5-Aryl-pyrazole-3-carboxylic acids as selective inhibitors of human carbonic anhydrases IX and XII

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		Percentage of inhibition at 10 ⁻⁷ (10 ⁻⁴) M						
Compound	R-	hCA I	hCA II	hĆA IX	hCA XII			
1	4-Me	20 (30)	34 (35)	29 (44)	33 (48)			
2	4-Et	21 (37)	35 (37)	29 (46)	41 (52)			
3	4- <i>i</i> -Pr	17 (36)	31 (45)	24 (47)	33 (47)			
4	4- <i>n</i> -Bu	25 (39)	35 (45)	33 (51)	42 (52)			
5	4- <i>t</i> -Bu	20 (39)	36 (45)	30 (52)	41 (51)			
6	2,4-di-Me	15 (32)	36 (38)	33 (50)	42 (49)			
7	3,4-di-Me	19 (30)	33 (37)	28 (50)	35 (46)			
8	2,4,5-tri-Me	20 (33)	33 (39)	30 (54)	35 (48)			
9	2,3,5,6-tetra-Me	28 (34)	34 (41)	29 (50)	36 (46)			
10	2,4,6-tri-Et	24 (33)	35 (45)	30 (49)	39 (47)			
11	2,4-di- <i>i</i> -Pr	22 (44)	35 (43)	27 (52)	33 (49)			
12	2,4,6-tri- <i>i</i> -Pr	17 (39)	34 (49)	26 (52)	37 (52)			
13	β -Tetralinyl	24 (33)	19 (25)	33 (49)	43 (51)			
14	β -Naphtyl	21 (36)	35 (38)	29 (48)	42 (54)			
15	4-Phenyl	19 (34)	34 (45)	32 (48)	42 (55)			
16	4-Pyrollidinyl	20 (36)	34 (48)	33 (46)	35 (52)			
17	4 - F	20 (29)	32 (36)	29 (45)	36 (51)			
18	4-C1	17 (30)	36 (38)	31 (45)	42 (50)			
19	3-Br	20 (32)	32 (35)	30 (54)	38 (49)			
20	4 - OH	23 (30)	35 (41)	30 (45)	35 (51)			
21	2-OMe	21 (30)	28 (40)	27 (44)	35 (50)			
22	4-OMe	25 (30)	36 (40)	33 (51)	34 (47)			
23	4-OMe-2,5-diMe	22 (35)	35 (40)	33 (52)	42 (47)			

Table S1. Percentage of CAs I, II, IX and XII inhibition on two concentrations of compounds 1-23

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compounds 1-23 in its anionic form.											
Compound	R-	SA*	PSA*	ASA*	Volume	VLogP*					
1	4-Me-	224.8130	64.0790	160.7340	177.4143	1.4671					
2	4-Et-	243.2491	64.0790	179.1701	192.8189	2.0007					
3	4- <i>i</i> -Pr-	264.0426	64.0790	199.9636	209.9865	2.0888					
4	4- <i>n</i> -Bu-	292.9753	65.6070	227.3683	230.5539	2.8327					
5	4- <i>t</i> -Bu-	282.4734	63.6160	218.8573	228.9744	2.6183					
6	2,4-di-Me-	246.8261	65.3018	181.5243	194.0475	1.9798					
7	3,4-di-Me-	242.2963	65.0251	177.2712	193.6693	1.9467					
8	2,4,5-tri-Me-	264.9485	64.5335	200.4150	212.3705	2.3428					
9	2,3,5,6-tetra-Me-	284.3445	65.9355	218.4090	228.3398	2.6140					
10	2,4,6-tri-Et-	327.5655	63.9959	263.5696	261.0485	3.4748					
11	2,4-di- <i>i</i> -Pr-	326.4613	64.9063	261.5551	261.1596	2.9713					
12	2,4,6-tri- <i>i</i> -Pr-	387.3279	64.1751	323.1528	311.0422	3.9021					
13	β -Tetralinyl-	268.1217	65.0251	203.0966	218.2483	2.3040					
14	β -Naphtyl-	251.6167	64.3173	187.2994	206.9182	1.9691					
15	4-Phenyl-	273.0847	64.9809	208.1038	228.0558	2.2108					
16	4-Pyrrolidinyl-	281.5775	67.1797	214.3978	229.2079	1.7434					
17	4-F-	205.8496	64.0790	141.7706	163.2093	1.2486					
18	4-Cl-	217.0917	64.0790	153.0126	174.1838	1.6192					
19	3-Br-	224.0955	64.6011	159.4944	179.5580	1.9400					
20	4-OH-	211.6867	87.4045	124.2822	168.2015	0.4326					
21	2-OMe-	236.4103	76.4652	159.9451	186.9544	0.9978					
22	4-OMe-	232.9842	75.3840	157.6001	185.6467	1.0959					
23	4-OMe-2,5-di-Me-	280.0472	75.7126	204.3346	222.4115	1.9971					

 Table S2. Calculated 3D-dependent whole-molecular properties of

 compounds 1 23 in its anionic form

*SA- Surface area, PSA - Polar surface area, ASA - Apolar surface area, VlogP - Virtual logP



11.5 9.5 8.0 6.5 5.0 3.5 2.0 Figure S1. NOESY spectrum of 4-Me-derivative (1) in DMSO upon addition of the 1 molar equivalent of piperidine.



b) Figure S2. FT-IR spectra of a) 4-Et- (2), and b) 3,4-di-Me-derivative (7). In the IR spectrum of derivative 7 broadening of N-H bands in region of \sim 3100 cm⁻¹ is observed, due to aggregation by intermolecular H-bonding.



Figure S3. Estimated pK_a values of derivative 1. Tautomers with protonated pyrazole nitrogens were used for prediction.



a)



Figure S4. Best-ranked solutions of compounds 15 (a) and 1(b) docked into CA I.



Figure S5. Best-ranked solutions of compounds 15 (a) and 1(b) docked into CA II.



Figure S6. Shape complementarity of active and inactive compounds toward CA IX. a) Surfaces of compound 9 (red transparent) and the cleft of CAIX (gray semi-transparent) are shown. b) 2D depiction of shape complementarity of compound 9. c) 2D depiction of shape complementarity of compound 11. e) 2D depiction of shape complementarity of compound 11. e) 2D depiction of shape complementarity of compound 13.



b)



Figure S7. Best-ranked solutions of compounds 4 (a), 2 (b), and 9 (c) docked into CA XII.



Figure S8. Molecular interaction fields of HBA probe (O) around residue 67 of carbonic anhydrase (hCA I - His, hCA II - Asn, hCA IX - Gln, hCA XII - Lys), depicted on isocontour level of -6.2 kcal/mol. Color of fields: hCA I - red, hCA II - orange, hCA IX - yellow, hCA XII - blue.



Figure S9. Labeled residues within the box used for GRID molecular-interaction fields calculation, exemplified on the hCA XII (PDB entry 1JD0). Active site Zn^{2+} ion is depicted as an orange sphere.



Figure S11. ¹³C NMR spectrum of compound 4.











Figure S21. ¹³C NMR spectrum of compound 11.



Figure S23. ¹³C NMR spectrum of compound 12.







Figure S29. ¹³C NMR spectrum of compound 22.



Figure S31. ¹³C NMR spectrum of compound 23.