
	0.012	10		0.003	11

	0.0039	11		<10	12
	0.82	11		n.a.	13
	1.4	11		n.a.	14
	5.2	11		n.a.	15
	0.38	11		n.a.	15
	<100	16		n.a.	15
	n.a.	17		n.a.	15
	32	18		n.a.	19

	n.a.	19		6.8	20
	13.1	20			

Structure characterization of the active compounds

General informations.

All the derivatives were purchased by National Cancer Institute (NCI) and used as received. ^{1}H NMR spectra were recorded with a Varian 400 spectrometer, operating at 400 MHz. Chemical shifts are reported in δ values (ppm) relative to internal Me4Si, and J values are reported in hertz (Hz). Direct infusion ESI-MS spectra were recorded on Waters ZQ 4000 apparatus.

290956;8-[3-(2-chloro-10H-phenothiazin-10-yl)propyl]-1-thia-4,8-diazaspiro[4.5]decan-3-one; ^{1}H NMR (DMSO-d_6) δ 10.39 (bs, 1 H), 7.26-6.95 (m, 7H), 3.93 (t, J = 7.01 Hz, 2H), 3.52 (s, 2H), 3.21-2.85 (m, 6H), 2.32-2.15 (m, 4H), 2.13-1.92 (m, 2H). MS found (M + H)$^+$ (m/z): 446. Calcd for $C_{22}H_{24}ClN_3OS_2$ m/z: 445.10.

299209 4-[(quinolin-3-ylmethyl)amino]benzenesulfonamide ^{1}H NMR (DMSO-d_6) δ 8.89 (s, 1H), 8.21 (s, 1H), 7.98 (d, J = 8.29 Hz, 1H), 7.92 (d, J = 7.96 Hz, 1H), 7.70 (t, J = 7.33 Hz, 1H), 7.56 (t, J = 7.55 Hz, 1H), 7.47 (d, J = 8.42 Hz, 2H), 7.11 (bs, 1H), 7.87 (bs, 2H), 6.68 (d, J = 8.46 Hz, 2H), 4.55 (d, J = 5.61 Hz, 2H). MS found (M + Na)$^+$ (m/z): 336. Calcd for $C_{16}H_{15}N_3O_2S$ m/z: 313.09.

263220; 3-benzyl-6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide; ^{1}H NMR (DMSO-d_6) δ 8.11 (s, 1H), 7.95 (s, 1H), 7.34-7.28 (m, 4H), 7.27-7.23 (m, 1H), 7.01 (s, 1H), 4.98-4.92 (m, 1H), 3.16-3.08 (m, 1H), 3.02-2.93 (m, 1H). MS found (M + Na)$^+$ (m/z): 410. Calcd for $C_{14}H_{14}ClN_3O_2S$ m/z: 387.01.

372280; 2-[5-(phenoxymethyl)-1*H*-indol-3-yl]2,4-dihydrofuro[2,3,*b*]quinoxalin-3-ol; ^{1}H NMR (DMSO-d_6) δ 12.01 (bs, 1H), 9.86 (s, 1H), 8.20 (s, 1H), 7.68-7.64 (m, 1H), 7.50-7.27 (m, 10H), 6.98-6.92 (m, 1H), 5.10 (m, 3H).

116490; 12-[(4-methylpiperidin-1-yl)acetyl]-12*H*-benzo[*b*]phenothiazine; ^{1}H NMR (DMSO-d_6) δ 8.20 (s, 1H), 8.10 (s, 1H), 7.95-7.86 (m, 2H), 7.66 (d, J = 8.27 Hz, 1H), 7.59-7.48 (m, 3H), 7.37 (t, J = 7.35 Hz, 1H), 7.28 (t, J = 7.14 Hz, 1H), 2.35 (m, 1H), 1.82 (m, 4H), 1.35 (m, 4H), 0.73 (d, J= 6.7 Hz, 3H), 0.87-0.54 (m, 5H). MS found (M + Na)$^+$ (m/z): 389. Calcd for $C_{24}H_{24}N_2OS$ m/z: 388.16.

162404; 3-methyl-2-oxo-2*H*-chromen-7-yl 4-{[amino(imino)methyl]amino}benzoate; ^{1}H NMR (DMSO-d_6) δ 8.16 (d, J = 8.77 Hz, 2H), 7.89-7.80 (m, 4H), 7.46-7.40 (m, 2H), 7.36-7.31 (m, 1H), 6.41(s, 1H), 2.45 (s, 3H). MS found $(M + H)^+$ (m/z): 338. Calcd for $C_{18}H_{15}N_3O_4$ m/z: 337.11

59349; 8-[3-(2-chloro-10*H*-phenothiazin-10-yl)propyl]-8-azabicyclo[3.2.1]octan-3-ol; ^{1}H NMR (DMSO-d_6) δ 7.29-6.94 (m, 7H), 3.98-3.79 (m, 5H), 3.04-2.91 (m, 2H), 2.11-1.59(m, 10H). MS found $(M + H)^+$ (m/z): 401. Calcd for $C_{22}H_{25}ClN_2OS$ m/z: 400.14

126710; 5-(3,4-dichlorophenyl)-6-[(3-methyl-4-nitrophenoxy)methyl]pyrimidine-2,4-diamine; ^{1}H NMR (DMSO-d_6) δ 7.96 (d, J = 10.13 Hz, 1H), 7.57 (d, J = 8.07 Hz, 1H), 7.40 (s, 1H), 7.16 (d, J = 8.22 Hz, 1H), 6.80 (s, 2H), 6.15 (s, 2H), 6.02 (bs, 4H), 4.63 (s, 3H). MS found $(M + H)^+$ (m/z): 420. Calcd for $C_{18}H_{15}Cl_2N_5O_3$ m/z: 419.06

13316; [2-(4-chlorophenyl)quinolin-4-yl](piperidin-2-yl)methanol; ^{1}H NMR (DMSO-d_6) δ 8.28-8.19 (m, 3H), 8.12 (s, 1H), 8.07 (d, J = 8.54 Hz, 1H), 7.75 (t, J = 7.47 Hz, 1H), 7.63-7.56 (m, 3H), 5.66 (bs, 1H), 5.26-5.21 (m, 1H), 2.92-2.78(m, 2H), 2.40 (t, J = 11.90 Hz, 1H), 1.71-1.63 (m, 1H), 1.52-1.36 (m, 2H), 1.28-1.06 (m, 3H). MS found $(M + H)^+$ (m/z): 353. Calcd for $C_{21}H_{21}ClN_2O$ m/z: 352.13

150117; (2Z)-2-benzylidene-3-(cyclohexylamino)indan-1-one; ^{1}H NMR (DMSO-d_6) δ 8.14-8.09 (m, 1H), 8.04-7.97 (m, 1H), 7.94-7.80 (m, 4H), 7.77-7.67 (m, 1H), 7.56-7.45 (m, 3H), 2.68 (m, 1H), 1.89 (m, 4H), 1.61-0.86 (m, 6H). MS found $(M + H)^+$ (m/z): 318. Calcd for $C_{22}H_{23}NO$ m/z: 317.18

19976; 10-[3-(1-azaspiro[4.5]dec-1-yl)propyl]-2-chloro-10*H*-phenothiazine; ^{1}H NMR (DMSO-d_6) δ 7.21-6.99 (m, 5H), 6.97-6.88 (m, 2H), 3.89 (t, J= 6.7 Hz, 2H), 2.68 (m, 2H), 1.75-1.40 (m, 12H), 1.27-0.83 (m, 6H). MS found $(M + H)^+$ (m/z): 413. Calcd for $C_{24}H_{29}ClN_2S$ m/z: 412.17

309874; 3-chloro-N-{2-[(2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)methyl]phenyl}-1-benzothiophene-2-carboxamide; ^{1}H NMR (DMSO-d_6) δ 8.17-8.11 (m, 1H), 7.98-7.90 (m, 1H), 7.68-7.57 (m, 3H), 7.20-6.88 (m, 7H), 3.81 (m, 1H), 3.18-2.92 (m, 2H), 2.67-2.25 (m, 4H), 2.15 (s, 3H). MS found $(M + H)^+$ (m/z): 447. Calcd for $C_{26}H_{23}ClN_2OS$ m/z: 446.12

88852; 1-{[(3,5-dibromo-2-hydroxybenzyl)(methyl)amino]methyl}-2-naphthol; ^{1}H NMR (DMSO-d_6) δ 7.99 (d, J = 8.72 Hz, 1H), 7.82-7.72 (m, 2H), 7.54-7.45 (m, 2H), 7.33-7.17 (m, 3H), 4.08 (s, 2H), 3.82 (s, 2H), 2.17 (s, 3H). MS found $(M + H)^+$ (m/z): 452. Calcd for $C_{19}H_{17}Br_2NO_2$ m/z: 450.96

170561; 1-methyl-2-[(4-methylpiperazin-1-yl)methyl]indeno[1,2,3-de]quinazolin-1-ium; ^{1}H NMR (DMSO-d_6) δ 8.29 (d, J = 7.68 Hz, 1H), 8.08 (t, J = 6.95 Hz, 2H), 7.86-7.74 (m, 2H), 7.60 (t, J = 7.39 Hz, 1H), 7.50 (t, J = 7.56 Hz, 1H), 4.03 (s, 2H), 2.76-2.61 (m, 4H), 2.39-2.25 (m, 4H), 2.13 (s, 3H). MS found $(M + H)^+$ (m/z): 333. Calcd for $C_{20}H_{20}N_4O$ m/z: 332.16

62914; 2,6-Bis(2-hydroxy-3-tert-butyl-5-methylbenzyl)-4-methylphenol; ^{1}H NMR (DMSO-d_6) δ 6.79 (s, 2H), 6.70 (s, 2H), 6.56 (s, 2H), 3.79 (s, 4H), 2.07 (s, 9H), 1.32 (s, 18H). MS found $(M + Na)^+$ (m/z): 483. Calcd for $C_{31}H_{40}O_3$ m/z: 460.30.

79563; 1,3-bis(4-chlorobenzyl)-2-(5,8-dihydrophenanthren-9-yl)hexahydropyrimidine; ^{1}H NMR (DMSO-d_6) δ 8.04-7.90 (m, 2H), 7.78-7.58 (m, 5H), 7.23-7.12 (m, 5H), 7.06-6.94 (5H), 4.08 (s, 1H), 3.34 (s, 4H), 2.11-2.04 (m, 4H), 1.54 (m, 2H). MS found $(M + H)^+$ (*m*/*z*): 513. Calcd for $C_{32}H_{30}Cl_2N_2$ *m*/*z*: 512.18

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