Bioorganic & Medicinal Chemistry Letters 20 (2010) 6797–6801

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of *N*-benzyl-2-[(4*S*)-4-(1*H*-indol-3-ylmethyl)-5-oxo-1-phenyl-4,5-dihydro-6*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-6-yl]-*N*-isopropylacetamide, an orally active, gut-selective CCK1 receptor agonist for the potential treatment of obesity

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ARTICLE INFO

Article history:
Received 29 June 2010
Revised 22 August 2010
Accepted 24 August 2010
Available online 28 September 2010

Keywords:
CCK
Cholecystokinin
CCK1R
Gut selective
Triazolobenzodiazepinone

ABSTRACT

We describe the design, synthesis, and structure–activity relationships of triazolobenzodiazepinone CCK1 receptor agonists. Analogs in this series demonstrate potent agonist activity as measured by in vitro and in vivo assays for CCK1 agonism. Our efforts resulted in the identification of compound **4a** which significantly reduced food intake with minimal systemic exposure in rodents.

Cholecystokinin (CCK) is an endogenous gastrointestinal hormone that is released from the gut in response to ingestion of a meal. CCK has numerous physiological functions, including stimulation of gallbladder contraction, slowing of gastric emptying and promotion of satiety resulting in suppression of food intake.[1] CCK interacts with two G protein-coupled receptor subtypes, CCK1R and CCK2R. The CCK1R is localized primarily in the periphery (gastrointestinal tract) whereas the CCK2R is predominantly located in the brain and gastric mucosa.[2] Numerous studies support the hypothesis that CCK mediates its satiety effect through the CCK1R[3] which relays the post-prandial satiety signal via the vagal afferent neurons to the central nervous system (CNS).[4] These data suggest that CCK1R agonists may be effective anorectic agents for the treatment of obesity. In addition, recent reports suggest that activation of the CCK1R may also be beneficial for the management of diabetes.[5]

Small molecule CCK1R agonists (e.g., 1,5-benzodiazepine GI181771X (**1**),[6] thiazole SR-146131 (**2**),[7] and imidazole carboxamide (**3**))[8] have been disclosed (Fig. 1). The results of phase I and IIA clinical trials conducted with **1** have also been reported.[9] Despite statistically significant reduction in food intake following oral administration of **1**, no weight loss was discerned in subsequent Phase II trials.[9b,c] Dose-limiting gastrointestinal events including emesis were reported which may have precluded a thorough analysis of the CCK1 mechanism in the clinic. Herein we describe our efforts to design a novel series of triazolobenzodiazepinone-based CCK1R agonists, leading to the discovery of our clinical candidate **4a**, recently evaluated in Phase IIA clinical trials for the treatment of obesity and management of glycemic control.[10]

Our strategy for the design of an orally active, small molecule CCK1R agonist was based on the premise that systemic exposure is not required for anorectic activity and that robust efficacy could be achieved solely through activation of receptors within the enteric nervous system (ENS).[4] This hypothesis is supported by preclinical studies with both **1** and **3**, wherein despite poor systemic

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doi:10.1016/j.bmcl.2010.08.115

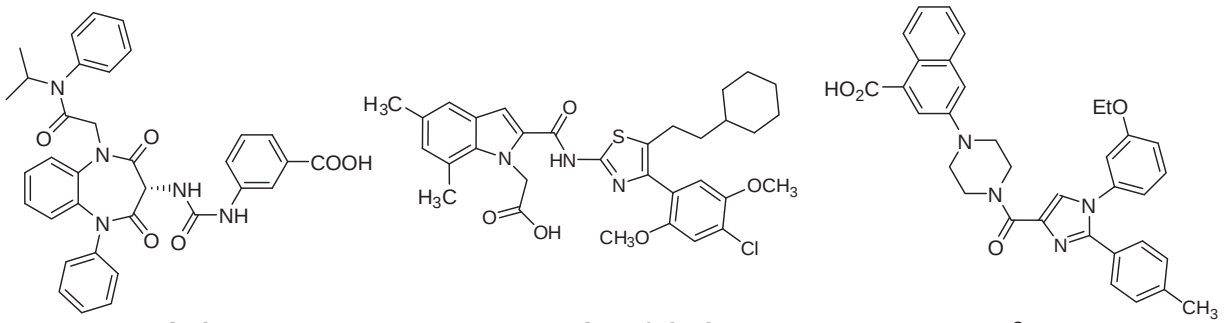


Figure 1. Structures of CCK1R agonists.

exposure, significant suppression of food intake was observed in rodents.[6c,8] Therefore we desired a gut-selective CCK1R agonist with the appropriate balance of solubility and membrane permeability to achieve enough free drug exposure to stimulate CCK1R activation within the ENS, yet rapid hepatic clearance to minimize plasma exposure. This approach offers a key advantage in that potential off-target pharmacology associated with mechanisms that require peripheral or CNS exposure would be avoided. In addition, a CCK1R agonist with this profile has the potential to better mimic the normal gallbladder physiology of contraction and refilling.

To achieve this profile, we deliberately diverged from Lipinski's 'rule of 5' for orally bioavailable drugs[11] seeking high molecular weight, lipophilic compounds to enhance intestinal permeability and increase hepatic clearance. Given the encouraging reports on suppression of food intake by **1**, we focused on the 1,5-benzodiazepine template as a starting point for design.[6,9a] Compound **1** is a CCK1 in vitro partial agonist with molecular weight 605, moderate rat clearance driven primarily by biliary clearance, and poor oral bioavailability. Shifting the clearance mechanism from biliary to hepatic was desirable to avoid the potential for enterohepatic recirculation. Because of their physical properties, 1,5-benzodiazepine CCK1 agonists generally suffered from poor oral absorption. Attempts to correlate efficacy as a function of absorptive permeability using the Caco-2 in vitro assay were not informative[6c] although solubility limitations may have confounded interpretation of these in vitro data. We utilized log *D* in the design of analogs to drive to a physicochemical property space appropriate for enhancing absorptive permeability and hepatic clearance. Relative to **1**, our focus for design was to maximize hepatic clearance by increasing lipophilicity and to improve permeability by decreasing the number of hydrogen bond donors and acceptors while maintaining CCK1R potency and selectivity to achieve the desired pharmacological profile.

Given the importance of the *N*1-isopropylanilidoacetamide moiety for CCK1R agonism in 1,5-benzodiazepine analogs,[6b] we chose to conserve this motif and focused on structural modifications to the benzodiazepine core. Key to our success was the discovery that a fused triazolobenzodiazepinone core could mimic the hydrogen bond acceptor properties of the carbonyl group present at C-4 in the 1,5-benzodiazepinone core while maintaining CCK1R binding and functional activity (Fig. 2).[12]

Initial synthetic attempts to access the core failed; our breakthrough came with the discovery of a novel, one-step condensation approach, thus enabling the development of this series. The synthesis of triazolobenzodiazepinone analogs is illustrated in Scheme 1. Acid-catalyzed condensation of 1,2-phenylenediamine (**5**) with 3,3-diethoxy-acrylic acid ethyl ester (**6**) followed by condensation of **7** with benzoyl hydrazine provided the triazolobenzodiazepinone scaffold **8**. N-Alkylation with the appropriate α-bromo-acetamide (**9**) afforded key intermediate **10**. Oximation at the C-3 position in **10** with isoamyl nitrite followed by reduction of the oxime provided the amine intermediate **11** which was readily functionalized to generate the corresponding amide, urea or carbamate analogs **4b–f**. Alternatively, condensation of **10** with an aromatic aldehyde followed by reduction, or alkylation with the appropriate arylmethyl halide afforded compounds **4a**,[13] **4g**, and **4h**. Chiral separation of either the racemic products or amine intermediates (**11a** and **11b**) provided the desired active enantiomers. The more active enantiomer was identified by preparing and testing both in vitro. As an example, data for the enantiomers of the indol-3-ylmethyl derivative, **4a** and **4h**, are provided (Table 1). Binding, using ^{125}I-CCK-8 as the radiolabel, and functional activity were assessed using a CHO cell line expressing either the human (Table 1) or rat CCK1R (Table 2). Compounds were determined to be agonists by their ability to stimulate the release of calcium using a FLIPR assay.

Preparation of **4b** where the *N*1-isopropylphenylacetamide moiety and the urea side-chain found in **1** were maintained provided a CCK1R partial agonist with improved binding (~6×) and comparable functional activity relative to **1**, thus confirming the

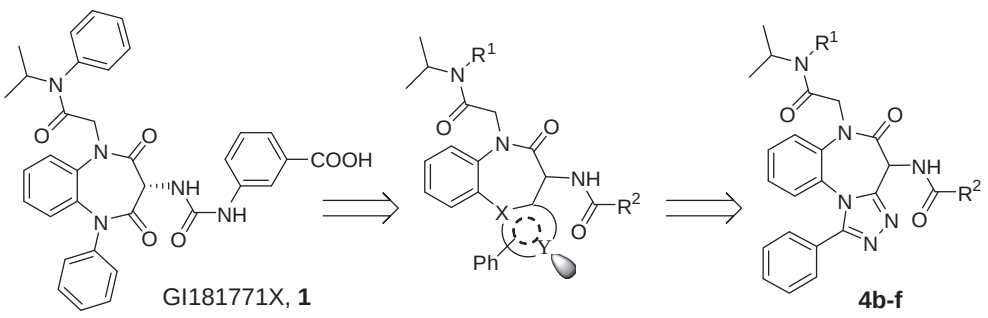
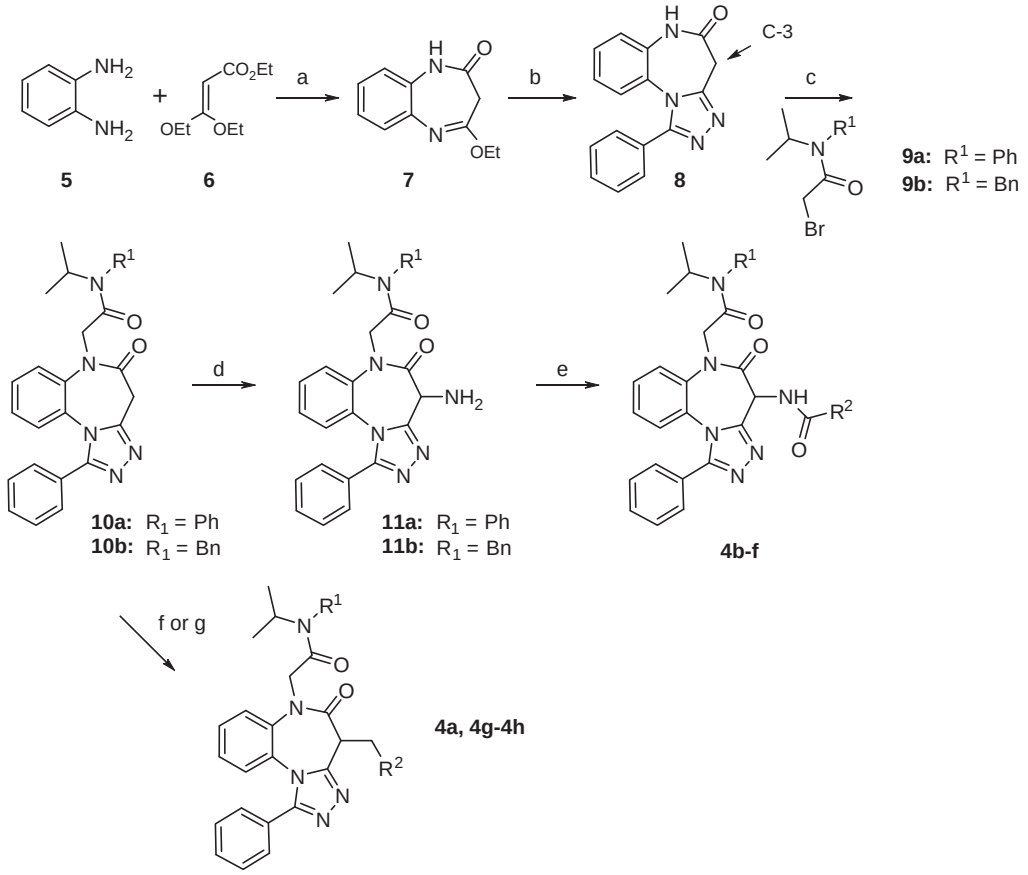


Figure 2. Design principle.



Scheme 1. Synthesis of CCK1R Agonists. Reagents: (a) AcOH, xylenes, reflux; (b) AcOH, benzoyl hydrazine, reflux; (c) NaHMDS, DMF, **9a** or **9b**; (d) (i) NaH, isoamyl nitrite; (ii) H_2, Pd/C, NH_4CO_2H; (iii) optional chiral separation; (e) (i) CDI, DMAP, R^2NH_2; or pyridine, R^2COCl; or $NaHCO_3$, R^2OCOCl; (ii) optional chiral separation; (f) (i) R^2CHO, cat. piperidine, toluene, reflux; (ii) H_2, Pd/C, NH_4CO_2H; (iii) Chiral separation; (g) (i) NaH, R^2CH_2X; (ii) Chiral separation.

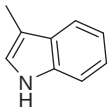
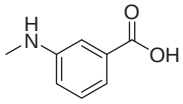
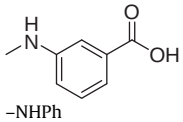
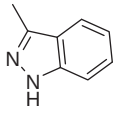
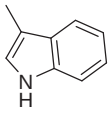
bioactivity of our new template. Replacement of the aniline functionality with benzyl provided **4c**, which led to 2.5× and 4× losses in binding and functional potencies as compared to **4b**. Removal of the acid (**4d**) and alternatives to the urea (**4e** and **4f**) were explored for improved potency as well as to increase log *D* and microsomal clearance. With the exception of **4e**, these analogs maintained moderate to good in vitro potency at the CCK1R. As expected, higher intrinsic clearance and decreased solubility were observed with these non-acidic, more lipophilic analogs. Replacement of the C-3 amine derivatives with indol-3-ylmethyl or indazol-3-ylmethyl tryptophan mimetics[14] (**4a** and **4g**) provided further increases in log *D* and clearance while maintaining in vitro potency. In addition, we observed good in vitro CCK1R agonist selectivity[15] with both analogs; **4a** also demonstrated very high intrinsic clearance, albeit at a solubility cost. Interestingly, the 3(*R*) antipode **4h** had decreased (∼8×) binding affinity as compared to **4a** with no detectable agonism.

The mouse gallbladder emptying model[16] was used to rapidly assess in vivo functional activity. With the exception of **4c**, compounds tested in this assay demonstrated complete gallbladder emptying at oral doses of 6 mg/kg, confirming target activity (Table 1). By 24 h, **4a** demonstrated nearly complete refilling of the gallbladder, whereas sustained gallbladder contraction was observed after dosing with **1**.

Food intake efficacy was assessed at 6 mg/kg in a rat spontaneous feeding model during the normal, nighttime feeding cycle (Table 2). No significant efficacy was observed with our more polar analogs (**4c–d**) despite having good in vitro potency and functional activity at the rat receptor. On the other hand, the more lipophilic compounds (**4a**, **4f,** and **4g**; log *D* >3) exhibited robust efficacy in this model. The added lipophilicity presumably enhanced intestinal permeability of these compounds, providing sufficient drug exposure at the site of action. Interestingly, no gastrointestinal side-effects were observed in this model with any of the CCK1R agonists tested. Confidence in CCK1R mediated efficacy observed in the feeding model was established by pre-treating rats with a CCK1R antagonist followed by administration of **4a**, resulting in the loss of food intake efficacy.[17] Because compound **4a** demonstrated robust efficacy along with high intrinsic clearance in microsomes, we progressed this compound forward to more detailed PK studies to support our gut-selective hypothesis.

Unfortunately, no inhibition of food intake was observed in rats dosed with crystalline **4a**, likely due to inadequate absorption of the less soluble crystalline form (no detectable solubility in phosphate buffered saline). Reformulation of crystalline **4a** as a solid spray dried dispersion (SDD)[18] led to significant improvements in solubility (∼20×) and consequently robust food intake efficacy over 12 h (Fig. 3). Independent PK studies with **4a** assessed intestinal and plasma exposure. Despite the solubility enhancement of the SDD formulation, oral exposure of **4a** in rats remained poor (*F* = 1%, Table 3). Unbound plasma concentrations remained well below the CCK1 rat IC_{50} of 12 nM (C_{max} = 0.98 ± 0.30 nM). In addition, exposure in the small intestine (normalized for non-specific binding) was determined. A maximal unbound gut exposure of 136 nM was measured at 0.25 h post-dose, after which time, gut concentrations steadily declined (95 nM, 14 nM and 3.0 nM at 1, 2 and 4 h post-dose, respectively). Overall, these data indicated that sufficient free gut concentrations (⩾CCK1R binding IC_{50}) of

Table 1
hCCK1R in vitro and mouse in vivo results for **1** and triazolobenzodiazepinone analogs **4a–h**[a]

	R^1	R^2	log D[b]	IC_{50}[c] (nM)	EC_{50} (nM) (% agonism vs CCK-8)[c]	Human microsomes Clint (mL/min/kg)	pH 6.5 kinetic solubility (μM)	Mouse % GBE[d] at 1 h 6 mg/kg	Mouse % GBE at 24 h 2 mg/kg
1	See Figure 1		1.09	131 ± 7.7	283 ± 7.3 (62.8 ± 0.5)	<8.0	499	82	91.4[d]
4a[e]	Bn		3.39	31.7 ± 4.3	315 ± 34.9 (53 ± 2)	199	0.94	91[f]	27.5[f,g]
4b	Ph		0.92	19.7 ± 0.9	160 ± 30.6 (67.8 ± 3.0)	<8.0	395	NT	NT
4c	Bn		0.44	49.3 ± 8.4	645 ± 158 (55.5 ± 4.4)	33.3	390	64	NT
4d	Bn	–NHPh	2.82	24.3 ± 2.3	91.3 ± 34.1 (73.7 ± 2.6)	85.4	6.0	90.4	NT
4e	Ph	–Bn	3.13	201 ± 32	1810 ± 451 (40.1 ± 2.5)	259	4.6	NT	NT
4f	Bn	–OPh	3.08	15 ± 4	628 ± 138 (57.7 ± 6.4)	29.0	12.1	90	NT
4g	Bn		3.16	48.5 ± 11	522 ± 187 (72.3 ± 2.1)	39.5	2.26	86.3	NT
4h[h]	Bn		3.39	246 ± 39	>10,000	NT	NT	NT	NT

[a] h = Human; NT = not tested; NS = not significant; μsomes = liver microsomes; GBE = gallbladder emptying; compounds tested in vivo were amorphous unless noted.
[b] Measured at pH 7.4.
[c] Mean ± standard error of at least *n* of three independent experiments.
[d] P <0.001 versus vehicle.
[e] 3(*S*)-isomer.
[f] Dosed in SDD formulation.
[g] P <0.003 versus **1**.
[h] 3(*R*)-isomer.

Table 2
Rat CCK1R in vitro and in vivo results[a]

	IC_{50}[b] (nM)	EC_{50}[b] (nM)	Rat SPFI %[c] reduction at 6 mg/kg
1	52.6 ± 10.4	63.8 ± 8.8	−52
4a	12.2 ± 3.8	445.8 ± 43.6	−56[d]
4c	17.0 ± 3.0	428 ± 23	NS
4d	2.0 ± 1.0	294 ± 6	NS
4f	NT	NT	−41
4g	21 ± 3	481.2 ± 154.9	−43
4h	NT	NT	NS

[a] NT = not tested; NS = not significant; SPFI = spontaneous food intake; compounds tested in vivo were amorphous unless noted.
[b] Mean ± standard error of at least two independent experiments.
[c] % Food intake at 4 h post-dose; P <0.05 versus vehicle unless noted.
[d] Dosed in SDD formulation.

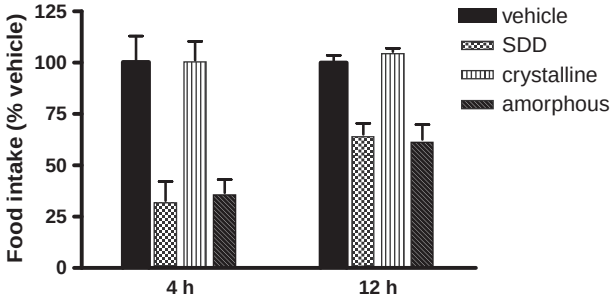


Figure 3. The effects of **4a** (6 mg/kg) on spontaneous rat food intake at 4 h and 12 h post-dose.

4a were achieved for up to 2 h following oral administration and likely accounted for the oral food intake activity observed in rat. Food intake effects were observed for up to 12 h (Fig. 3) suggesting that sufficient receptor activation was achieved to trigger downstream events leading to sustained efficacy.

Additional PK studies in rat indicated that **4a** has moderate plasma clearance (41.7 mL/min/kg, Table 3) and low biliary clearance (0.0277 mL/min/kg). The in vivo rat clearance data was in good agreement with the predicted blood clearance of 47 mL/min/kg generated from rat liver microsomes[19] and suggested that the primary clearance mechanism of **4a** was via oxidative metabolism in the liver. Published data on compound **1** indicated that biliary clearance significantly contributed to the overall clearance of the compound in rat (65% of parent is excreted into the bile after iv administration).[6c] Differences in the clearance mechanisms between **1** and **4a** may explain the observed disparate rates of gallbladder refilling. The presence of a CCK1R agonist in the bile may trigger activation of receptors present on the gallbladder leading to sustained contraction.

Table 3
PK parameters of **4a** in male Sprague-Dawley rats[a]

Dose (mg/kg)	Route	C_{max} (ng/mL)	T_{max} (h)	Cl_p (mL/min/kg)	Vd_{ss} (L/kg)	$AUC_{(0\text{-}tlast)}$ (ng h/mL)	$t_{1/2}$ (h)	F (%)
3.0	iv	NA	NA	41.7 ± 8.8	3.70 ± 0.62	1190 ± 253	1.37 ± 0.13	NA
6.0[b]	po	14.6 ± 4.4	1.33 ± 0.58	NA	NA	42.5 ± 15.7	NA	1.0 ± 0.4

[a] NA = not applicable, mean ± standard deviation, *n* of 3.
[b] SDD formulation.

In summary, the design and synthesis of CCK1R agonists with physicochemical properties suited for limited systemic exposure has led to the identification of our clinical candidate, **4a**. Compound **4a** demonstrated sustained food intake efficacy in our preclinical model with complete gallbladder refilling 24 h after dosing. Additionally, PK studies with **4a** to assess intestinal and systemic exposure suggest that efficacy is primarily derived from activation of the intestinal CCK1 receptors. Unfortunately, after 12 weeks of dosing in overweight and obese subjects with type-2 diabetes, **4a** demonstrated inadequate efficacy (HbA1c and body weight) and was discontinued from clinical development. Additional details will be reported in subsequent publications.

Acknowledgments

We thank Andrew Butler, Jon Bordner, Richard Hank, Sharon Matis, Daniel Virtue, Robert DePianta, Marlys Hammond, Terrell Patterson, Li She, Jim Coutcher, and Lucy Rogers, for their contributions to this work. We thank Larry Miller for providing the CHO hCCK1R cell line.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.115.

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13. Stereochemistry at C-3 for the active enantiomer **4a** was determined to be in the *S* configuration based on the following experiment: (1) single X-ray analysis of *N*-benzyl-2-{(4*S*)-4-[(5-bromo-1*H*-indol-3-yl)methyl]-5-oxo-1-phenyl-4,5-dihydro-6*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-6-yl}-*N*-isopropylacetamide; (2) removal of bromide via hydrogenation provided **4a**. The crystal structure has been deposited at the Cambridge Crystallographic Data Centre and was allocated the following deposition number: CCDC 789675.
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15. Because CCK2R agonist activity is undesired due to the potential for inducing anxiety,[2b] CCK2R stimulation was evaluated for **4a** and **4g** in a calcium mobilization assay (FLIPR) while CCK2R inhibition was examined by GTPγ35S binding. Compounds **4a** and **4g** had no significant activity at 10,000 nM in either assay. A full account of the triazolobenzodiazepinone SAR will be published in a separate manuscript.
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18. (a) Compound **4a** was formulated as a 25 wt % drug: 75 wt % hydroxypropyl methylcellulose acetate succinate-high granular (HPMCAS-HG) SDD in 0.5% methylcellulose.; (b) Rasenack, N.; Müller, B. W. *Pharm. Res.* **2002**, *19*, 1894; (c) Friesen, D. T.; Shanker, R.; Crew, M.; Smithey, D. T.; Curatolo, W. J.; Nightingale, J. A. S. *Mol. Pharm.* **2008**, *5*, 1003; (d) Curatolo, W.; Nightingale, J. A.; Herbig, S. M. *Pharm. Res.* **2009**, *26*, 1419.
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