Supporting Information

Rational Design of Benzylidenehydrazinyl-Substituted Thiazole Derivatives as Potent Inhibitors of Human Dihydroorotate Dehydrogenase with *in vivo* Anti-arthritic Activity

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Table S1. HPLC analysis data of the commercially obtained compounds **7-14**. The purity of the 8 bought compounds in the paper was determined by HPLC equipped with a Diamonsil-C18 column (250×4.6 mm, 5 μm particle size) and a UV/VIS detector setting of λ = 254 nm. All compounds were eluted with the two solvent systems (CH_3CN as organic phase in Method I and CH_3OH as organic phase in Method II) listed in the table at a flow rate of 1 mL/min.

Compound	Method I	Method II
7	CH_3CN:0.01M $(NH_4)_2CO_3$ = 50:50 (v/v) retention time: 8.42 min relative purity: 99.8%	CH_3OH:0.01M $(NH_4)_2CO_3$ =60:40 (v/v) retention time: 5.72 min relative purity: 99.9%
8	CH_3CN:0.01M $(NH_4)_2CO_3$ = 50:50 (v/v) retention time: 18.11 min relative purity: 97.7%	CH_3OH:0.01M $(NH_4)_2CO_3$ =98:2 (v/v) retention time: 3.01 min relative purity: 99.1%
9	CH_3CN:0.01M $(NH_4)_2CO_3$ = 50:50 (v/v) retention time: 22.86 min relative purity: 97.1%	CH_3OH:0.01M $(NH_4)_2CO_3$ =98:2 (v/v) retention time: 3.72 min relative purity: 97.3%
10	CH_3CN:0.01M $(NH_4)_2CO_3$ = 96:4 (v/v) retention time: 5.15 min relative purity: 99.9%	CH_3OH:0.01M $(NH_4)_2CO_3$ =98:2 (v/v) retention time: 2.34min relative purity: 99%
11	CH_3CN:0.01M $(NH_4)_2CO_3$ = 50:50 (v/v) retention time: 21.47 min relative purity: 94.0%	CH_3OH:0.01M $(NH_4)_2CO_3$ =98:2 (v/v) retention time: 3.51 min relative purity: 98.1%
12	CH_3CN:0.01M $(NH_4)_2CO_3$ = 50:50 (v/v) retention time: 21.80 min relative purity: 97.1%	CH_3OH:0.01M $(NH_4)_2CO_3$ =98:2 (v/v) retention time: 3.55 min relative purity: 96.5%
13	CH_3CN:0.01M $(NH_4)_2CO_3$ = 50:50 (v/v) retention time: 18.24 min relative purity: 95.4%	CH_3OH:0.01M $(NH_4)_2CO_3$ =98:2 (v/v) retention time: 3.42 min relative purity: 95.0%
14	CH_3CN:0.01M $(NH_4)_2CO_3$ = 50:50 (v/v)	CH_3OH:0.01M $(NH_4)_2CO_3$ =60:40 (v/v)

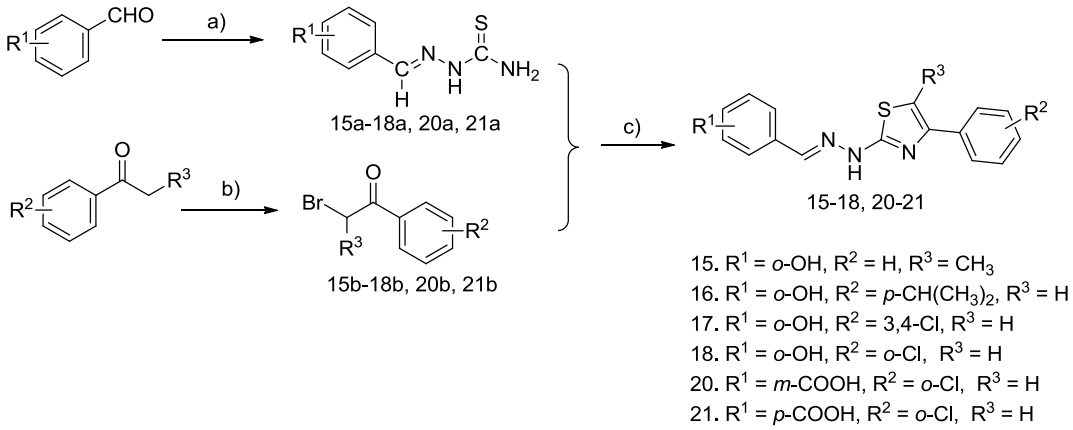
	retention time: 11.48 min relative purity: 95.3%	retention time: 7.08 min relative purity: 96.2%

Table S2. Information about the 35 crystal structures of *h*DHODH deposited in the Protein Data Bank.

No.	PDB ID	No. of buried water molecules in the ubiquinone-binding site	Resolution (Å)
1	1D3G	1	1.6
2	1D3H	2	1.8
3	2B0M	1	2.0
4	2BXV	1	2.15
5	2FPT	1	2.4
6	2FPV	1	1.8
7	2FPY	1	2.0
8	2FQI	1	1.95
9	2PRH	1	2.4
10	2PRL	1	2.1
11	2PRM	1	3.0
12	2WV8	1	1.9
13	3F1Q	2	2
14	3FJ6	3	1.8
15	3FJL	2	1.9
16	3G0U	2	2.0
17	3G0X	1	1.8
18	3KVJ	0	1.94
19	3KVK	2	2.05
20	3KVL	1	1.85
21	3KVM	2	2.0
22	3U2O	1	2.18
23	3ZWS	0	1.6
24	3ZWT	0	1.55
25	3W7R	0	1.68
26	4JTU	1	1.90
27	4OQV	2	1.23
28	4IGH	1	1.3
29	4JGD	2	2.05
30	4JS3	0	2.0
31	4JTS	0	2.21
32	4JTT	0	2.10
33	4LS0	3	2.07
34	4LS1	3	2.20
35	4LS2	2	2.27
total		43	

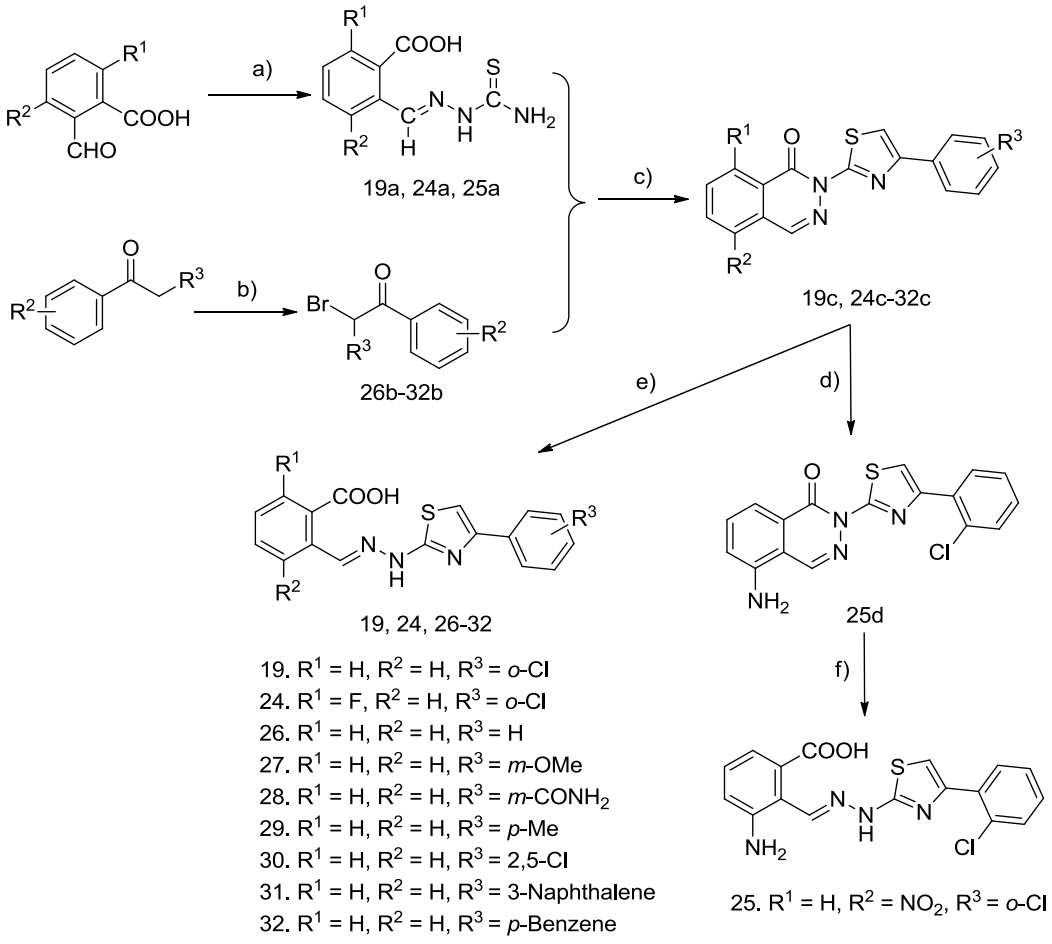
Schemes

Scheme S1. Synthesis of the benzylidenehydrazinyl-substituted thiazole derivatives **15-18**, **20-21** [1-3]



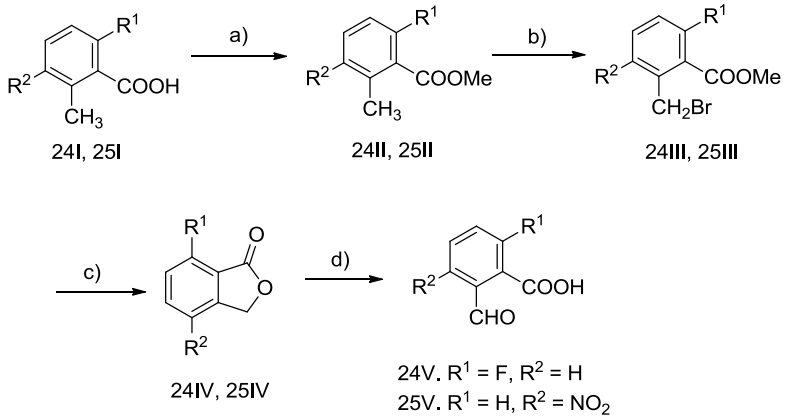
Gerenal conditions: a) Thiosemicarbazide, ethanol/acetic acid, 40 ^{o}C 4-5 h; b) Br_2, $AlCl_3$, anhydrous ether, overnight at 0 ^{o}C; c) Ethanol, reflux for 3 h.

Scheme S2. Synthesis of the benzylidenehydrazinyl-substituted thiazole derivatives **19**, **24-32** [1,2,4,5]



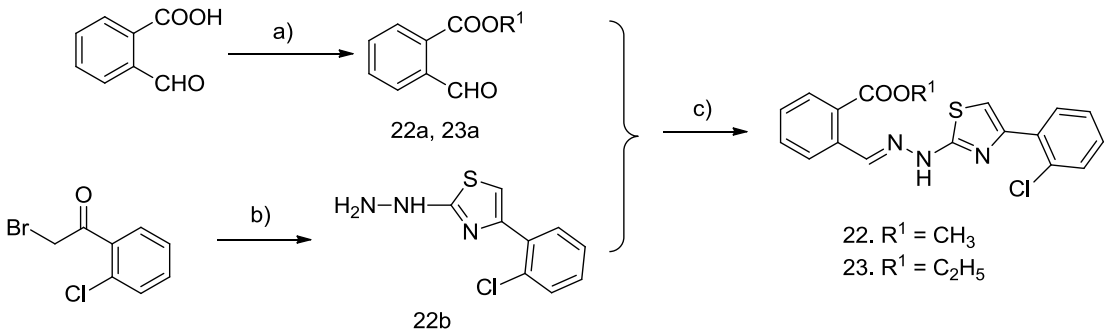
Gerenal conditions: a) Thiosemicarbazide, ethanol/acetic acid, 40 ^{o}C for 4-5 h; b) Br_2, $AlCl_3$, anhydrous ether, overnight at 0 ^{o}C; c) Ethanol, reflux for 3 h; d) $SnCl_2$, HCl, 80 ^{o}C for 5 h; e) NaOH, dry THF, overnight at rt; f) NaOH, dry THF, overnight at rt;

Scheme S3. Synthesis of the o-carboxybenzaldehyde intermediates **24V-25V** [6-12]



Gerenal conditions: a) 98% H_2SO_4, methanol, reflux overnight; b) NBS, AIBN, CCl_4, reflux 4-6 h; c) $CaCO_3$, H_2O/1,4-dioxane (1:1, v/v), 90 ^oC 5 h; d) 1. NBS, AIBN, $ClCH_2CH_2Cl$, reflux 4 h; 2. H_2O, reflux 5-6 h.

Scheme S4. Synthesis of the benzylidenehydrazinyl-substituted thiazole derivatives **22-23** [2,3,13-15]



Gerenal conditions: a) Iodomethane/Iodoethane, potassium carbonate, dry DMF, reflux for 3 h; b) Thiosemicarbazide, 1,4-dioxane, overnight at rt; c) ethanol/acetic acid, rt for 5 h.

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