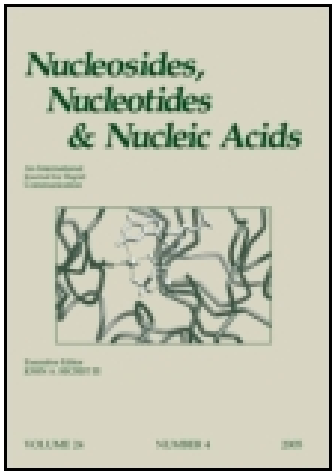

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Human Uric Acid Transporter 1 (hURAT1): An Inhibitor Structure-Activity Relationship (SAR) Study

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HUMAN URIC ACID TRANSPORTER 1 (hURAT1): AN INHIBITOR STRUCTURE–ACTIVITY RELATIONSHIP (SAR) STUDY

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□ *The current study describes the chemical synthesis of a series of (2-ethylbenzofuran-3-yl)(substituted-phenyl)methanone compounds and their subsequent in vitro testing via oocytes expressing hURAT1. The experimental data support the notion that a potent hURAT1 inhibitor requires an anion (i.e., a formal negative charge) to interact with the positively charged hURAT1 binding pocket. An anion appears to be a primary requirement in order to be a hURAT1 substrate (i.e., urate) or inhibitor. We discuss the inhibitor structure–activity relationship and how electronically donating or withdrawing groups attached to the B-ring can decrease or increase inhibitory potency, respectively.*

Keywords Human uric acid transporter 1 (hURAT1; SLC22A12); inhibitor study; structure–activity relationship (SAR)

INTRODUCTION

At the 14th International Symposium of the Purine and Pyrimidine Society (PP11) held in Japan, we presented on a series of compounds and their ability to inhibit urate transport mediated via human uric acid transporter 1 (hURAT1; SLC22A12). Humans metabolically degrade purine nucleotides, nucleosides, and bases to produce urate (uric acid **1**, Figure 1)

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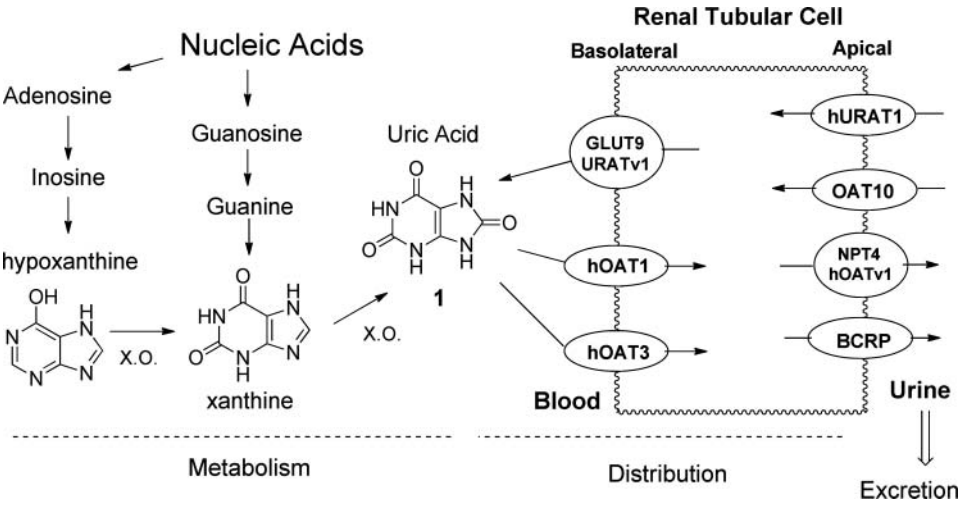

FIGURE 1 Uric acid formation and the transporter-mediated distribution in humans.

and eliminate in the urine. Various renal transporter proteins are critical in regulating uric acid serum levels.[1–3] Important transporter proteins in the kidney are located at both the urine and blood interfaces. The urine interface (apical side) includes hURAT1, the human organic anion transporter 10 (hOAT10; SLC22A13), the natrium-dependent phosphate transporter 4 (NPT4, hOATv1; SLC17A3),[4–7] and the breast cancer resistance protein (BCRP/ABCG2).[8,9] The basolateral transporters (blood interface) include the facilitative glucose transporter 9 (GLUT9, URATv1; SLC2A9) and human organic anion transporter proteins 1 (hOAT1; SLC22A6) and 3 (hOAT3; SLC22A8).[7,10–13]

Elevated uric acid (hyperuricemia) may lead to gout and produce episodes of acute inflammatory arthritis.[4] Diet, lifestyle, and age (renal function declines as people age) are known contributors to the production of elevated serum urate.[14] Elevated uric acid levels throughout the body are believed to also play a pivotal role in other diseases such as hypertension, diabetes, chronic renal disease, and cardiovascular disease.[14–16] Therefore, we believe that the development of uric acid transporter inhibitors will lead to novel and therapeutically important drugs for combating various diseases. Hence, this requires that we obtain a reliable inhibitor structure–activity relationship. We recently published a chemical investigation where we probed a variety of different (3,5-dibromo-4-hydroxyphenyl) (2-ethylbenzofuran-(#)-yl)methanone regioisomers (# = 3, 4, or 7 position) and their ability to inhibit ^{14}C-urate uptake in oocytes expressing hURAT1.[17] The current work describes the preparation and in vitro testing of additional compounds and further illustrates important chemical and structural requirements for potent hURAT1 inhibitor activity.

MATERIALS AND METHODS

Benzoyl chloride, 4-fluorobenzoyl chloride, 4-cyanobenzoyl chloride, 3-chloro-4-methoxybenzoic acid, 3-bromo-4-methoxy-benzoic acid, and 1,2-dimethyl-1H-indole were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). The 3-fluoro-*p*-anisic acid was purchased from TCI America (Chicago, IL, USA). All other reagents were procured as previously described.[17]

Chemical Synthesis

Compounds *1-(benzofuran-2-yl)ethanone* **3**, *2-ethylbenzofuran* **4**, *(2-ethylbenzofuran-3-yl)(4-methoxyphenyl)methanone* **6**, *(2-ethylbenzofuran-3-yl)(4-hydroxyphenyl)methanone* **7**, *(3,5-dibromo-4-hydroxyphenyl)(2-ethylbenzofuran-3-yl)methanone* **16**, *(3,5-dibromo-4-hydroxyphenyl)(2-ethyl-6-methoxybenzofuran-3-yl)methanone* **17**, and *(3,5-dibromo-4-hydroxyphenyl)(2-ethyl-6-hydroxy-benzofuran-3-yl)methanone* **18** were prepared as previously described.[17]

(2-ethylbenzofuran-3-yl)(phenyl)methanone **5**: Benzofuran (**4**; 0.200 g, 1.37 mmol) was diluted with CS_2 (4.0 mL), stirred, and cooled in an ice bath (30 minutes). Next, benzoyl chloride (0.21 mL, 1.78 mmol) was added drop-wise followed by tin (IV) chloride (0.21 mL, 1.78 mmol). The contents were stirred (3.0 hours) and warmed to room temperature (RT) (21 hours). The reaction mixture was diluted with water and extracted with EtOAc (4 × 20 mL). The combined organic layers were washed with HCl (0.5 N, 20 mL), water (20 mL), $NaHCO_3$ (20 mL), and brine (20 mL). The organic phase was dried (Na_2SO_4), filtered, concentrated under reduced pressure, and purified via SiO_2 chromatography (100% Hex to 5% EtOAc in Hex) to give **5** (220 mg, 0.879 mmol, 64% yield). ^{1}H-NMR (400 MHz) $CDCl_3$: 7.84–7.82 (d, 2H), 7.63–7.59 (t, 1H), 7.51–7.47 (m, 3H), 7.39–7.37 (d, 1H), 7.31–7.26 (t, 1H), 7.21–7.17 (t, 1H), 2.94–2.88 (q, 2H), 1.36–1.32 (t, 3H); ^{13}C-NMR (100 MHz) $CDCl_3$: 192.0, 166.5, 153.7, 139.4, 132.6, 129.1, 128.4, 127.0, 124.3, 123.5, 121.4, 116.1, 110.9, 21.8, 12.3. LC/MS-MS: 251.1 → 105.0 *m/z*; GS1 and GS2 at 25, DP = 56, CE = 29, CXP = 6, t_R = 4.99 minutes.

(2-ethylbenzofuran-3-yl)(4-fluorophenyl)methanone **8** was prepared with analogous methods used with compound **5**, except that it was stirred at RT for 3 days. Compound **8** (160 mg, 0.60 mmol, 43%) was an orange oil. ^{1}H-NMR (400 MHz) $CDCl_3$: 7.88–7.84 (t, 2H), 7.49–7.47 (d, 1H), 7.33–7.25 (m, 2H), 7.21–7.13 (m, 3H), 2.94–2.89 (q, 2H), 1.35–1.32 (t, 3H); ^{13}C-NMR (100 MHz) $CDCl_3$: 190.3, 166.8, 166.4, 164.3, 153.7, 135.6, 131.8 (d), 126.8, 124.4, 123.6, 121.2, 115.8 (t), 111.1, 21.8, 12.3. LC/MS-MS: 269.0 → 123.1 *m/z*; GS1 and GS2 at 25, DP = 61, CE = 29, CXP = 6, t_R = 4.85 minutes.

4-(2-ethylbenzofuran-3-carbonyl)benzonitrile **9** was prepared using analogous methods as compound **8**. Compound **9** (16.6 mg, 0.060 mmol, 5% yield) was a yellow oil. ^{1}H-NMR (400 MHz) $CDCl_3$: 7.92–7.89 (d, 2H), 7.81–7.79

(d, 2H), 7.52–7.50 (d, 1H), 7.33–7.29 (t, 1H), 7.25–7.20 (m, 2H), 2.96–2.90 (q, 2H), 1.37–1.33 (t, 3H); ^{13}C-NMR (100 MHz) $CDCl_3$: 190.2, 167.8, 153.7, 142.9, 132.4, 129.4, 126.1, 124.8, 123.9, 121.0, 118.0, 115.9, 115.4, 111.3, 22.0, 12.2. LC/MS-MS: 276.0 → 123.0 *m/z*; GS1 and GS2 at 25, DP = 71, CE = 27, CXP = 8, t_R = 4.57 minutes.

(2-ethylbenzofuran-3-yl)(3-fluoro-4-methoxyphenyl)methanone **10** was prepared with analogous methods used with compound **8** except stirred at RT for 16 hours. Compound **10** (218 mg, 0.731 mmol, 54% yield) was a yellow oil. ^{1}H-NMR (400 MHz) $CDCl_3$: 7.65–7.63 (d, 2H), 7.49–7.47 (d, 1H), 7.40–7.37 (d, 1H), 7.31–7.26 (t, 1H), 7.22–7.19 (t, 1H), 7.03–6.99 (t, 1H), 3.98 (s, 3H), 2.95–2.89 (q, 2H), 1.37–1.33 (t, 3H); ^{13}C-NMR (100 MHz) $CDCl_3$: 189.5, 165.8, 153.6, 153.2, 151.8, 150.7, 132.1, 126.7 (t), 124.4, 123.5, 121.1, 116.8 (d), 115.8, 112.3, 111.0, 56.3, 21.8, 12.3. LC/MS-MS: 298.7 → 153.1 *m/z*; GS1 and GS2 at 25, DP = 41, CE = 29, CXP = 10, t_R = 4.77 minutes.

(3-chloro-4-methoxyphenyl)(2-ethylbenzofuran-3-yl)methanone **11** was prepared with analogous methods used with compound **10**. Compound **11** (0.140 g, 0.445 mmol, 40% yield) was a light yellow oil. ^{1}H-NMR (400 MHz) $CDCl_3$: 7.92 (s, 1H), 7.78–7.75 (d, 1H), 7.50–7.48 (d, 1H), 7.41–7.39 (d, 1H), 7.31–7.27 (t, 1H), 7.23–7.19 (t, 1H), 7.00–6.98 (d, 1H), 3.99 (s, 3H), 2.94–2.88 (q, 2H), 1.37–1.33 (t, 3H); ^{13}C-NMR (100 MHz) $CDCl_3$: 189.3, 165.9, 158.6, 153.6, 132.5, 131.5, 129.8, 126.9, 124.4, 123.5, 122.8, 121.1, 115.8, 111.2, 111.0, 56.4, 21.8, 12.3. LC/MS-MS: 315.0 → 169.1 *m/z*; GS1 and GS2 at 25, DP = 56, CE = 31, CXP = 10, t_R = 4.99 minutes.

(3-bromo-4-methoxyphenyl)(2-ethylbenzofuran-3-yl)methanone **12** was prepared with analogous methods used with compound **10**. Compound **12** (0.195 g, 0.542 mmol, 40%) was a yellow oil. ^{1}H-NMR (400 MHz) $CDCl_3$: 8.10 (s, 1H), 7.83–7.80 (d, 1H), 7.50–7.48 (d, 1H), 7.42–7.40 (d, 1H), 7.29–7.27 (t, 1H), 7.23–7.21 (t, 1H), 6.97–6.94 (d, 1H), 3.99 (s, 3H), 2.94–2.88 (q, 2H), 1.37–1.33 (t, 3H); ^{13}C-NMR (100 MHz) $CDCl_3$: 189.2, 165.9, 159.5, 153.7, 134.7, 132.9, 130.6, 126.9, 124.4, 123.6, 121.2, 115.8, 111.8, 111.1, 111.0, 56.5, 21.9, 12.3. LC/MS-MS: 361.0 → 214.8 *m/z*; GS1 and GS2 at 25, DP = 71, CE = 31, CXP = 14, t_R = 5.20 minutes.

(2-ethylbenzofuran-3-yl)(3-fluoro-4-hydroxyphenyl)methanone **13**: Compound **10** (90.0 mg, 0.302 mmol) was diluted with DMF (5.0 mL). NaSEt (0.127 g, 1.51 mmol) was added and warmed (105–110°C; 16 hours). The mixture was then quenched with 2 volumes of NH_4Cl aq and extracted with EtOAc (4 × 20 mL). The organic phase was washed with brine, water, and then dried (Na_2SO_4), filtered, and concentrated under reduced pressure, and purified via SiO_2 chromatography (4:1; Hex:EtOAc) to give **13** as a brown oil (46 mg, 0.162 mmol, 54% yield). ^{1}H-NMR (400 MHz) $CDCl_3$: 7.68–7.64 (d, 1H0, 7.60–7.57 (d, 1H), 7.50–7.48 (d, 1H), 7.40–7.38 (d, 1H), 7.31–7.26 (t, 1H), 7.23–7.19 (t, 1H), 7.10–7.06 (t, 1H), 6.45 (bs, 1H), 2.95–2.90 (q, 2H), 1.37–1.33 (t, 3H); ^{13}C-NMR (100 MHz) $CDCl_3$: 189.9, 166.1, 153.6, 152.0, 149.6, 148.4 (d), 132.1, 127.3, 126.8, 124.4, 123.6, 121.1, 117.1 (t),

115.8, 111.0, 21.8, 12.3. LC/MS-MS: 285.0 → 139.0 *m/z*; GS1 and GS2 at 25, DP = 61, CE = 29, CXP = 8, t_R = 4.49 minutes.

(3-chloro-4-hydroxyphenyl)(2-ethylbenzofuran-3-yl)methanone **14** was prepared with analogous methods used with compound **13**. Compound **14** (21.1 mg, 0.070 mmol, 44% yield) was a brown oil. ^{1}H-NMR (400 MHz) $CDCl_3$: 7.90 (s, 1H), 7.71–7.69 (d, 1H), 7.50–7.48 (d, 1H), 7.40–7.38 (d, 1H), 7.31–7.26 (t, 1H), 7.21–7.19 (t, 1H), 7.11–7.09 (d, 1H), 6.11 (bs, 1H), 2.95–2.89 (q, 2H), 1.37–1.33 (t, 3H); ^{13}C-NMR (100 MHz) $CDCl_3$: 189.3, 166.0, 155.3, 153.7, 132.9, 130.7, 130.4, 126.8, 124.4, 123.6, 121.1, 120.3, 116.0, 115.8, 111.1, 21.8, 12.3. LC/MS-MS: 300.9 → 155.0 *m/z*; GS1 and GS2 at 25, DP = 66, CE = 29, CXP = 8, t_R = 4.59 minutes.

(3-bromo-4-hydroxyphenyl)(2-ethylbenzofuran-3-yl)methanone **15** was prepared with analogous methods used with compound **13**. Compound **15** (23.2 mg, 0.079 mmol, 57% yield) was a light yellow oil. ^{1}H-NMR (400 MHz) $CDCl_3$: 8.06–8.05 (d, 1H), 7.76–7.74 (dd, 1H), 7.50–7.48 (d, 1H), 7.41–7.39 (d, 1H), 7.31–7.29 (t, 1H), 7.23–7.19 (t, 1H), 7.10–7.08 (d, 1H), 6.21 (bs, 1H), 2.94–2.89 (q, 2H), 1.37–1.33 (t, 3H); ^{13}C-NMR (100 MHz) $CDCl_3$: 189.2, 166.0, 156.3, 153.7, 133.8, 133.2, 131.1, 126.8, 124.5, 123.6, 121.1, 115.9, 115.8, 111.1, 110.5, 21.8, 12.3. LC/MS-MS: 344.9 → 198.8 *m/z*; GS1 and GS2 at 25, DP = 61, CE = 33, CXP = 12, t_R = 4.59 minutes.

(2-ethyl-5-fluorobenzofuran-3-yl)(4-methoxyphenyl)methanone **19** was prepared using analogous methods as compound **5** to produce a white solid (1.60 g, 5.36 mmol, 44% yield). *1-(5-fluorobenzofuran-2-yl)ethanone* and *2-ethyl-5-fluorobenzofuran* were prepared using analogous methods as previously described.[15] Compound **19**: ^{1}H-NMR (400 MHz) $CDCl_3$: 7.84–7.81 (d, 2H), 7.41–7.38 (d, 1H), 7.09–7.06 (d, 1H), 7.01–6.96 (m, 3H), 3.90 (s, 3H), 2.93–2.87 (q, 2H), 1.35–1.31 (t, 3H); ^{13}C-NMR (100 MHz) $CDCl_3$: 190.0, 167.1, 163.6, 160.7, 158.3, 149.8, 131.6, 128.2 (d), 116.5, 113.8, 112.0, 111.7 (t), 107.0 (d), 55.5, 21.9, 12.2. LC/MS-MS: 299.0 → 135.0 *m/z*; GS1 and GS2 at 30, DP = 11, CE = 31, CXP = 8, t_R = 4.69 minutes.

(2-ethyl-5-fluorobenzofuran-3-yl)(4-hydroxyphenyl)methanone **20** was prepared with analogous methods used with compound **13**. Compound **20** (550 mg, 1.93 mmol, 58% yield) was a white solid. ^{1}H-NMR (400 MHz) $CDCl_3$: 7.79–7.77 (d, 2H), 7.42–7.38 (dd, 1H), 7.08–7.06 (d, 1H), 7.02–6.95 (t, 1H), 6.95–6.92 (d, 2H), 6.57 (bs, 1H), 2.93–2.88 (q, 2H), 1.35–1.31 (t, 3H); ^{13}C-NMR (100 MHz) $CDCl_3$: 190.7, 167.4, 160.6 (d), 158.3, 149.8, 132.0, 131.4, 128.1 (d), 115.5, 112.1, 111.9, 111.6 (t), 107.1 (d), 21.9, 12.2. LC/MS-MS: 285.0 → 121.2 *m/z*; GS1 and GS2 at 30, DP = 4, CE = 29, CXP = 6, t_R = 4.31 minutes.

(3,5-dibromo-4-hydroxyphenyl)(2-ethyl-5-fluorobenzofuran-3-yl)methanone **21**: In a RBF/SB, NBS (0.250 g, 1.41 mmol) diluted with DCM (12.0 mL) was mixed with DMF (0.4 mL) and the mixture was cooled in an ice bath (10 minutes). Compound **20** (0.200 g, 0.704 mmol) in DCM (1.0 mL) was added and warmed to RT (17 hours). The reaction mixture was

quenched with H_2O and diluted with DCM (30 mL). The organic phase was washed four times with H_2O, NaCl aq, and then dried (Na_2SO_4), filtered, and concentrated under reduced pressure, and purified twice via SiO_2 chromatography (Hex:EtOAc; 4:1) to give **21** (176 mg, 0.398 mmol, 57% yield) as a yellow solid. ^{1}H-NMR (400 MHz) $CDCl_3$: 7.97 (s, 2H), 7.43–7.40 (dd, 1H), 7.19–7.11 (d, 1H), 7.04–7.01 (t, 1H), 6.53 (bs, 1H), 2.88–2.84 (q, 2H), 1.36–1.33 (t, 3H); ^{13}C-NMR (100 MHz) $CDCl_3$: 187.4, 167.9, 160.9, 158.5, 153.3, 149.9, 133.3, 127.5 (d), 115.6, 112.5, 112.3, 111.8 (t), 110.1, 107.0 (d), 22.1, 12.1. LC/MS-MS: 442.9 → 278.8 *m/z*; GS1 and GS2 at 30, DP = 21, CE = 35, CXP = 18, t_R = 4.59 minutes.

(1,2-dimethyl-1H-indol-3-yl)(4-methoxyphenyl)methanone **22** was prepared with analogous methods used with compound **5** except stirred at room temperature for 16 hours. Compound **22** (215 mg, 0.770 mmol, 28% yield) was a light brown solid. ^{1}H-NMR (400 MHz) $CDCl_3$: 7.80–7.78 (d, 2H), 7.38–7.36 (d, 1H), 7.33–7.31 (d, 1H), 7.23–7.19 (t, 1H), 7.10–7.06 (t, 1H), 6.95–6.93 (d, 2H), 3.89 (s, 3H), 3.74 (s, 3H), 2.60 (s, 3H); ^{13}C-NMR (100 MHz) $CDCl_3$: 191.7, 162.5, 143.8, 136.5, 133.8, 131.6, 127.1, 121.8, 121.1, 120.9, 113.8, 113.4, 109.1, 55.4, 29.6, 12.4. LC/MS-MS: 280.0 → 135.0 *m/z*; GS1 and GS2 at 30, DP = 21, CE = 29, CXP = 8, t_R = 4.25 minutes.

(1,2-dimethyl-1H-indol-3-yl)(4-hydroxyphenyl)methanone **23** was prepared with analogous methods used with compound **13**. Compound **23** (40 mg, 1.50 mmol, 84% yield) was a white solid. ^{1}H-NMR (400 MHz) $CDCl_3$: 7.75–7.73 (d, 2H), 7.37–7.32 (t, 2H), 7.23–7.19 (t, 1H), 7.10–7.06 (t, 1H), 6.89–6.87 (d, 2H), 5.57 (bs, 1H), 3.75 (s, 3H), 2.61 (s, 3H); ^{13}C-NMR (100 MHz) $CDCl_3$: 191.8, 158.9, 144.0, 136.5, 133.9, 131.9, 127.1, 121.9, 121.2, 120.9, 115.0, 113.7, 109.1, 29.7, 12.4. LC/MS-MS: 266.0 → 121.1 *m/z*; GS1 and GS2 at 30, DP = 21, CE = 31, CXP = 6, t_R = 3.96 minutes.

(3,5-dibromo-4-hydroxyphenyl)(1,2-dimethyl-1H-indol-3-yl)methanone **24** was prepared with analogous methods used with compound **21**. Compound **24** (17.0 mg, 0.040 mmol 54% yield) was a white solid. ^{1}H-NMR (400 MHz) DMSO-d_6: 10.7 (bs, 1H), 7.78 (s, 2H), 7.53–7.55 (d, 1H), 7.31–.7.32 (d, 1H), 7.18–7.22 (t, 1H), 7.07–7.11 (t, 1H), 3.77 (s, 3H), 2.50 (s, 3H); ^{13}C-NMR (100 MHz) DMSO-d_6: 187.9, 154.2, 145.6, 136.8, 135.2, 133.4, 126.8, 122.4, 121.8, 120.1, 112.2, 111.8, 110.7, 30.3, 12.9. LC/MS-MS: 423.9 → 278.8 *m/z*; GS1 and GS2 at 30, DP = 21, CE = 37, CXP = 18, t_R = 4.26 minutes.

Functional Analysis via Oocytes Expressing hURAT1

The methods used to test compounds via oocytes expressing hURAT1 were the same as those previously described.[17]

Computational Analysis

Chemical structures were drawn using CS Chem-Draw Ultra® (version 6.0.1; Cambridge Soft Corporation, Cambridge, MA, USA). The structures were copied into CS Chem3D Ultra® and molecular geometries were

generated in the Gaussian Z-matrix style via the CS MOPAC application. For each compound, an Austin-Model (AM1) semiempirical calculation (closed shell, tight convergence criteria) was conducted using Gaussian© software packet (Gaussian, Inc., Carnegie, PA, USA).[18] Next, gas phase ab initio geometry optimizations were performed using Gaussian 03 at the Hartree–Fock (HF) level of theory using the 6-311G basis set.[19,20]

RESULTS AND DISCUSSION

As summarized in Figure 2 and Table 1, we prepared and tested a series of (2-ethylbenzofuran-3-yl) (3,4,5-substituted-phenyl)methanone compounds not previously described.[17] These compounds and the in vitro data illustrate very important structural requirements in order for a molecule to become a potent hURAT1 inhibitor. We chemically modified the para-position of the C-ring[17] and subsequently probed ^{14}C-urate (10.0 μM) transport inhibition (50 μM test compound). The functional group transformation from hydrogen **5**, to methoxy **6**, to phenol **7** afforded a profound inhibitory effect. Phenol (**7**) was fairly potent and subsequently tested at a variety of concentrations to generate an in vitro IC_{50} value (2.80 $\pm$ 0.18 μM; Table 1). The insightful modification of methoxy **6** to phenol **7** suggested the notion that a good inhibitor required a hydrogen bonding interaction in this region of the molecule. However, the fabrication and testing of fluoro **8** and nitrile **9**—two molecules that may participate in hydrogen bonding—showed that they were not appreciable hURAT1 inhibitors. From

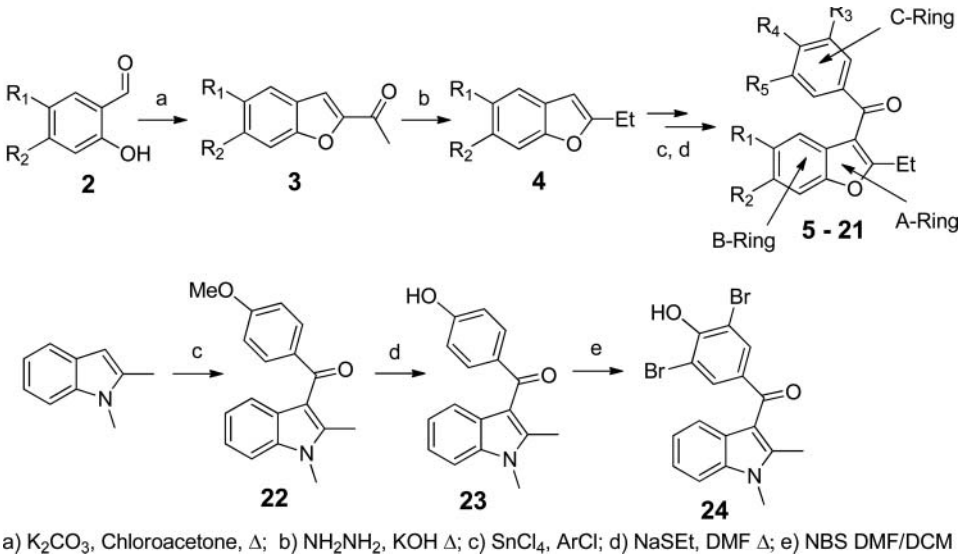

FIGURE 2 General synthesis of (2-ethylbenzofuran-3-yl) (3,4,5-(substituted)-phenyl)methanones and (3,5-dibromo-4-hydroxyphenyl) (1,2-dimethyl-1H-indol-3-yl)methanone.

TABLE 1 Compound and inhibition data summary

Entry	R_1	R_2	R_3	R_4	R_5	% Inhibition at 50 μM	IC_{50}
5	H	H	H	H	H	13.5	ND
6	H	H	H	O-Me	H	13.3	ND
7	H	H	H	OH	H	90.8	2.80 ± 0.18 μM
8	H	H	H	F	H	10.4	ND
9	H	H	H	CN	H	23.6	ND
10	H	H	H	O-Me	F	29.4	ND
11	H	H	H	O-Me	Cl	52.4	ND
12	H	H	H	O-Me	Br	22.2	ND
13	H	H	H	OH	F	97.9	2.41 ± 0.09 μM
14	H	H	H	OH	Cl	98.8	874 ± 56 nM
15	H	H	H	OH	Br	99.1	814 ± 160 nM
16	H	H	Br	OH	Br	99.9	26 ± 3 nM
17	H	O-Me	Br	OH	Br	99.9	111 ± 14 nM
18	H	OH	Br	OH	Br	99.8	138 ± 88 nM
19	F	H	H	O-Me	H	27.2	ND
20	F	H	H	OH	H	98.0	1.59 ± 0.11 μM
21	F	H	Br	OH	Br	100	6 ± 4 nM
22*	H	H	H	O-Me	H	74.0	45.21 ± 0.39 μM
23*	H	H	H	OH	H	95.6	2.05 ± 0.12 μM
24*	H	H	Br	OH	Br	100	401 ± 116 nM

*See Figure 2 for the complete chemical structure.

these data, the concept of hydrogen bonding was clearly not the predominate interaction to afford a potent inhibitor. Next, we continued to probe these concepts by modifying the C-Ring and preparing monohalogen methoxy analogues where X = F, Cl, and Br; compounds **10**, **11**, and **12**, respectively. Interesting enough, while there was a moderate inhibitory enhancement by incorporating a monohalogen (chloro-methoxy performed the best at 50 μM), these methoxy analogues were still weak inhibitors. These data also support the idea that a phenol may be required in the C-ring. This concept becomes very clear after preparing and testing phenol analogues **13**, **14**, and **15**. These compounds demonstrate a halogen/steric effect where modification to the chloro **14** and bromo **15** analogues produced submicromolar (<1.0 μM) hURAT1 inhibitors (874 and 814 nM, respectively). Lastly, the incorporation of an additional bromine atom ortho to the phenol to afford dibromo-analogue **16** (benzbromarone) generated a very potent inhibitor (26 nM). While also potent inhibitors, 6-methoxy **17** and 6-hydroxy **18** (the major in vivo metabolite of **16**) were less potent than **16**. Thus, it appears that an electron donating group on the B-ring decreases inhibitor potency. This notion appears to be supported by comparing 5-fluoro analogues **19**, **20**, and **21**. As was the case with going from **6** to **7** and then to **16**, going from methoxy analogue **17** to phenol **18**, and subsequently to dibromo **21**, produced a dramatic increase in inhibitory potency. The inductively withdrawn 5-fluoro analogue **21** became superior to **16**, having an IC_{50} of 6 ±

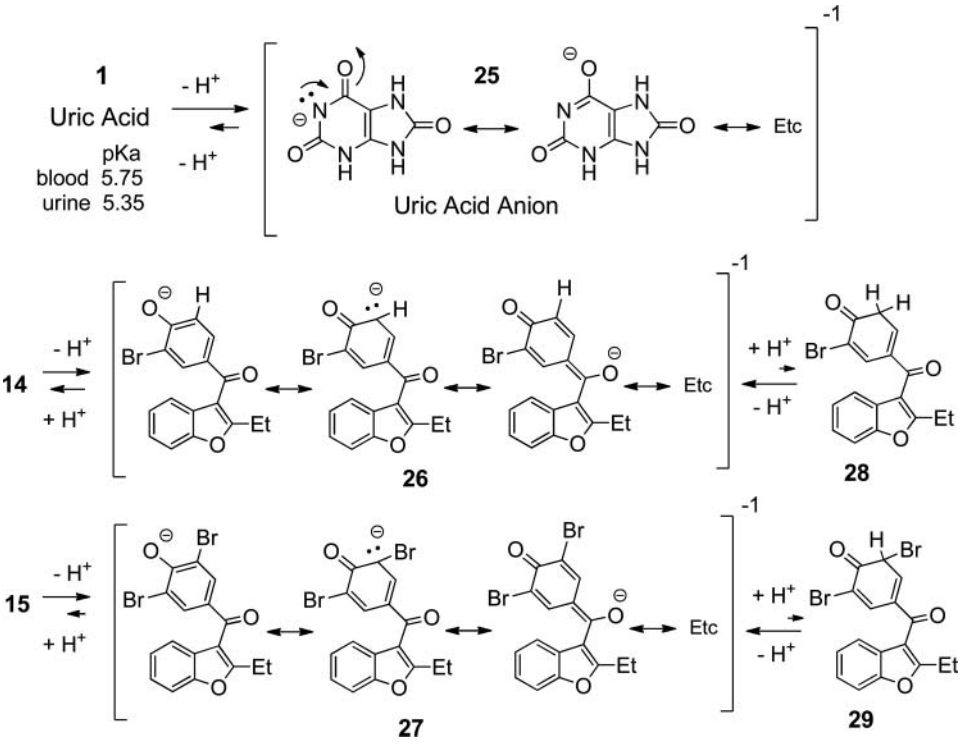

FIGURE 3 Resonance-stabilized anions of urate and compounds **15** and **16**.

4 nM (Table 1). In addition, we investigated the influence of replacing the oxygen atom within the benzofuran functionality with a nitrogen atom to afford (1,2-dimethyl-1H-indol-3-yl)methanone compounds **22**, **23**, and **24**. Interestingly enough, compounds **22** and **23** were more potent inhibitors than **6** and **7**, respectively. However, compound **24** was not as potent as **16**. These examples also illustrate the importance of the A-ring heteroatom and the influence of electronics and substituted groups.

As depicted in Figure 3, urate, a weak acid in blood and urine, has a p*K*a of approximately 5.75 and 5.35, respectively, and therefore predominates in the anionic form. As an anion, **25** functions as a substrate of various organic anion transporters such as hOAT1 and hOAT3, as well as NPT4 and hURAT1. Furthermore, the data supports the notion that hURAT1 has a binding pocket possessing a positive charge, and that Coulombic interactions are required for substrate and/or inhibitor recognition. Comparing the increase in inhibitory potential going from monobromo **15** to dibromo **16**, the change in molecular acidity (more acidic, lower p*K*a) produces a shift in equilibrium (i.e., Le Chatelier's principle) to afford resonance-stabilized anions **26** and **27**, respectively. The more acidic **16** produces a higher concentration of anion **27**, and thus is a better inhibitor. Furthermore, phenols are well known to undergo phenol-keto tautomerism and

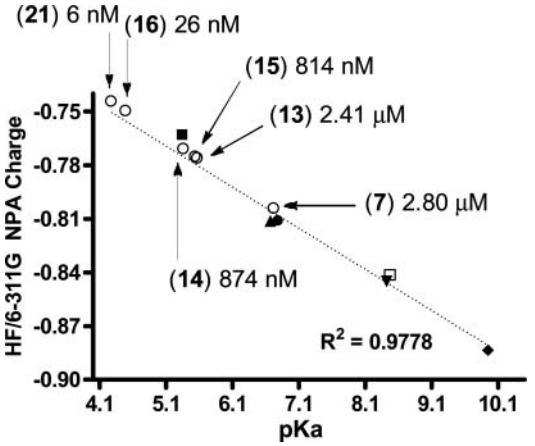

FIGURE 4 Computed HF/6-311G natural population analysis (NPA) charge versus p*K*a and hURAT1 inhibitory potential; ♦ = phenol; □ = chlorophenol; ▼ = bromophenol; • = dichlorophenol; ▲ = dibromophenol; ■ = urate; ○ = test compounds.

thus, their equilibrium may be influenced by the chemical environment.[21] We compared the gas phase energies of **15** and **16** and their corresponding keto tautomers **28** and **29**, respectively. The keto tautomers were computed (HF/6-311G) to be 23.4 and 24.6 kcal/mol higher in energy than their corresponding phenols, respectively. To further illustrate the importance of the anion phenomena, we conducted additional computational modeling using various phenols (phenol, *o*-chloro-phenol, *o*-bromo-phenol, 2,6-dichlorophenol, and 2,6-dibromophenol) with well-known p*K*a values (9.95, 8.48, 8.42, 6.79, and 6.67, respectively). The anions were computed and the charges (Natural Population Analysis; obtained via Pop=NPA and SCF=Tight) of the phenolic oxygen at the HF/6-311G basis set were plotted (Figure 4). A linear relationship between the charge and experimental p*K*a exists. The charge corresponding to the urate nitrogen anion **25** was also included. The theoretical charges for compounds **7**, **13–16**, and **21** were then plotted. These data (Figure 4) further illustrate the importance of the phenol functionality and its intrinsic acidity to afford a higher concentration of anions, and thus enhance inhibitor potency.

In conclusion, the experimental data support the notion that a potent hURAT1 inhibitor requires an anion (i.e., a formal negative charge) to interact with the positively charged binding pocket. An anion appears to be a primary requirement in order to be a hURAT1 substrate (i.e., urate) or inhibitor (i.e., **16**). The difference between the molecules being a substrate as opposed to an inhibitor relates to molecular size. For example, **1** has a computed Connolly Solvent-Excluded Volume (CSEV) of 92.3 Å,^{3} while compound **16** has a CSEV of 253.1 Å^3. In our hands, compound **16** was not readily transported by hURAT1 and inhibitor interactions occurred at

the apical (urine) side.[17] Lastly, electronically withdrawing group(s) on the B-ring appear to enhance inhibitor potency and are likely helpful for avoiding and/or reduceing the P450 metabolism associated with the B-ring. For example, hydroxy **18** is the major metabolite of compound **16** in vivo. Our current efforts are focused on developing B-ring-modified analogs and blocking unwanted the CYP-catalyzed metabolism believed to be responsible for unwanted drug–drug interactions with respect to this chemical template. Furthermore, we are studying other transporter proteins known to be involved in urate re-absorption and secretion (e.g., URATv1, hOAT's).

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