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SYNTHESIS AND STRUCTURE–ACTIVITY RELATIONSHIPS FOR A SERIES OF SUBSTITUTED PYRROLIDINE NK_1/NK_2 RECEPTOR ANTAGONISTS

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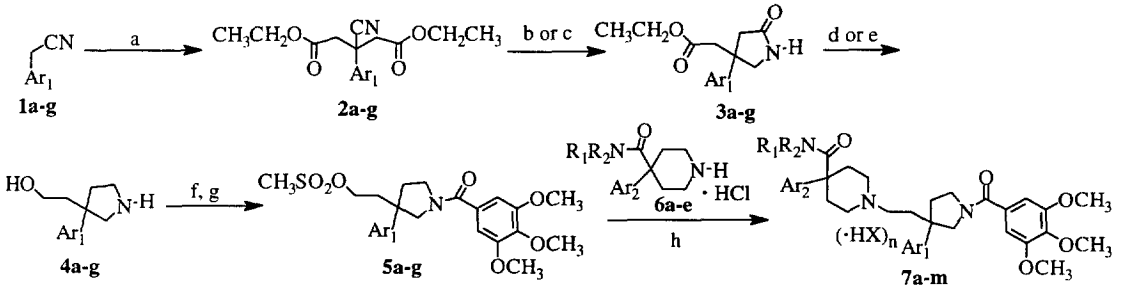
Abstract: We recently described the synthesis and characterization of MDL 105,212, a non peptide tachykinin antagonist with high affinity for NK_1 and NK_2 receptors.[1] Here we report the synthesis and structure–activity relationships for a series of analogs of MDL 105,212 with regards to: NK_1 and NK_2 receptor binding affinity, physical–chemical characterization; in vitro absorption potential; in vitro metabolic stability; and efficacy in a capsaicin-challenge conscious guinea pig model after oral administration. © 1997 Elsevier Science Ltd.

Substance P (SP) and neurokinin A (NKA) are peptide neurotransmitters that regulate vascular and bronchial tone, vascular permeability, mucus secretion, cell proliferation, and immune responses through action at their preferred receptors, NK_1 and NK_2, respectively.[2,3] Recognition of the similarities between tachykinin-mediated effects and asthmatic symptoms has led to the postulation that SP and NKA participate in neuro-inflammatory processes in the airways of asthmatics.[4] Based on the complementary nature of the airway effects of SP and NKA, and since both are released upon sensory nerve stimulation, we have designed, synthesized, and characterized a series of 1-[2-(pyrrolidin-3-yl)-ethyl]piperidines as part of our program directed toward development of dual NK_1/NK_2 receptor antagonists as potential therapeutic agents for the treatment of asthma.

Initially, we synthesized a series of racemic analogs to investigate the structure–activity relationships for this series with respect to receptor binding affinity. The information from these studies led us to synthesize a series of chiral analogs which were further characterized. The compounds were evaluated in terms of physical–chemical characteristics, then ranked according to absorption potential and metabolic stability relative to MDL 105,212. The compounds were then evaluated for their ability to inhibit the respiratory effects induced by capsaicin aerosol in conscious guinea pigs after oral administration.[5]

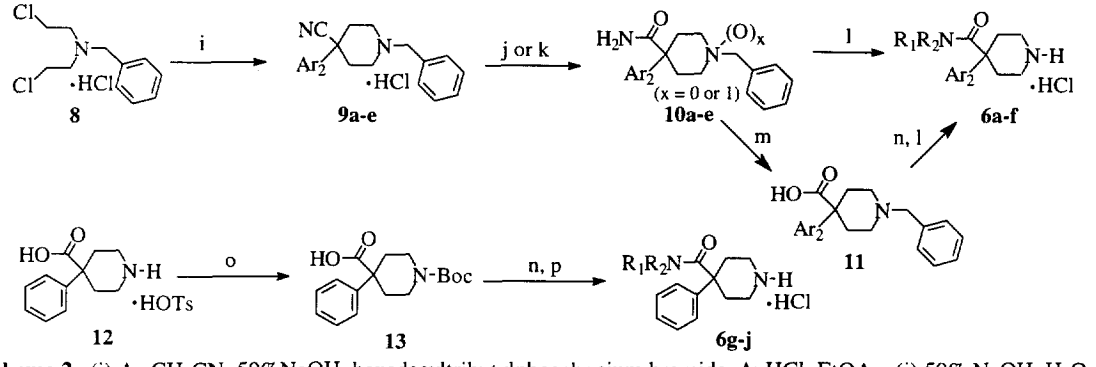


Scheme 1. (a) 2 equiv NaHMDS, 2 equiv ethyl bromoacetate; (b) Raney Ni, H_2, EtOH/NH_4OH; (c) $NaBH_4$, $CoCl_2 \cdot 6H_2O$, CH_3OH; (d) $LiAlH_4$, THF 50 °C; (e) AlH_3, THF, 50 °C; (f) 3,4,5-trimethoxybenzoyl chloride, $NaHCO_3$, acetone/H_2O; (g) methanesulfonyl chloride, DIEA, CH_2Cl_2; (h) K_2CO_3, THF/H_2O; HX.

The synthesis of the racemic analogs **7a–m** is outlined in Scheme 1. Aryl acetonitriles **1a–g** were treated with sodium bistrimethylsilylamide (2 equiv), and the resulting solutions were added to ethyl bromoacetate (2 equiv) in THF

to afford, after purification by crystallization or chromatography, **2a–g** in 58–95% yield. Nitriles **2a–g** were hydrogenated over Raney nickel (70–99% yield) or reduced with $CoCl_2/NaBH_4$ in methanol[6] to give lactam esters **3a–g** (32–85%yield). The Raney nickel method was more convenient in terms of work up. The lactam esters containing a halogenated Ar_1 moiety (**3a–e**) were reduced to the corresponding amino alcohols using AlH_3 (28–90% yield).[7] When Ar_1 was not substituted with halogen (**3f–g**) $LiAlH_4$ was used for the reduction (47–70% yield). The amino alcohols **4a–g** were acylated with 3,4,5-trimethoxybenzoyl chloride using Schotten–Baumann conditions (46–98% yield) and the resulting amide alcohols were converted to the methanesulfonates **5a–g** (95–99% yield). Nucleophilic displacement of the mesylates with the requisite substituted 4-arylpiperidine carboxamide hydrochloride **6a–e** provided after purification by chromatography and conversion to the salt (hydrochlorides **7a–l**, **n** and **o** or oxalate **7m**) the desired 1-[2-(pyrrolidin-3-yl)-ethyl]piperidines in 26–90% yield.

The synthesis of the substituted 4-arylpiperidine carboxamide hydrochlorides **6a–j** are outlined in Scheme 2. The primary carboxamide of 4-phenylpiperidine (**6a**) was prepared from commercially available N-benzyl-4-cyano-4-phenyl-piperidine hydrochloride (**9a**). The hydrochloride salt was neutralized, the free base was crystallized from heptane, and the nitrile was hydrolyzed with basic peroxide to afford the carboxamide N-oxide **10a** (x = 1) in 81% yield. Alternatively, the nitrile **9a** was hydrolyzed under acidic conditions to afford the primary carboxamide **10a** (x=0) in quantitative yield.[8] The N-oxide **10a** (x = 1) was reduced and the benzyl group was removed by catalytic hydrogenation over 5% Pd/C in acetic acid. The residue was treated with HCl in ethyl acetate to give the hydrochloride salt **6a** in 72% yield.



Scheme 2. (i) Ar_2CH_2CN, 50%NaOH, hexadecyltributylphosphonium bromide, Δ; HCl, EtOAc; (j) 50% NaOH, H_2O_2, EtOH, 50°C; (k) H_2SO_4, H_2O, 100°C; (l) H_2, 10%Pd/C; HCl, EtOAc; (m) HSO_4NO/H_2O; (n) R_1R_2NH, EDC, HOBt, DIEA, CH_2Cl_2; (o) $[Boc]_2O$, DIEA, DMF; (p) 4N HCl, dioxane.

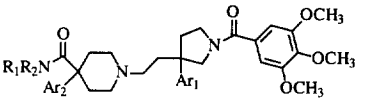
The 4-arylpiperidine carboxamides that are not commercially available were synthesized from the aryl acetonitriles and N-benzyl-bis(2-chloroethyl)amine **8**[9] to give **9b–e** in 33–93% yield using phase-transfer conditions.[10] The nitrile was hydrolyzed with basic hydrogen peroxide and the benzyl group was removed by hydrogenation over 5% Pd/C in ethanol or methanol.

The carboxamide analogs of the 4-phenylpiperidines were synthesized from 4-phenylpiperidine carboxylic acid *p*-toluenesulfonate salt **12**. The piperidine nitrogen was protected as the *tert*-butylcarbamate (84% yield) and the carboxylic acid **13** was coupled with the desired primary or secondary amine in the presence of EDC and HOBT. Chromatography and treatment with 4N HCl in dioxane gave the substituted 4-arylpiperidine carboxamide hydrochlorides **6g–j** in 55–85% yield.

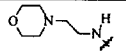
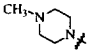
4-(Pyrid-3-yl)-piperidine **10d** (x = 0) was treated with nitrosyl sulfuric acid[11] (75% yield) to give the carboxylic acid **11** which was coupled with N-methylpiperazine using EDC/HOBT to afford the N-methylpiperazine carboxamide analog in 79% yield. The benzyl group was removed by catalytic hydrogenation over 5% Pd/C in methanol to give the piperidine **6f** (85% yield).

The NK_1/NK_2 receptor binding affinity data for the racemic analogs are summarized in Table 1. Previously, we described the design rationale that led to the 3,4,5-trimethoxyphenyl benzamide as the best benzamide in terms of dual NK_1/NK_2 receptor binding affinity for this series.[1] Several aryl-3-yl-substituted pyrrolidine analogs (**7a–f**) had good affinity for the NK_1 receptor; however, the 3,4-dichlorophenyl substituent Ar_1 of the pyrrolidine (**7d**) was found to be optimal in terms of dual receptor affinity. A secondary or tertiary carboxamide moiety at the 4-position of the piperidine was found to improve NK_2 receptor binding affinity compared to the primary carboxamide (**7h–k** compared to **7d**). Modifications of Ar_2 (**7l–o**) had less of an effect in terms of receptor binding affinity; however, incorporation of the pyridyl moiety (**7n–o**) at this position was found to effect other properties, vide infra.

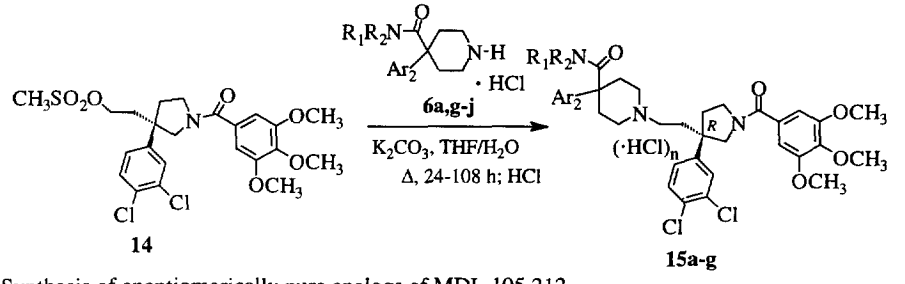
Table 1. NK_1 and NK_2 receptor binding affinity for racemic analogs of MDL 105,212[12]



7a-o

Compound	R_1R_2N	Ar_1	Ar_2	NK_1 IC_{50} (nM)	NK_2 IC_{50} (nM)
7a	H_2N	3-chlorophenyl	phenyl	10.7	190
7b	H_2N	4-chlorophenyl	phenyl	8.62	57.9
7c	H_2N	4-fluorophenyl	phenyl	23.3	235
7d	H_2N	3,4-dichlorophenyl	phenyl	5.88	21.0
7e	H_2N	3,4-difluorophenyl	phenyl	9.76	74.5
7f	H_2N	3,4-dimethoxyphenyl	phenyl	36.7	2,770
7g	H_2N	3-pyridyl	phenyl	567	>10,000
7h		3,4-dichlorophenyl	phenyl	7.53	11.8
7I		3,4-dichlorophenyl	phenyl	23.2	9.39
7j		3,4-dichlorophenyl	phenyl	8.72	3.64
7k		3,4-dichlorophenyl	phenyl	4.51	3.13
7l	H_2N	3,4-dichlorophenyl	3-methoxyphenyl	8.49	17.1
7m	H_2N	3,4-dichlorophenyl	4-fluorophenyl	13.5	12.0
7n	H_2N	3,4-dichlorophenyl	3-pyridyl	8.14	16.2
7o	H_2N	3,4-dichlorophenyl	4-pyridyl	4.88	16.4

Based on the receptor binding affinity for the racemic analogs, several compounds were selected for synthesis in optically pure form. The piperidines **6a, g–j** were allowed to react with the previously reported mesylate **14**[1] to afford the enantiomerically pure products (**15a–g**) after purification and salt formation in 41–90% yield (Scheme 3).



Scheme 3. Synthesis of enantiomerically pure analogs of MDL 105,212.

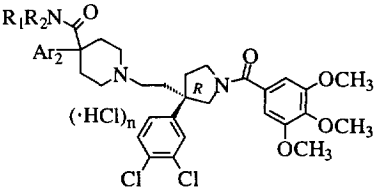
The compounds in Table 2 (**15a–f**) have high binding affinity for NK_1 and NK_2 receptors. The compounds that contain a second or third basic nitrogen (**15b,c,e,f**) were found to have increased solubility (6 to 17–fold) in pH 7.4 phosphate buffer compared to MDL 105,212A (**15a**). The absorption potential with Caco-2 cells correlated with the clogP for this series of analogs (r^2=0.74). The in vitro metabolic stability with rat liver (10S) fraction was slightly improved for analogs **15b–f** compared to MDL 105,212A (**15a**). While improvements in in vitro absorption and metabolic stability were made, these effects did not translate to improved *in vivo* efficacy and duration of action (results not shown) in the capsaicin challenge guinea pig model. For example, **15d** and **15e** were predicted to be absorbed better than **15a** (3.7 and 2.1, respectively); however, they inhibited the capsaicin-induced respiratory effects by 39% and 31%, respectively, compared to 60% for **15a**.[13]

In summary, a versatile synthetic route for the preparation of substituted 1-[2-(pyrrolidin-3-yl)-ethyl]piperidines has been developed. Analysis of the NK_1/NK_2 receptor binding affinity for a series of racemic analogs of MDL 105,212 revealed that the 3,4-dichlorophenylpyrrolidine moiety was optimal for dual affinity. Incorporation of other piperidines containing different carboxamide and 4-aryl substitutents were found to have increased aqueous solubility, and improved metabolic stability. The absorption potential as predicted from in vitro studies with Caco-2 cells did not correlate with the *in vivo* activity after oral administration using HPβCD as vehicle.[14]

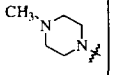
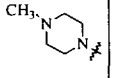
Acknowledgments

The authors wish to thank Dr. A. A. Carr for helpful discussions during the course of this work and R.J. Barbuch, D. J. Robke, Dr. E. Huber and Dr. D. Friedrich for providing spectral and analytical data used in the structural characterization of the compounds described in this manuscript.

Table 2. Characterization of MDL 105,212 and analogs.



15a-g

MDL	R_1R_2N	Ar_2	NK_1 IC_{50} (nM)[8]	NK_2 IC_{50} (nM)[8]	pKa_1; pKa_2; pKa_3; clogP; logD[15]	solubility (μg/mL)[16]	Relative Permeability[17]	Relative Metabolic Stability[18]	% inhibition at 10 mg/kg, p.o.[19]
105,212 (A) **15a**	H_2N	phenyl	3.11	8.40	7.64; NA; NA; 3.4; 3.02	70	1	1	60%
15b	H_2N	3-pyridyl	4.01	7.77	7.60; 3.97; NA; 1.9; 2.59	548	0.1	0.72	38%
15c	H_2N	4-pyridyl	2.49	10.2	7.53; 4.14; NA; 1.9; 2.64	408	0.1	0.78	20%
15d		phenyl	4.34	2.05	7.51; NA; NA; 4.3; 3.02	25	3.7	0.77	39%
15e		phenyl	3.94	2.19	7.92; 6.86; NA; 4.7; 3.71	426	2.1	0.43	31%
15f		3-pyridyl	2.46	4.51	11.41; 7.76; 6.71; 3.2; 2.41	1200	1.2	ND	13%

NA = not applicable, ND = not determined

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12. NK_1 IC_{50} determined using [^{125}I]-Bolton Hunter labeled SP and NK_1 receptors from guinea pig lung. NK_2 IC_{50} determined using [^{125}I]-Iodohistidyl NKA and NK_2 receptors in HSKR-1 cells. Each value is the mean of at least 3 determinations. Receptor binding affinity has been determined by the experimental methods previously described (Kudlacz, E. M., Logan, D. E., Shatzer, S. A., Farrell, A. M., Baugh, L. E. *Eur. J. Pharm.* **1993**, *241*, 17).

13. The oral bioavailability of MDL 105,212 in the guinea pig with 40% HPβCD as vehicle was found to be 46%.

14. Other analogs that permeate Caco-2 cells better and show improved efficacy after oral administration, compared to MDL 105,212, will be reported in due course.

15. We thank C. M. Berger, (pION Inc.) for determining the pK_a and log D (log P, pH 7.4) values for these compounds using a Sirius PCA101 instrument equipped with an Orion Ross™ semimicro pH electrode, which was standardized daily. The pK_a was determined by titrating the compound in an aqueous solution (ISA). If the compound did not dissolve in ISA water (*15a* and *15d*), an organic co-solvent was added and the apparent pK_a determined using the Yasuda–Shedlovsky procedure (*Anal. Chem.* **1993**, *65*, 42). A minimum of three apparent pK_as were collected and extrapolated to the zero-co-solvent aqueous pK_a value. The log D was determined by titrating the compound in the presence of octanol and water (ISA). The apparent pK_a was obtained and compared to the aqueous pK_a, from which the log D was determined.

16. The solubility of MDL 105,212 analogs were determined in a 50 μM phosphate buffer solution at pH 7.4. Saturated solutions were obtained by sonicating the compounds in buffer for five min and then filtering through a Millex HV13 filter (Millipore). The concentrations of the compounds in the filtrate were quantified by HPLC, utilizing peak areas in comparison to an external standard.

17. The Caco-2 cells were cultured in supplemented Dulbecco's modified eagle medium with 10% fetal bovine serum and seeded onto polycarbonate membranes for test compound transport studies. The Caco-2 cell seeded membranes were grown and maintained for 21 to 30 days for growth curves and transport experiments. Caco-2 cells were maintained on Snapwell® supports and kept in an incubator at 37 °C with 5% CO_2/95%O_2 and approximately 95% humidity. For transport experiments apical samplings were taken at 0, 15, 30, 45, 60, 90, 120, 180, and 240 min after addition of test compound. MDL 105,212 analogs were snap frozen on dry ice/methanol and analyzed by HPLC. Phenytoin and Mannitol were evaluated as positive and negative controls and they permeated 8.5 times and 0.2 times as much as MDL 105,212A, respectively.

18. Each test substrate was incubated at an initial concentration of approximately 10 μM with rat liver 10S fortified with NADPH. The liver 10S was a pooled preparation so that each analog was incubated with the same preparation to factor out any intersample variability. The incubation took place for 60 min. The rate of disappearance of the test substrates was calculated from the slope of its disappearance curve over the initial linear portion of that curve. Metabolic stability was assessed by comparing the rates of each individual analog; compounds with the lowest rates are predicted to have the greatest metabolic stability.

19. This value is the average of the % inhibition of the capsaicin induced respiratory effects, as measured by the number of significant respiratory events (coughs and gasps), maximum pressure, and final pressure, from studies with conscious guinea pigs in a whole body plethysmograph one hour after oral administration of 10 mg/kg of test compound in HPβCD (see ref 1c for further details using this model with MDL 105,212).

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