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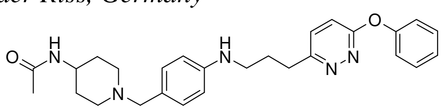

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Design, synthesis and evaluation of MCH Receptor 1 antagonists – Part II: Optimization of pyridazines toward reduced phospholipidosis and hERG inhibition

Gerald J. Roth [a, *], Armin Heckel[a], Jörg Kley[a], Thorsten Lehmann[a], Stephan G. Müller[a], Thorsten Oost[a], Klaus Rudolf[a], Kirsten Arndt[b], Ralph Budzinski[b], Martin Lenter[b], Ralf R. H. Lotz[c], Marcus Schindler[b], Leo Thomas[b], and Dirk Stenkamp[a]

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ABSTRACT

Despite recent success there remains a high therapeutic need for the development of drugs targeting diseases associated with the metabolic syndrome. As part of our search for safe and effective MCH-R1 antagonists for the treatment of obesity, a series of 3,6-disubstituted pyridazines was evaluated. During optimization several issues of the initial lead structures had to be resolved, such as selectivity over related GPCRs, inhibition of the hERG channel as well as the potential to induce phospholipidosis. Utilizing property-based design, we could demonstrate that all parameters can significantly be improved by consequently increasing the polarity of the compounds. By this strategy, we succeeded in identifying potent and orally available MCH-R1 antagonists with good selectivity over M1 and 5-HT_{2A} and an improved safety profile with respect to hERG inhibition and phospholipidosis.

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Obesity is a major risk factor in the modern world and associated with many serious diseases of the metabolic syndrome such as type 2 diabetes, dyslipidemia, coronary heart disease, and stroke.[1] The melanin-concentrating hormone (MCH), a cyclic 19-amino acid polypeptide, has been in the focus of obesity research over the recent years.[2] It is expressed in the lateral hypothalamus of the brain and the natural ligand for the seven-transmembrane G-protein-coupled receptors MCH-R1 and MCH-R2. MCH-R1 is involved in the regulation of feeding and energy homeostasis and has therefore been considered an interesting target for the treatment of obesity over the years. While MCH-R1 is expressed in humans and rodents, less is known about the exact function of MCH-R2 which is also expressed in humans and other species, but not in rodents. Despite many efforts of the pharmaceutical industry to develop MCH-R1 antagonists as potential anti-obesity agents, only few compounds advanced to the phase 1 clinical stage.[3] Safety concerns such as hERG inhibition or phospholipidosis[4] were among the prominent reasons for the discontinuation of preclinical research programs in the past.[5,6] Thus, there remains a high therapeutic need for the development of safe and effective MCH-R1 antagonists.

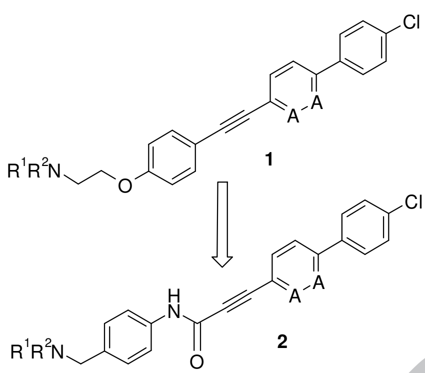


Figure 1. Design of the 4-atom linker series

As part of our ongoing research program aimed at the identification of potent MCH-R1 antagonists[7], compounds such as **2** were designed with a modified topology that, compared to compound **1**, positions the left-hand side aryl moiety differently (Figure 1). We reasoned that modifying the position of the aryl moiety could help overcome problems with respect to hERG inhibition and phospholipidosis (PL) discovered in series **1,** as the position of lipophilic moieties is often an integral part of the hERG and PL pharmacophore[8].

Compound **4** is a potent MCH-R1 binder, but insufficient selectivity over M1 was an initial concern for this probe (Table 1). Several related 4-atom linkers were synthesized to screen for improved lead compounds (Scheme 1). Reducing the triple bond (compounds **5** and **7**) as well as introducing additional hetero atoms (compounds **8** and **10**) into the linker led to reduced affinity to the receptor. In addition, selectivity over M1 remained poor for these derivatives and binding to 5-HT_{2A} was identified as additional off-target activity. Removing the carbonyl group from **7** finally resolved the problem and furnished compound **3** with single-digit nanomolar potency and improved selectivity over M1 and 5-HT_{2A} as a new lead to the MCH-R1 program. Of note, *N*-methylated derivatives such as **6** or **9** showed reduced affinity and were not followed up further.

Compound **3** displayed a rather unattractive clogP of 7.7 and did show strong inhibition of the hERG channel at a concentration of 1 µM (Table 2). In order to reduce the lipophilicity of the compounds a series of derivatives based on a central pyridazine scaffold rather than the phenyl moiety was synthesized (Scheme 2). Starting from pyridazine **17**, Sonogashira coupling and subsequent hydrogenation furnished building block **18**. 2-Chloro pyridazine **18** could be further modified by either Suzuki couplings utilizing aryl boronic esters or nucleophilic displacement reactions giving rise to Boc-protected 3-pyridazinyl-propylamines **21**. Alternatively, the order of reactions can be reversed using triflates **19**[9] or iodides **20**[9] as starting material. After deprotection, Buchwald coupling with aryl bromides **22** yielded the final products **23** in acceptable to good yield.[10]

Pyridazine **24** displays a significantly reduced calculated lipophilicity and is still a highly potent and selective MCH-R1 binder (Table 2). Gratifyingly, no hERG inhibition was observed for this compound at 1 µM. However, first phospholipidogenic effects[4] were observed for this compound at a concentration of 6.25 µM in a cellular test system.[11] According to Ploemen at al.[8] the phospholipidogenic potential can depend on the polarity and basicity of the compounds. We therefore decided to synthesize additional compounds with further reduced lipophilicity (as judged by the clogP) and basicity. Modifying R^3 (Table 2) led to compounds **25**-**29** with improved polarity but in most cases reduced potency. Only the 4-methoxy-substituted derivative **26** showed comparable activity and was used for further explorations. As a next step we explored the SAR around the basic moiety R^1R^2N with a special emphasis on improving the polarity further. Replacing the piperidine moiety with smaller residues such as pyrrolidine **30** or dimethyl amine **31** led to reduced affinity. While neutral compounds did not show binding to the MCH-R1, compounds with reduced basicity such as morpholine **32** retained potency. We decided to keep 6-membered amines as preferred residues and explored the effect of ring substitution further. In combination with starting point **24** compounds **32**-**35** differ in up to 3 clogP units and represent a remarkable example on how to improve compound characteristics by property-based design. All compounds display single-digit nanomolar potency and excellent selectivity over the M1 and 5-HT_{2A} receptors. By consequently reducing the clogP to a value of 2.2 a reduction of the phospholipidogenic potential into a concentration range of 100 µM could be achieved. We reasoned that polarity is clearly the decisive factor influencing phospholipidosis in our series of compounds rather than the basicity (see Table 2). Using the 4-substituted piperidines **34** and **35** as a basis for further modifications, additional derivatives such as **36**, **37**, and **38** with an overall excellent profile with respect to potency, selectivity, hERG inhibition (IC_{50} >10 µM for **38**) and phospholipidosis (first effects >100 µM for **38**) could be identified.

Parallel to the 3-pyridazinyl-propylamine series we explored the SAR of the related 3-pyridazinyl-propylethers (Table 3). The synthesis of the compounds is described in Scheme 3. Employing propargylic ether **48**[8] in the Sonogashira reaction/hydrogenation sequence, central intermediates **49** could be derived. Introduction of the basic moieties R^1R^2N was achieved by either mesylation of the benzylic alcohol and subsequent nucleophilic substitution reaction or by transformation into the aldehyde and reductive amination furnishing compound series **50**. SAR in the 3-pyridazinyl-propylether series were comparable to those in the propylamine series. Substituted piperidines such as **39** and **40** were identified as potent and selective binders to MCH-R1. Modification of R^3 delivered additional potent compounds such as **42**, **43**, and **45-47**, however the correct combination of optimal left-hand and right-hand side is crucial for good potency (for comparison see **41** and **44**). Selectivity over M1 and 5-HT_{2A}, as well as inhibition of the hERG channel was not an issue within this series. The potential for phospholipidosis followed the same trends as before: while less polar compounds such as **39** and **40** induced phospholipidogenic effects in the cellular assay at concentrations of 6-12 µM, more polar compounds such as **45-47** had a reduced potential for phospholipidosis (first effects at 100 µM).

Compounds **35**, **38** and **47** display high affinity to MCH-R1, are selective over M1 and 5-HT_{2A} and show an improved safety profile with respect to inhibition of hERG and phospholipidosis. In addition, these compounds exhibited favorable exposure in rats after oral administration (Table 4) and are candidates for further characterization in *in vivo* experiments.

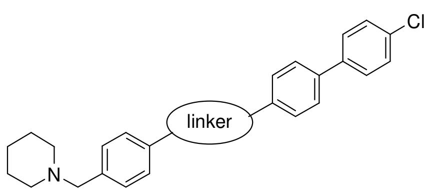
Table 4. Pharmacokinetic data after oral application to Wistar rats

	dose [µmol/kg]	c_{max} [nM][a]	t_{max} [h]	MRT [h]	AUC[a] [nM∗h]
35	11.9	83	1.5	4.0	476
38	10.9	24	2.8	6.4	123
47	6.7	531	0.8	2.9	175

[a] Dose-normalized for 1 µmol/kg

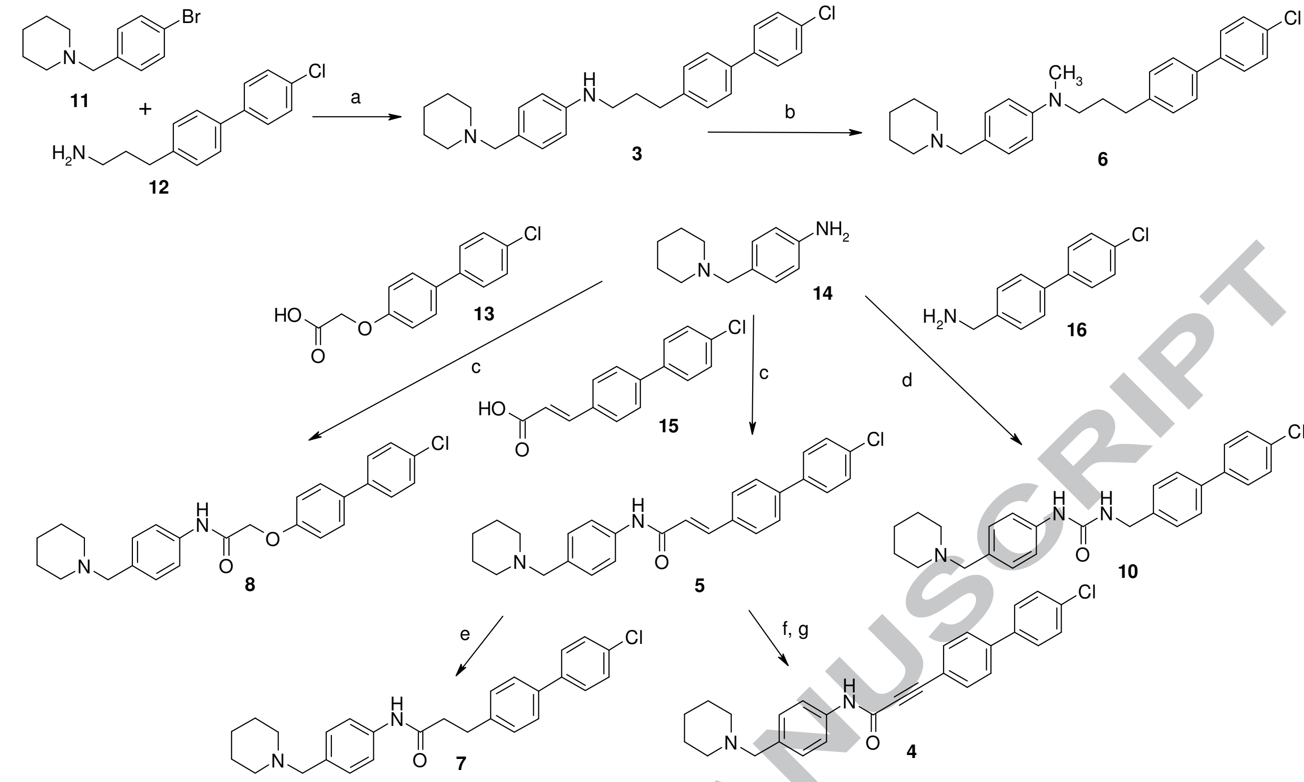
In summary, starting from a potent but non-selective lead, we managed to identify potent and orally available MCH-R1 binders with good selectivity over M1 and 5-HT_{2A} and a clearly improved safety profile with respect to the inhibition of the hERG channel and the induction of phospholipidosis. A key finding during optimization was that especially the potential for phospholipidosis could significantly be lowered by increasing the polarity of the compounds into a clogP range of 2-3.

Table 1. Representative SAR of 4-atom-linker series leading to improved M1 selectivity

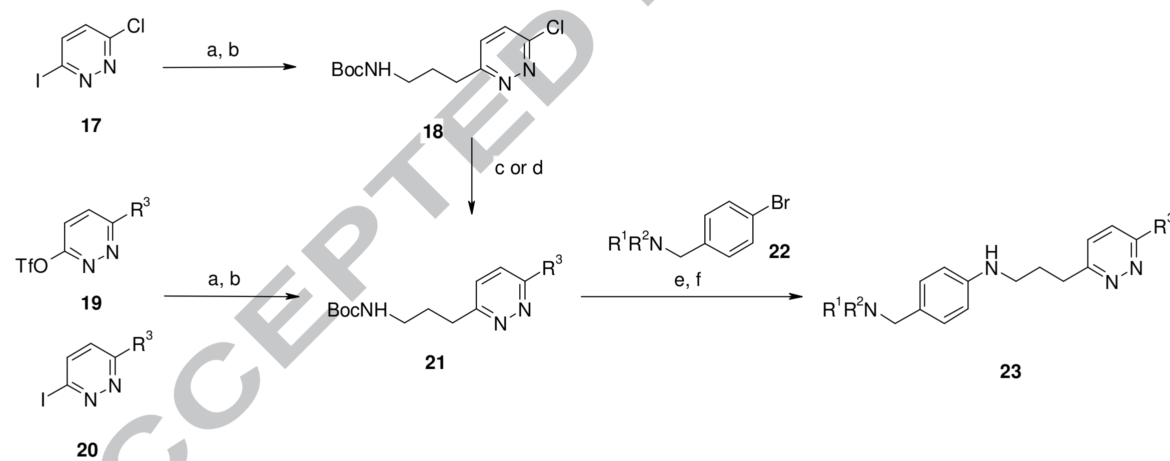


	linker	MCH-R1[a] IC_{50} [nM]	M1 IC_{50} [nM]	Selectivity M1/MCH	5-HT_{2A} IC_{50} [nM]	Selectivity 5-HT_{2A}/MCH
3	-$NHCH_2CH_2CH_2$-	3	325	110	680	230
4	-NHCOCC-	10	225	23	NT[b]	NT
5	(*E*)-NHCOCHCH-	16	401	25	1932	121
6	-(NCH_3)$CH_2CH_2CH_2$-	17	419	25	646	38
7	-$NHCOCH_2CH_2$-	38	89	2	138	4
8	-$NHCOCH_2O$-	94	446	5	91	1
9	-(NCH_3)COCHCH-	634	625	1	362	1
10	-$NHCONHCH_2$-	899	511	<1	29	<1

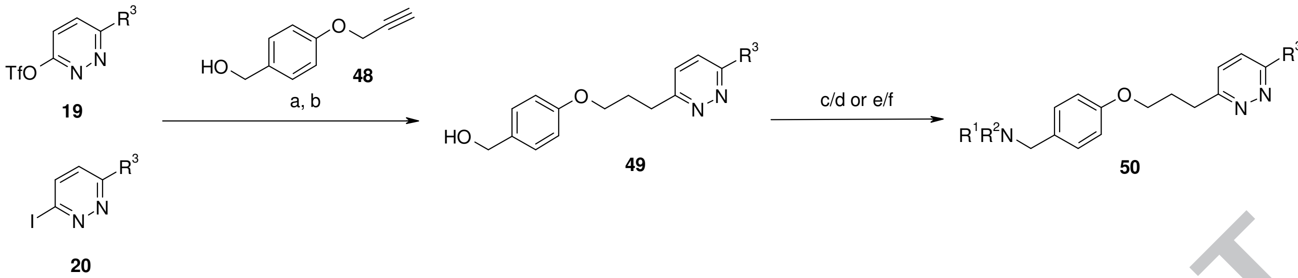
[a] For the description of biological methods, see reference 9. [b]Not tested.



Scheme 1. Reagents and conditions: (a) $Pd_2(dba)_3$, 2-(di-*tert*-butylphosphino)biphenyl, NaO*t*Bu, toluene, 80°C, 29%; (b) $NaCNBH_3$, formalin, HOAc, THF/CH_3CH, r.t., 49%; (c) TBTU, HOBt, NEt_3, DMF, r.t.; (d) CDT, THF, 0°C -> r.t., 63%, 90%; (e) H_2, Ra-Ni, EtOAc, r.t./50 psi, 27%;(f) Br_2, CH_2Cl_2, r.t., 92%; (g) KO*t*Bu, butanol, 40°C, 24%.

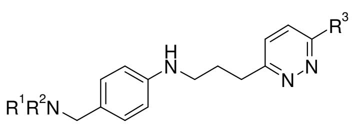


Scheme 2. Reagents and conditions: (a) *N*-Boc-propargylamine, $Pd(PPh_3)_2Cl_2$, CuI, HN*i*Pr_2, THF, -10°C -> r.t.; (b) H_2, Ra-Ni, EtOAc/EtOH, r.t./50 psi; (c) R^3-$B(OH)_2$, $Pd(PPh_3)_2Cl_2$, $NaHCO_3$, dioxane, 110°C; (d) phenol, K_2CO_3, CH_3CN, reflux; (e) TFA, CH_2Cl_2, r.t.; (f) **22**, $Pd_2(dba)_3$, 2-(di-*tert*-butylphosphino)biphenyl, NaO*t*Bu, dioxane, 80°C.



Scheme 3. Reagents and conditions: (a) **48**, $Pd(PPh_3)_2Cl_2$, CuI, HN*i*Pr_2, THF, -10°C -> r.t.; (b) H_2, Ra-Ni, THF/EtOH, r.t./50 psi; (c) MsCl, NEt_3, CH_2Cl_2, -10°C -> r.t.; (d) R^1R^2NH, NEt_3, THF or DMF, reflux; (e) MnO_2, CH_2Cl_2, r.t.; (f) R^1R^2NH, $NaBH(OAc)_3$, NaOAc, CH_2Cl_2, r.t..

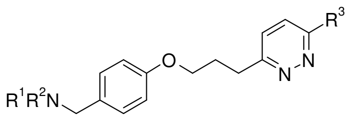
Table 2. SAR and optimization of 3-pyridazinyl-propylamine series toward increased polarity



	R^1R^2N	R^3	clogP	pKa	MCH-R1[a] IC_{50} [nM]	M1 IC_{50} [nM]	5-HT_{2A} IC_{50} [nM]	Phospholipidosis First effect conc. [µM]	hERG Patch Clamp Blockage
3 (see Table 1)			7.7	NT[b]	3	325	680	NT	76% @ 1µM
24	piperidin-1-yl	4-chloro-phenyl	5.3	NT	4	310	138	6.25	8% @ 1µM
25	piperidin-1-yl	4-fluoro-phenyl	4.7	NT	21	3737	34	NT	NT
26	piperidin-1-yl	4-methoxy-phenyl	4.6	9.0	7	962	1810	6.25	31% @ 1µM
27	piperidin-1-yl	4-cyano-phenyl	4.0	NT	151	1920	1460	NT	NT
28	piperidin-1-yl	4-pyridyl	3.2	NT	2530	NT	NT	NT	NT
29	piperidin-1-yl	methoxy	3.1	NT	>3000	NT	NT	NT	NT
30	pyrrolidin-1-yl	4-methoxy-phenyl	4.0	NT	95	1860	4540	NT	NT
31	dimethylamino	4-methoxy-phenyl	3.4	NT	154	1030	4580	NT	NT
32	morpholin-4-yl	4-methoxy-phenyl	3.3	7.6	20	>10000	>10000	12.5	5% @ 1µM
33	3-hydroxy-piperidin-1-yl	4-methoxy-phenyl	3.3	8.7	6	>10000	>10000	25	10% @ 1µM
34	4-hydroxy-piperidin-1-yl	4-methoxy-phenyl	2.5	8.6	20	>10000	8820	50	10% @ 1µM
35	4-acetylamino-piperidin-1-yl	4-methoxy-phenyl	2.2	8.8	10	>10000	>10000	100	7% @ 1µM
36	4-hydroxy-piperidin-1-yl	benzyloxy	2.8	NT	3	>10000	>10000	50	4% @ 1µM
37	4-hydroxy-piperidin-1-yl	phenoxy	2.4	9.1	24	>10000	>10000	50	35% @ 10µM
38	4-acetylamino-piperidin-1-yl	phenoxy	2.1	8.6	9	>10000	>10000	>100	IC_{50} >10 µM

[a] For the description of biological methods, see reference 9. [b]Not tested.

Table 3. SAR and optimization of 3-pyridazinyl-propylether series



	R^1R^2N	R^3	clogP	MCH-R1[a] IC_{50} [nM]	M1 IC_{50} [nM]	5-HT_{2A} IC_{50} [nM]	Phospholipidosis First effect conc. [µM]	hERG Patch Clamp Blockage
39	(*R*)-3-hydroxy-piperidin-1-yl	4-chloro-phenyl	4.4	15	5470	595	6.25	13% @ 1µM
40	4-hydroxymethyl-piperidin-1-yl	4-chloro-phenyl	4.2	4	1650	790	12.5	NT[b]
41	(*R*)-3-hydroxy-piperidin-1-yl	phenoxy	3.7	92	>10000	>10000	NT	NT
42	4-hydroxy-piperidin-1-yl	4-chloro-phenyl	3.6	13	>10000	1410	25	38% @ 1µM
43	4-hydroxymethyl-piperidin-1-yl	phenoxy	3.4	13	2110	>10000	100	8% @ 1µM
44	4-hydroxy-piperidin-1-yl	4-fluoro-phenyl	3.0	186	>10000	242	NT	NT
45	4-acetylamino-piperidin-1-yl	4-fluoro-phenyl	2.7	15	4470	713	100	2% @ 1µM
46	4-acetylamino-piperidin-1-yl	benzyl	2.5	22	>10000	>10000	100	13% @ 1µM
47	4-acetylamino-piperidin-1-yl	phenoxy	2.5	10	>10000	9650	100	13% @ 10µM

[a] For the description of biological methods, see reference 9. [b]Not tested.

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10. **Buchwald coupling reaction - representative procedure:** *N*-(1-{4-[3-(6-Phenoxy-pyridazin-3-yl)-propylamino]-benzyl}-piperidin-4-yl)-acetamide (Example **38**)
6.00 g (26.2 mmol) 3-(6-Phenoxy-pyridazin-3-yl)-propylamine and 8.09 g (26.0 mmol) *N*-[1-(4-Bromo-benzyl)-piperidin-4-yl]-acetamide are dissolved in 60 mL of dioxane and 390 mg (1.30 mmol) 2-(di-*tert*-butylphosphino)biphenyl, 715 mg (0.78 mmol) tris(dibenzylideneaceton)dipalladium(0) and 3.50 g (36.4 mmol) sodium *tert*-butoxide are added. The mixture is stirred for 2 hours at 50°C under argon atmosphere. After cooling, the mixture is poured onto 1N HCl/ice water. After neutralizing with ammonia, the mixture is extracted three times with ethyl acetate. The combined organic layers are dried over sodium sulfate. The solvent is removed and the residue is purified by column chromatography (aluminum oxide, act. 2-3, ethyl acetate/ethanol 20:1). Yield: 7.55 g (63% of theory), R_f value: 0.40 (silica gel, methylene chloride/methanol/ammonia = 9:1:0.1); ESI mass spectrum: m/z = 460 $[M+H]^+$; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.72 (d, J = 7.7 Hz, 1H), 7.68 (d, J = 9.0 Hz, 1H), 7.48 – 7.41 (m, 2H), 7.37 (d, J = 9.0 Hz, 1H), 7.29 – 7.22 (m, 1H), 7.21 – 7.16 (m, 2H), 6.95 (d, J = 8.5 Hz, 2H), 6.52 – 6.45 (m, 2H), 5.56 (t, J = 5.6 Hz, 1H), 3.49 – 3.44 (m, 1H), 3.03 (q, J = 6.6 Hz, 2H), 2.95 (t, J = 7.7 Hz, 2H), 2.75 – 2.64 (m, 2H), 2.50 (p, J = 1.9 Hz, 3H), 1.97 – 1.85 (m, 4H), 1.76 (s, 3H), 1.65 (dd, J = 12.8, 3.8 Hz, 2H), 1.35 – 1.27 (m, 2H).
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