Supplemental Information

Synthesis and inhibition potency of novel ureido
benzenesulfonamides incorporating GABA as tumor-
associated carbonic anhydrase IX and XII inhibitors.

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Experimental and spectroscopic details for the characterization of compounds **12-20** described in the paper are here provided.

General Procedure for the preparation of compounds 6-20.

The 4-amino-N-(4-sulfamoyl-benzyl)-butyramide hydrochloride **4** or the 4-amino-N-[2-(4-sulfamoyl-phenyl)-ethyl]-butyramide hydrochloride **5** (1.0 eq) were suspended in dry acetonitrile (10 ml) and then treated with stoichiometric amount of arylisocyanate **A1-8** (Table 1 reported in the paper) under anhydrous conditions followed by dropwise addition of diisopropylethylamine (1.05 eq). The mixture was stirred at room temperature for 3 hours or overnight under nitrogen flow until completion (TLC monitoring). At the end of the reaction time the mixture was quenched with slush (2-3 g) and 3.0 M HCl (5 ml). The formed precipitate was then filtered off and dried in vacuo or extracted with ethyl acetate, the combined organic layers dried over anhydrous sodium sulphate, filtered and the solvent removed under reduced pressure. Further purification such as crystallization from water or trituration with diethylether was required to obtain pure products. All compounds described here were characterized by spectroscopic methods.

Synthesis of 4-(3-Phenethyl-ureido)-N-[2-(4-sulfamoyl-phenyl)-ethyl]-butyramide **12**:

4-(3-Phenethyl-ureido)-N-[2-(4-sulfamoyl-phenyl)-ethyl]-butyramide **12**: pale yellow solid; 56.5% yield; δ_{H} (400 MHz, DMSO- d_6): 7.94 (t, 1H, NH, J 5.6, exchange with D₂O), 7.77 (d, 2H, CH, J 8), 7.42 (d, 2H, CH, J 8.4), 7.34 (s, 2H, NH₂, exchange with D₂O), 7.32 (d, 2H, CH, J 8),

7.24 (m, 3H, CH), 5.92 (bt, 1H, NH, exchange with D₂O), 5.84 (bt, 1H, NH, exchange with D₂O), 3.32 (td, 2H, CH₂, J 6.6), 3.24 (brm, 2H, CH₂), 2.97 (brm, 2H, CH₂), 2.81 (t, 2H, CH₂, J 7), 2.71 (t, 2H, CH₂, J 7.2), 2.06 (t, 2H, CH₂, J 7.4), 1.58 (tt, 2H, CH₂, J 7.2); δ_c (100 MHz, DMSO-*d*₆): 172.82 (C=O), 158.97 (C=O), 144.70 (qC aromatic), 142.93 (qC aromatic), 140.69 (qC aromatic), 130.02 (2 CH aromatic), 129.57 (2 CH aromatic), 129.22 (2 CH aromatic), 126.89 (1 CH aromatic), 126.58 (2 CH aromatic), 41.89 (CH₂), 40.61 (CH₂), 39.81 (CH₂), 37.11 (CH₂), 35.79 (CH₂), 33.84 (CH₂), 27.28 (CH₂); *m/z* (ESI positive), 433.2 [M-H]⁺.

Synthesis of 4-(3-Cyclopentyl-ureido)-N-(4-sulfamoyl-benzyl)-butyramide 13:

4-(3-Cyclopentyl-ureido)-N-(4-sulfamoyl-benzyl)-butyramide 13: white solid; 65.4% yield; δ_H (400 MHz, DMSO-*d*₆): 8.47 (t, 1H, NH, J 6, exchange with D₂O), 7.80 (d, 2H, CH, J 8.4), 7.45 (d, 2H, CH, J 8.4), 7.33 (s, 2H, NH₂, exchange with D₂O), 5.81 (d, 1H, NH, J 7.6, exchange with D₂O), 5.72 (t, 1H, NH, J 5.6, exchange with D₂O), 4.35 (d, 2H, CH₂, J 6), 3.86 (m, 1H, CH cyclopentyl), 3.01 (td, 2H, CH₂, J 6.4), 2.18 (t, 2H, CH₂, J 7.6), 1.79 (m, 2H, CH₂), 1.63 (m, 4H, 2 CH₂), 1.52 (m, 2H, CH₂), 1.30 (m, 2H, CH₂); δ_c (100 MHz, DMSO-*d*₆): 173.05 (C=O), 158.73 (C=O), 144.78 (qC aromatic), 143.47 (qC aromatic), 128.38 (2 CH aromatic), 126.61 (2 CH aromatic), 51.91 (CH cyclopentyl), 42.63 (CH₂), 39.86 (CH₂), 33.88 (2 CH₂), 33.77 (CH₂), 27.37 (CH₂), 24.09 (2 CH₂); *m/z* (ESI positive), 383.2 [M-H]⁺.

Synthesis of 4-(3-Cyclopentyl-ureido)-N-[2-(4-sulfamoyl-phenyl)-ethyl]-butyramide 14:

4-(3-Cyclopentyl-ureido)-N-[2-(4-sulfamoyl-phenyl)-ethyl]-butyramide 14: pale yellow solid; 73.2% yield; δ_{H} (400 MHz, DMSO- d_6): 7.96 (t, 1H, NH, J 5.6, exchange with D₂O), 7.77 (d, 2H, CH, J 8.4), 7.42 (d, 2H, CH, J 8.4), 7.32 (s, 2H, NH₂, exchange with D₂O), 5.81 (appd, 1H, NH, exchange with D₂O), 5.70 (appd, 1H, NH, exchange with D₂O), 3.86 (m, 1H, CH cyclopentyl), 3.32 (td, 2H, CH₂, J 6.7), 2.96 (td, 2H, CH₂, J 6.8), 2.82 (t, 2H, CH₂, J 7.2), 2.06 (t, 2H, CH₂, J 7.6), 1.79 (m, 2H, CH₂), 1.58 (m, 4H, 2 CH₂), 1.51 (m, 2H, CH₂), 1.29 (m, 2H, CH₂); δ_{C} (100 MHz, DMSO- d_6): 172.78 (C=O), 158.66 (C=O), 144.67 (qC aromatic), 142.91 (qC aromatic), 130.0 (2 CH aromatic), 126.56 (2 CH aromatic), 51.86 (CH cyclopentyl), 42.60 (CH₂), 39.74 (CH₂), 35.77 (CH₂), 33.84 (3 CH₂), 27.30 (CH₂), 24.04 (2 CH₂); m/z (ESI positive), 397.21 [M-H]⁺.

Synthesis of 4-[3-(4-Ethyl-phenyl)-ureido]-N-(4-sulfamoyl-benzyl)-butyramide 15:

4-[3-(4-Ethyl-phenyl)-ureido]-N-(4-sulfamoyl-benzyl)-butyramide 15: pale pink solid; 67.2% yield; δ_{H} (2 atropisomers were detected in 1/0.31 ratio. Only the major one is reported herein) (400 MHz, DMSO- d_6): 8.48 (t, 1H, NH, J 5.8, exchange with D₂O), 8.34 (s, 1H, NH, exchange with D₂O), 7.80 (d, 2H, CH, J 8.4), 7.45 (d, 2H, CH, J 8), 7.34 (s, 2H, NH₂, exchange with D₂O), 7.32 (d, 2H, CH, J 8.4), 7.08 (d, 2H, CH, J 8.4), 6.13 (t, 1H, NH, J 5.6, exchange with D₂O), 4.36 (d, 2H, CH₂, J 6.0), 3.12 (td, 2H, CH₂, J 6.5), 2.55 (q, 2H, CH₂ overlapping DMSO- d_6), 2.23 (t, 2H, CH₂, J 7.4), 1.72 (tt, 2H, CH₂, J 7.2), 1.17 (t, 3H, CH₃, J 7.6); δ_{C} (100 MHz, DMSO- d_6): 172.92 (C=O), 156.26 (C=O), 144.73 (qC aromatic), 143.47 (qC aromatic), 139.12 (qC aromatic), 137.15 (2 CH aromatic), 128.71 (2 CH aromatic), 128.36 (2 CH aromatic), 126.59 (1

CH aromatic), 118.73 (2 CH aromatic), 42.62 (CH₂), 39.66 (CH₂), 33.68 (CH₂), 28.38 (CH₂), 27.07 (CH₂), 16.72 (CH₃); *m/z* (ESI positive), 419.1 [M-H]⁺.

Synthesis of 4-[3-(4-Ethyl-phenyl)-ureido]-N-[2-(4-sulfamoyl-phenyl)-ethyl]-butyramide 16:

4-[3-(4-Ethyl-phenyl)-ureido]-N-[2-(4-sulfamoyl-phenyl)-ethyl]-butyramide **16**: pale brown solid; 82% yield; δ_{H} (400 MHz, DMSO-*d*₆): 8.36 (s, 1H, NH, exchange with D₂O), 7.98 (t, 1H, NH, J 5.4 exchange with D₂O), 7.77 (d, 2H, CH, J 8.4), 7.42 (d, 4H, CH, J 8.4), 7.32 (s, 2H, NH₂, exchange with D₂O), 7.31 (d, 2H, CH, J 8.4), 7.08 (d, 1H, CH, J 8.8), 6.12 (appt, 1H, NH, exchange with D₂O), 3.33 (td, 2H, CH₂, J 6.5), 3.07 (brm, 2H, CH₂), 2.82 (t, 2H, CH₂, J 7.2), 2.55 (q, 2H, CH₂ overlapping DMSO-*d*₆), 2.11 (t, 2H, CH₂, J 7.6), 1.66 (tt, 2H, CH₂, J 7.2), 1.17 (t, 3H, CH₃, J 7.6); δ_{C} (100 MHz, DMSO-*d*₆): 172.78 (C=O), 156.29 (C=O), 144.71 (qC aromatic), 142.94 (qC aromatic), 139.13 (qC aromatic), 137.17 (qC aromatic), 130.03 (2 CH aromatic), 128.74 (2 CH aromatic), 126.60 (2 CH aromatic), 118.75 (2 CH aromatic), 40.66 (CH₂), 39.68 (CH₂), 35.80 (CH₂), 33.82 (CH₂), 28.40 (CH₂), 27.08 (CH₂), 16.75 (CH₃); *m/z* (ESI positive), 433.2 [M-H]⁺.

Synthesis of 4-[3-(4-Methyl-benzyl)-ureido]-N-(4-sulfamoyl-benzyl)-butyramide 17:

4-[3-(4-Methyl-benzyl)-ureido]-N-(4-sulfamoyl-benzyl)-butyramide **17**: white solid; 89.6% yield; δ_{H} (2 atropisomers were detected in 1/0.36 ratio. Only the major one is reported herein) (400 MHz, DMSO-*d*₆): 8.47 (t, 1H, NH, J 6, exchange with D₂O), 7.80 (d, 2H, CH, J 8.0), 7.45 (d, 2H,

CH, J 8.4), 7.34 (s, 2H, NH₂, exchange with D₂O), 7.16 (s, 2H, CH), 7.15 (s, 2H, CH), 6.28 (t, 1H, NH, J 5.8, exchange with D₂O), 5.97 (t, 1H, NH, J 5.8, exchange with D₂O), 4.35 (d, 2H, CH₂, J 6), 4.18 (d, 2H, CH₂, J 6), 3.05 (td, 2H, CH₂, J 6.5), 2.30 (s, 3H, CH₃), 2.19 (t, 2H, CH₂, J 7.4), 1.67 (tt, 2H, CH₂, J 7.2); δ_C (100 MHz, DMSO-*d*₆): 173.0 (C=O), 159.01 (C=O), 144.77 (qC aromatic), 143.46 (qC aromatic), 138.79 (qC aromatic), 136.42 (qC aromatic), 129.66 (2 CH aromatic), 128.35 (2 CH aromatic), 127.93 (2 CH aromatic), 126.59 (2 CH aromatic), 43.58 (CH₂), 42.60 (CH₂), 39.95 (CH₂), 33.74 (CH₂), 27.32 (CH₂), 21.57 (CH₃); *m/z* (ESI positive), 419.25 [M-H]⁺.

Synthesis of 4-[3-(4-Methyl-benzyl)-ureido]-N-[2-(4-sulfamoyl-phenyl)-ethyl]-butyramide 18:

4-[3-(4-Methyl-benzyl)-ureido]-N-[2-(4-sulfamoyl-phenyl)-ethyl]-butyramide 18: white solid; 94.6% yield; δ_H (2 atropisomers were detected in 1/0.27 ratio. Only the major one is reported herein) (400 MHz, DMSO-*d*₆): 7.96 (brt, 1H, NH, exchange with D₂O), 7.77 (d, 2H, CH, J 8), 7.42 (d, 2H, CH, J 8.4), 7.32 (s, 2H, NH₂, exchange with D₂O), 7.15 (s, 4H, CH), 6.27 (brt, 1H, NH, exchange with D₂O), 5.94 (brt, 1H, NH, exchange with D₂O), 4.18 (d, 2H, CH₂, J 5.6), 3.32 (td, 2H, CH₂, J 6.6), 3.01 (brm, 2H, CH₂), 2.81 (t, 2H, CH₂, J 7.2), 2.30 (s, 3H, CH₃), 2.07 (t, 2H, CH₂, J 7.6), 1.62 (tt, 2H, CH₂, J 7.2); δ_C (100 MHz, DMSO-*d*₆): 172.82 (C=O), 159.03 (C=O), 144.71 (qC aromatic), 142.94 (qC aromatic), 138.78 (qC aromatic), 136.44 (qC aromatic), 130.03 (2 CH aromatic), 129.67 (2 CH aromatic), 127.94 (2 CH aromatic), 126.59 (2 CH aromatic), 43.60 (CH₂), 40.76 (CH₂), 39.92 (CH₂), 35.80 (CH₂), 33.83 (CH₂), 27.30 (CH₂), 21.58 (CH₃); *m/z* (ESI positive), 433.25 [M-H]⁺.

Synthesis of 4-[3-(4-Iodo-phenyl)-ureido]-N-(4-sulfamoyl-benzyl)-butyramide 19:

4-[3-(4-Iodo-phenyl)-ureido]-N-(4-sulfamoyl-benzyl)-butyramide **19**: white solid; 36.3% yield; δ_{H} (2 atropisomers were detected in 1/0.23 ratio. Only the major one is reported herein) (400 MHz, DMSO- d_6): 8.60 (s, 1H, NH, exchange with D₂O), 8.48 (t, 1H, NH, J 5.8, exchange with D₂O), 7.80 (d, 2H, CH, J 8), 7.56 (d, 2H, CH, J 8.8), 7.45 (d, 2H, CH, J 8.4), 7.34 (s, 2H, NH₂, exchange with D₂O), 7.28 (d, 2H, CH, J 8.8), 6.24 (t, 1H, NH, J 5.8, exchange with D₂O), 4.36 (d, 2H, CH₂, J 6.0), 3.12 (td, 2H, CH₂, J 6.4), 2.23 (t, 2H, CH₂, J 7.4), 1.72 (tt, 2H, CH₂, J 7.2); δ_{C} (100 MHz, DMSO- d_6): 172.89 (C=O), 155.93 (C=O), 144.72 (qC aromatic), 143.46 (qC aromatic), 141.40 (qC aromatic), 138.05 (2 CH aromatic), 128.36 (2 CH aromatic), 126.59 (2 CH aromatic), 120.86 (2 CH aromatic), 84.36 (qC aromatic), 42.61 (CH₂), 39.72 (CH₂), 33.65 (CH₂), 26.94 (CH₂), m/z (ESI positive), 517.0 [M-H]⁺.

Synthesis of 4-[3-(4-Iodo-phenyl)-ureido]-N-[2-(4-sulfamoyl-phenyl)-ethyl]-butyramide **20**:

4-[3-(4-Iodo-phenyl)-ureido]-N-[2-(4-sulfamoyl-phenyl)-ethyl]-butyramide **20**: pale yellow solid, 30.41 % yield; δ_{H} (2 atropisomers were detected in 1/0.30 ratio. Only the major one is reported herein) (400 MHz, DMSO- d_6): 8.59 (s, 1H, NH, exchange with D₂O), 7.97 (t, 1H, NH, J 5.6, exchange with D₂O), 7.72 (d, 2H, CH, J 8.4), 7.56 (d, 2H, CH, J 8.8), 7.42 (d, 2H, CH, J 8.4), 7.32 (s, 2H, NH₂, exchange with D₂O), 7.28 (d, 2H, CH, J 8.8), 6.21 (t, 1H, NH, J 5.6, exchange with D₂O), 3.32 (td, 2H, CH₂, J 6.6), 3.08 (td, 2H, CH₂, J 6.4), 2.81 (t, 2H, CH₂, J 7.2), 2.11 (t, 2H, CH₂, J 7.6), 1.68 (tt, 2H, CH₂, J 7.4); δ_{C} (100 MHz, DMSO- d_6): 172.72 (C=O), 155.92

(C=O), 144.68 (qC aromatic), 142.93 (qC aromatic), 141.41 (qC aromatic), 138.05 (2 CH aromatic), 130.0 (2 CH aromatic), 126.58 (2 CH aromatic), 120.86 (2 CH aromatic), 84.35 (qC aromatic), 40.62 (CH₂), 39.67 (CH₂), 35.78 (CH₂), 33.77 (CH₂), 26.93 (CH₂); *m/z* (ESI positive), 531.08 [M-H]⁺.