Pyrazolopyrimidine-2,4-dione Sulfonamides: Novel and Selective Calcitonin Inducers

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A series of pyrazolo[4,3-*d*]pyrimidine sulfonamides and pyrazolo[3,4-*d*]pyrimidine sulfonamides have been synthesized. These compounds increase transcription of a calcitonin−luciferase promoter and production of cellular calcitonin in a calcitonin−secretion/RIA assay with minimized phosphodiesterase type 4 inhibitory activity at 30 μM as compared to structurally related xanthine methylene ketones such as denbufyllene. These two series are notable examples of small molecules that act as CT-inducers, a method to potentially treat bone loss diseases.

Introduction

Calcitonin (CT) is a 32 amino acid polypeptide hormone secreted by the thyroid that decreases blood calcium primarily by inhibiting bone resorption. Salmon calcitonin (s-CT) is a currently prescribed treatment for high-turnover bone loss, one of the results of postmenopausal osteoporosis.[1] Although s-CT has demonstrated efficacy in the prevention of high-turnover bone loss, a limitation for its widespread use is its lack of oral bioavailability, necessitating administration by parental (intramuscular), intrapulmonary,[2] or most commonly by intranasal routes.[3] In addition, the development of patient resistance to intranasal s-CT limits its use. References to oral delivery systems for s-CT have appeared in the patent literature (stable formulations,[4] polymer conjugation,[5] and a combination of polymer conjugation and non-native amino acids),[6] but currently none have been approved for clinical use. An orally bioavailable small organic CT mimetic that could increase endogenous CT would offer an important advance in the use of CT as an antiresorptive agent.[7]

We have previously communicated the discovery that xanthine sulfonamides **1**, based on known phosphodiesterase type 4 (PDE4) inhibitor denbufyllene **2**, increase CT gene transcription (CT gene promoter−luciferase reporter construct or CT-luci) and CT secretion (CTS) in vitro and possess bone-sparing activity in ovariectomized (OVX) rats.[8] Importantly, **1** showed only moderate to weak PDE4 inhibitory activity and thus would not be expected to display the same emetic side effects as **2**.[9] We now disclose that the related pyrazole series **3** and **4** display a similar in vitro profile as **1** and could also be useful as a class of CT inducers.

Chemistry

Series **3** and **4** are prepared according to Schemes 1 and 2. Key steps in the synthesis of **3** and **4**, respectively, are as follows: series **3**—diazotization of uracil **6** followed by cyclization of the resulting diazonium species gives the corresponding pyrazolo[4,3-*d*]pyrimidine **7**;[10] series **4**—conversion of uracil **9** with hydrazine gives the corresponding pyrazolo[3,4-*d*]pyrimidine **10**.[11]

Structural proof of series **3** was determined by two-dimensional (2D) nuclear magnetic resonance (NMR) $^{1}H-^{13}C$ and $^{1}H-^{15}N$ correlation experiments on compound **11**. The sulfonamide nitrogen was readily assigned via a three bond correlation from the sulfonamide methyl group in a 2D ^{15}N HMBC experiment. To differentiate between structures **11** and **11a**, we considered the relative magnitudes of the $^{15}N-H(5)$ long-range couplings. In pyrazole systems, the largest long-range couplings (J = 10−14 Hz) result from a cis orientation of the C−H to the lone pair of an sp2 ^{15}N atom, such as the H(5)−N(6) coupling in structure **11a** (Chart 1).[12] However, this is not seen as the cross-peak intensity from H(5) to this lone pair (N(7)) is substantially smaller. Thus, **11** must have a structure as shown. The assignment of H(5) in **3** was determined on the basis of long-range ^{13}C HMBC couplings, ^{13}C chemical shifts, and nuclear Overhauser effect (NOE) interactions. In particular, an NOE is observed between H(5) and H(1′) for **11**. Series **4** was assigned in a similar manner except that H(5) could not be assigned by an NOE to H(1′). The assignment of H(5) in **4** was verified by a three bond coupling from H(5) to C(4) in the ^{13}C HMBC spectrum of **19**.

Pharmacology

Newly synthesized compounds were evaluated in vitro using the following assays. A CT-luci reporter gene assay was used to measure the ability of a compound to increase transcription of a luciferase receptor gene linked to the human CT gene. CT-luci data are reported as a transcription activation ratio (TAR) of test luciferase activity/control luciferase activity at 30 μM. TAR values have an average precision error of ±10−15%. A CTS/radioimmunoassay (RIA) assay was used to measure the ability of a compound to increase cellular levels and secretion of human CT. CTS data are

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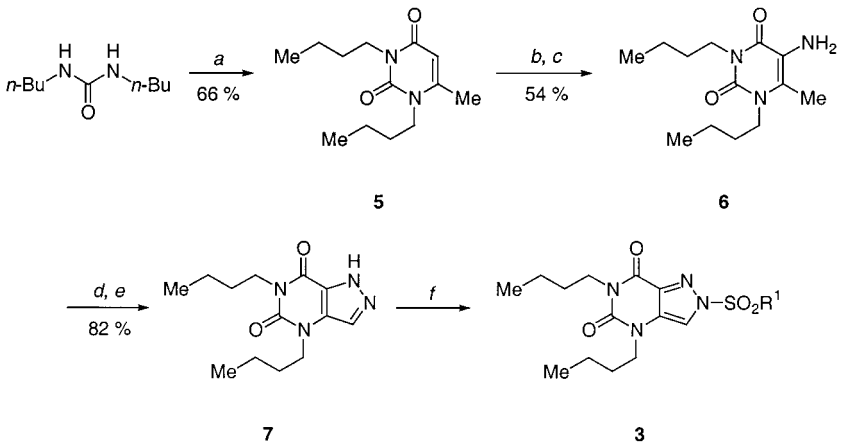
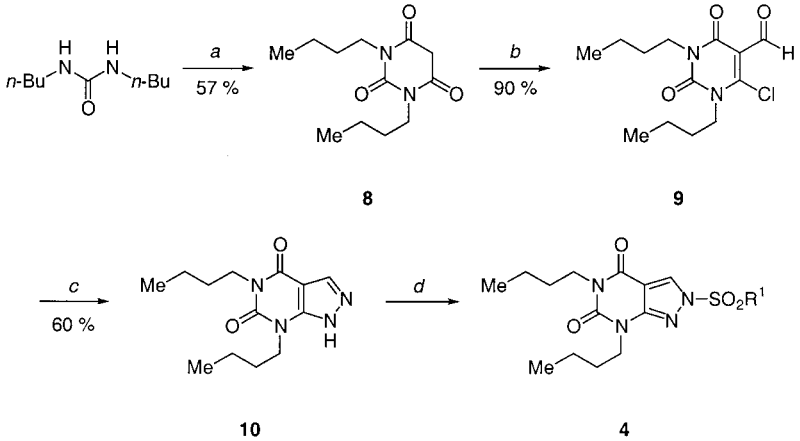
Scheme 1[a]



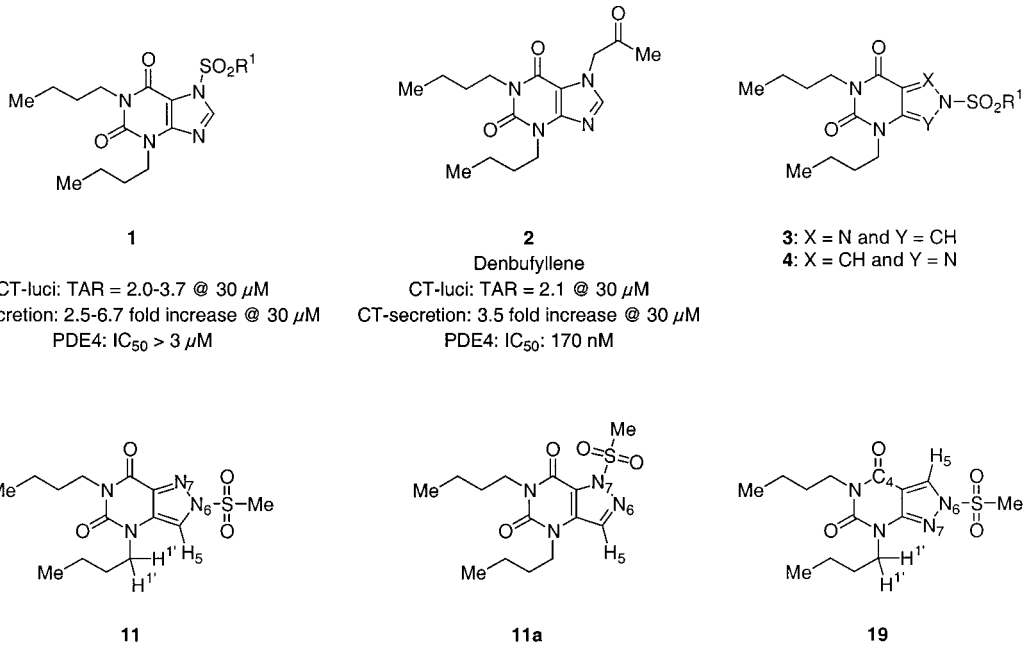
[a] (a) DMAP, Ac_2O, pyr, 100 °C; (b) HNO_3, H_2SO_4, 0 °C; (c) H_2, 5% Pd/C, EtOAc, 23 °C; (d) $NaNO_2$, HCl(aq), H_2O, 0 °C; (e) NaOH, H_2O, 0 °C; (e) R^1SO_2Cl, *i*-Pr_2NEt, CH_2Cl_2, 23 °C.

Scheme 2[a]



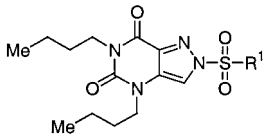
[a] (a) Malonic acid, Ac_2O, AcOH, 90 °C; (b) $POCl_3$, DMF, 105 °C; (c) $N_2H_4 \cdot H_2O$, MeOH, 23 °C; (d) R^1SO_2Cl, *i*-Pr_2NEt, CH_2Cl_2, 23 °C.

Chart 1



presented as a ratio of test CTS activity/control CTS activity at 30 μM. CTS values have an average precision error of ±10−33%. A standard U937 cell PDE4 enzyme assay was also used. In this assay, compounds were tested for PDE4 activity at 30 μM (PDE4 30) and scaled based on percent rolipram PDE4 activity (absence of

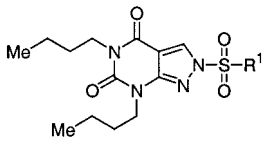
Table 1. In Vitro Profile of Pyrazolo[4,3-*d*]pyrimidine Sulfonamides **11**−**18**



no.	R^1	formula[a]	CT-luci TAR[b] (*n*)	CTS increase[c] (*n*)	PDE4 30% rolipram activity[d] (*n*)	PDE4 IC_{50}; μM[e] (*n*)
2 (denbufylline)			2.1 ± 0.3 (3)	3.5 ± 0.5 (3)	100 (3)	0.17 ± 0.05 (2)
11	Me	$C_{14}H_{22}N_4O_4S$	2.1 (3)	0.9 (1)	60 (1)	
12	3-Cl propyl	$C_{16}H_{25}ClN4O_4S$	1.3 (1)	4.4 (1)	69 (1)	4.9 (1)
13	3-CF_3 phenyl	$C_{20}H_{23}F_3N_4O_4S$	1.8 ± 0.3 (3)	3.8 (1)	32 ± 8 (2)	
14	4-*t*-Bu Ph	$C_{23}H_{32}N_4O_4S$	2.3 (1)	4.5 (1)	37 (1)	
15	2-naphthyl	$C_{23}H_{26}N_4O_4S$	1.8 (1)	4.9 (1)	66 (1)	
16	benzyl	$C_{20}H_{26}N_4O_4S$	1.5 (1)	2.6 (1)	65 (1)	
17	2-(4,5-dichloro)-thiophene	$C_{17}H_{20}Cl_2N_4O_4S_2$	1.7 (1)	3.4 (1)	59 (1)	
18	8-quinoline	$C_{22}H_{25}N_5O_4S$	2.2 ± 0.2 (2)	3.8 (1)	59 (1)	

[a] Structures of compounds confirmed by ^1H NMR, IR, and MS. Analytical results are within ±0.4% of the theoretical value. [b] CT-luci—TAR: transcription of a luciferase receptor gene linked to the human CT promoter gene expressed as a ratio of luciferase activity in the presence of test compound (at 30 μM) as compared to the luciferase activity in the untreated control. [c] CTS: stimulation of CT secretion as expressed as a ratio of secretion activity in the presence of test compound as compared to the secretion activity in the untreated control. [d] PDE4 30 assay: percent rolipram activity of the PDE4 assay at 30 μM concentration of test compound. [e] PDE4 assay: concentration at which 50% of the activity of the PDE4 assay is inhibited by test compound; *n*, number of experiments.

Table 2. In Vitro Profile of Pyrazolo[4,3-*d*]pyrimidine Sulfonamides **20**−**26**



no.	R^1	formula[a]	CT-luci TAR[b] (*n*)	CTS increase[c] (*n*)	PDE4 30% rolipram activity[d] (*n*)	PDE4 IC_{50}; μM[e](*n*)
2 (denbufylline)			2.1 ± 0.3 (3)	3.5 ± 0.5 (3)	100 (3)	0.17 ± 0.05 (2)
20	Me	$C_{14}H_{22}N_4O_4S$	1.8 ± 0.2 (2)	3.9 (1)	93 (1)	
21	3-Cl propyl	$C_{16}H_{25}ClN4O4S$	1.9 ± 0.0 (2)	2.7 (1)	66 (1)	4.9 (1)
22	4-*t*-Bu Ph	$C_{23}H_{32}N_4O_4S$	2.6 ± 0.05 (2)	2.9 (1)	57 (1)	
23	2-CN phenyl	$C_{20}H_{23}N_5O_4S$	3.6 ± 0.1 (2)	3.4 (1)	32 (1)	
24	benzyl	$C_{20}H_{26}N_4O_4S$	2.0 ± 0.05 (1)	3.4 (1)	52 (1)	
25	2-(4,5-dichloro)-thiophene	$C_{17}H_{20}Cl_2N_4O_4S_2$	3.2 ± 0.9 (1)	3.0 ± 0.9 (2)	16 (1)	
26	8-quinoline	$C_{22}H_{25}N_5O_4S$	2.9 ± 0.4 (3)	4.8 (1)	96 (1)	

[a] Structures of compounds confirmed by ^1H NMR, IR, and MS. Analytical results are within ±0.4% of the theoretical value. [b] CT-luci—TAR: transcription of a luciferase receptor gene linked to the human CT promoter gene expressed as a ratio of luciferase activity in the presence of test compound (at 30 μM) as compared to the luciferase activity in the untreated control. [c] CTS: stimulation of CT secretion as expressed as a ratio of secretion activity in the presence of test compound as compared to the secretion activity in the untreated control. [d] PDE4 30 assay: percent rolipram activity of the PDE4 assay at 30 μM concentration of test compound. [e] PDE4 assay: concentration at which 50% of the activity of the PDE4 assay is inhibited by test compound; *n*, number of experiments.

rolipram inhibition is set to 0% and full rolipram inhibition is set at 100%). PDE4 30 has an average precision error of ±10−25%. Desirable CT-inducing compounds are those that show elevated CT-luci and CTS activity (CT-luci TAR ≥ 1.5; CTS ≥ 2.5). Given the precision error stated above, "desirable compounds" that have elevated CT-luci/CTS values that show moderate to weak PDE30 values have the following profile: CT-luci TAR ≥ 1.5; CTS ≥ 2.5; PDE 30 ≤ 60.

Results and Discussion

Representative pyrazolo[4,3-*d*]pyrimidine sulfonamides from series **3** are shown in Table 1. Many of these compounds compare favorably with **1** and **2** in terms of CT-luci and CTS data. Additionally, the compounds in series **3** display only weak to moderate PDE4 inhibitory activity. It should also be noted that all compounds in Table 1 have PDE4 30 and/or PDE4 IC_{50} values substantially less potent than **2**. The best compounds in this series with respect to CT-luci and CTS data are aromatic sulfonamides **13**−**15**, **17**, and **18**, which show CT-luci values of approximately 2.0 and CTS values of 3.4−4.9 coupled with weak to moderate PDE4 inhibitory activity. In particular, **14** has an excellent profile (CT-luci, 2.3; CTS, 4.5; PDE4 30, 37%). The methyl sulfonamide **11** shows CT-luci activity but not CTS activity (CT-luci, 2.1; CTS, 0.9). The 3-Cl-propyl sulfonamide **12**, which was the optimal sulfonamide in series **1**, shows good CTS activity but poor CT-luci activity (CT-luci, 1.3; CTS, 4.4). Homologation of the aryl sulfonamides to the benzyl sulfonamide **16** shows CT-luci activity comparable to the other aromatic sulfonamides, but its CTS activity is comparably weaker (CT-luci, 1.5; CTS, 2.6).

The closely related pyrazolo[3,4-*d*]pyrimidine sulfonamides **4** are presented in Table 2. In general, these compounds compare favorably with the isomeric pyra-

zolo[4,3-*d*]sulfonamides **3** except that they generally appear to give better CT-luci values. Aryl sulfonamides **22**−**26** again appear to have the optimal in vitro profile with CT-luci values ranging from 2 to 3.6 and CTS values ranging from 2.9 to 4.8. The *o*-CN analogue **23** gives a particularly strong CT-luci TAR (CT-luci, 3.6). Thiophene analogue **25** has a particularly good in vitro profile as it shows notable CT-luci/CTS activity with very little PDE4 inhibition (CT-luci, 3.2; CTS, 3.0; PDE4 30, 16). Homologation of an aryl group to a benzyl, compound **24**, results in a slight loss of CT-luci activity as compared to the other aryl sulfonamides in Table 2. The same effect was seen for the benzyl sulfonamide **16** in Table 1. The 8-quinoline **26** analogue has substantial PDE4 inhibitory activity (similar to the activity of rolipram), which is not seen with the isomeric pyrazolo[4,3-*d*]pyrimidine analogue **18**.

Conclusion

In summary, we have discovered two new series of pyrazolopyrmidine sulfonamides, **3** and **4**, which increase CT-luci transcription as well as cellular CT production and secretion. Moreover, these series of compounds do not possess the same potent PDE4 inhibitory activity as the related xanthines such as denbufyllene **2**. These series of compounds are examples of interesting new chemical entities that may be useful as a new class of antiresorptive agents for the treatment of bone loss diseases.

Experimental Section

Melting points were determined on a Thomas-Hoover Melttemp apparatus and are uncorrected. The proton (^{1}H) NMR spectra were recorded at 300 MHz on a Bruker DPX-300 spectrometer using tetramethylsilane (δ 0.0) as an internal standard. Infrared (IR) spectra were obtained as KBr pellets. Combustion analyses were obtained using a Perkin-Elmer Series II 2400 CHNS/O analyzer. Mass spectra were obtained using a Micromass Platform Electrospray Ionization Quadrapole mass spectrometer. Flash chromatography was performed using EM Science 230−400 mess silica gel. Thin-layer chromatography (TLC) was performed in Analtech silica gel GHLF 250 μm prescored plates.

4,6-Dibutyl-2-methanesulfonyl-2,4-dihydro-pyrazolo-[4,3-*d*]pyrimidine-5,7-dione (11). To 281 mg (1.06 mmol) of **7** in 10 mL of CH_2Cl_2 were added 0.28 mL (206 mg, 1.60 mmol) of *i*-Pr_2NEt and 90 μL (134 mg, 1.17 mmol) of $MeSO_2Cl$. After this mixture was stirred at 23 °C for 2 h, the mixture was poured into 50 mL of brine and extracted with 2 × 25 mL of EtOAc. The combined organics were washed with 1 × 25 mL of H_2O and 1 × 25 mL of brine, dried over $MgSO_4$, filtered, and evaporated to a yellow solid. Flash chromatography on SiO_2, eluting with CH_2Cl_2/EtOAc (40/1 to 20/1), gave 259 mg (0.76 mmol, a 71% yield) of **11** as a white solid; mp 183−184 °C. ^{1}H NMR ($CDCl_3$): δ 7.87 (s, 1H), 4.05 (t, J = 7.4 Hz, 2H), 3.85 (t, J = 7.5 Hz, 2H), 1.72−1.57 (m, 4H), 1.45−1.31 (m, 4H), 0.98 (t, J = 7.5 Hz, 3H), 0.96 (t, J = 7.5 Hz, 3H). IR (cm^{-1}): 1714, 1675, 1624. MS (ES): 343 $(MH)^+$. Anal. ($C_{14}H_{22}N_4O_4S$) C, H, N.

5,7-Dibutyl-2-methanesulfonyl-2,7-dihydro-pyrazolo-[3,4-*d*]pyrimidine-4,6-dione (20). To a 23 °C solution of 350 mg (1.32 mmol) of **10** and 10 mL of CH_2Cl_2 were added 0.35 mL (257 mg, 1.99 mmol) of *i*-Pr_2NEt and 110 μL (167 mg, 1.46 mmol) of $MeSO_2Cl$. After the mixture was stirred at 23 °C for 2 h, the reaction mixture was poured into 50 mL of brine and extracted with 2 × 25 mL of EtOAc, and the combined organics were washed with 1 × 50 mL of H_2O and 1 × 50 mL of brine, dried over $MgSO_4$, filtered, and evaporated to a yellow oil. Flash chromatography on SiO_2, eluting with CH_2Cl_2/EtOAc (40/1 to 20/1), gave 346 mg (1.01 mmol, a 76% yield) of **20** as a white solid; mp 103−104 °C. ^{1}H NMR ($CDCl_3$): δ 8.53 (s, 1H), 4.05 (t, J = 7.4 Hz, 2H), 3.98 (t, J = 7.4 Hz, 2H), 3.42 (s, 3H), 1.78−1.67 (m, 2H), 1.63−1.54 (m, 2H), 1.39 (sept, J = 7.4 Hz, 4H), 0.99−0.91 (m, 6H). IR (cm^{-1}): 1724, 1668, 1608. MS (ES): 343 $(MH)^+$. Anal. ($C_{14}H_{22}N_4O_4S$) C, H, N.

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Supporting Information Available: Full experimentals and spectral data for compounds **5**−**10**, **12**−**18**, and **21**−**26** and pharmacological methods describing the CT-luci, CTS, and PDE4 30 assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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