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Identification of 3-substituted *N*-benzhydryl-nortropane analogs as nociceptin receptor ligands for the management of cough and anxiety

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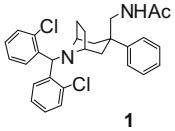
ABSTRACT

A series of nortropane analogs based on previously reported compound **1** have been synthesized and shown to bind to the nociceptin receptor with high affinity. The synthesis and structure–activity relationships around the C-3 nortropane substitution are described. From the SAR study and hPXR screening effort, compound **15** was identified to possess potent oral antitussive and anxiolytic-like activities in the guinea pig models.

The nociceptin receptor (NOP, ORL-1), first discovered in 1994, displays low binding affinity for the classical opioid ligands, albeit its sequence has ~50% homology to those of the opioid receptor family μ, κ, and δ (or MOP, KOP, and DOP, respectively).[1] NOP is widely distributed throughout the brain and spinal cord and thus is expected to participate in various physiological processes. Following the discovery of NOP, there has been remarkable advance toward understanding its pharmacological significance. The NOP endogenous ligand (nociceptin)[2] does not interact with the other opioid receptors, and has been reported to mediate various physiological processes, for instance, pain, cough, anxiety, cognition, feeding, sleep, substance abuse and urinary incontinence.[3] Thus, selective NOP agonists or antagonists might have clinical potential for the treatment of related diseases.

Nociceptin has been shown to display antitussive activity in the guinea pig model through peripheral (IV) or central (ICV) administration.[4] Thus, selective NOP agonists provide a novel therapeutic approach for the management of cough.



1

NOP Ki: 20 nM
NOP (GTPγS) EC_{50}: 269 nM
Antitussive activity:
ED_{50}: 0.19 mg/kg at 2 h

Genetic and pharmacological studies indicate that NOP modulates the anxiety response.[5,6] It was found that local (ICV) administration of nociceptin neuropeptide or IP administration of NOP agonist Ro64-6198 led to anxiolytic-like activity in rats.[6,7] Recently, NOP agonist SCH 221510, a nortropane analog structurally distinct from nociceptin and Ro64-6198, was disclosed to possess the oral anxiolytic-like activity in various animal models including the rat conditioned lick suppression (CLS) model and the separation-induced guinea pig pup vocalization (GPPV) model.[8] The reported data for SCH 221510 suggested that NOP agonists have the potential to be developed as a novel class of anxiolytic agents.

In our nociceptin agonist program, we have reported structure–activity relationships (SAR) based on the 4-hydroxy-4-phenyl piperidinyl scaffold.[9] Further SAR development was focused on the conformationally-restricted nortropane scaffold. Compound **1** was identified previously as a potent NOP agonist with potent in vivo antitussive activity in the guinea pig model from a series of 3-axial-aminomethyl-*N*-benzhydryl-nortropane analogs.[10] In this paper, we describe our continuing efforts to explore SAR at the C-3 position to modulate the binding affinity and selectivity profile (e.g., human pregnane X receptor (hPXR)). Synthesis and SAR of a new 3-axial-substituted-*N*-benzhydryl-nortropane series including carboxylamide, amine, and carbamate series are disclosed along with our strategy to identify potent dual antitussive and axiolytic agents.

The synthetic route to the C-3-α-substituted nitrile is summarized in Scheme 1. The commercially available tropinone (**2**) was

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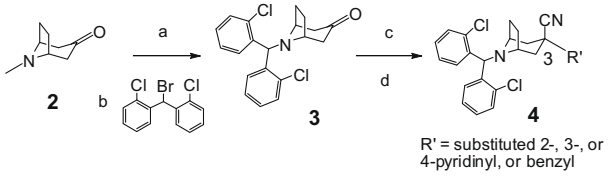
de-methylated and subsequently alkylated using previously established procedure to afford the ketone intermediate (**3**).[9,10] Transformation of **3** to the nitrile and subsequent nucleophilic addition of a benzyl or pyridyl group through the less hindered α face was achieved using the corresponding benzyl halides or pyridyl halides under the basic condition to give **4**.[10] The C-3 stereochemistry was confirmed with the NOE experiments of the hydrolyzed products (**5**). The NOE correlations were observed between the amide protons and the protons on the ethylene bridge in the NOESY experiment, confirming the β position of the amide group. The detail NMR and high-resolution mass data of one example of **5** are presented in the reference.[11]

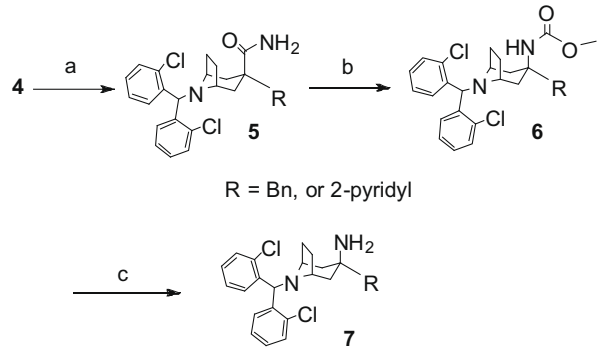
Further transformation of the C-3 nitrile (**4**) to the carboxylamide, carbamate, and amino functional groups is detailed in Scheme 2. Thus, the nitriles (**4**) were hydrolyzed to the carboxylamides (**5**) with concd H_2SO_4, and then rearranged through Hofmann rearrangement to afford the corresponding methyl carbamates (**6**) using bis(acetoxy)iodobenzene and KOH/MeOH. The 3-amino analogs (**7**) were obtained by removal of the $-CO_2Me$ group with TMSI in dichloromethane and then in MeOH under reflux.

The N-substituted analogs were synthesized as described in Scheme 3. Acetylation and methylation of **7** gave **8** and **9**, respectively.

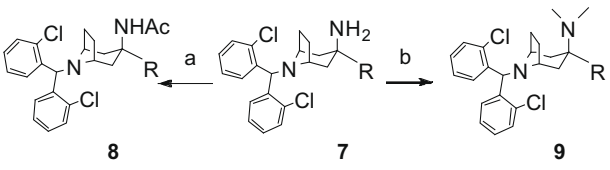
To prepare the amide homolog (**13**), a five-step synthesis was designed as shown in Scheme 4. The nitrile **4** was reduced to the aldehyde intermediate using DIBAL, and further reduced to the alcohol (**11**) with $NaBH_4$. Mesylation of the alcohol and the subsequent replacement with a cyano group gave nitrile **12**. Finally, the nitrile (**12**) was hydrolyzed to the carboxylamide (**13**) under the same acidic condition described above.



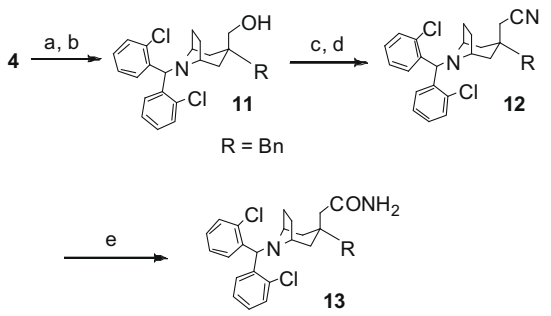
Scheme 1. Reagents and conditions: (a) α-chloroethyl chloroformate, DCE, reflux; then MeOH, reflux; (b) **2**, K_2CO_3, CH_3CN, reflux; (c) KO–*t*Bu, tosylmethyl isocyanide, DME, −40 °C to rt; (d) NaHMDS, RX, THF, −78 °C to rt.



Scheme 2. Reagents and conditions: (a) concd H_2SO_4, neat, rt; (b) $PhI(OAc)_2$, KOH, MeOH, 0 °C to rt; (c) TMSI, DCM, reflux, then MeOH reflux.



Scheme 3. Reagents and conditions: (a) Ac_2O, pyridine, rt; (b) HCHO, HCO_2H, rt; $NaBH(OAc)_3$, DCE, rt.



Scheme 4. Reagents and conditions: (a) DIBAL in toluene, 0 °C; (b) $NaBH_4$, MeOH, 0 °C; (c) MsCl, Et_3N, DCM, 0 °C; (d) KCN, 18-crown-6, DMF, 110 °C; (e) concd H_2SO_4, neat, rt.

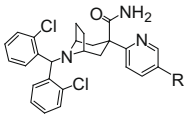
Target compounds were tested for their affinity at the cloned human nociceptin receptor expressed in CHO cell membranes by measuring their ability to compete with [^{125}I][Tyr^{14}]nociceptin. The opioid receptor binding assays were performed with CHO cell membranes expressing the human opioid receptors using [^{3}H]-diprenorphine as the radioligand. The functional activities of selected compounds were evaluated as their ability to enhance the binding of [^{35}S]GTPγS in the presence of GDP, using membranes isolated from CHO cells transfected with the human nociceptin gene. Since most of the nortropane analogs identified previously showed good selectivity over DOP and KOP,[10] only selectivity over MOP will be presented. To limit the number of the compounds for further evaluation, the compounds with the NOP K_i less than 15 nM and selectivity (NOP vs MOP) higher than ~10-fold were selected.

The C-3 2-pyridyl carboxylamide **14** showed potent NOP binding affinity with a K_i of 4 nM. Thus a series of the substituted pyridyl analogs were prepared. Introduction of a small substituent (e.g., F, Cl, Me, or OMe) at the 5 position of the pyridine ring slightly reduced NOP affinity (**15–19,** K_i between 6 and 22 nM, Table 1). Substitutions of the polar groups (OH or NH_2), cyano, or relatively large groups (e.g., Br, CF_3 or $CONH_2$) at the 5-position of the pyridine ring were found to decrease the NOP binding affinity significantly (**17**, **20–25**). The binding selectivity of this series of compounds for the NOP receptor versus MOP is around 4–17-fold.

The promising result of the 5-fluoro analog (**15**) triggered additional studies on the fluoro-substituted pyridyl analogs. Moving the fluoro atom from the 5-position to the 6-position retained NOP affinity and improved selectivity over MOP (**26**, 35-fold). Most of the di-fluoro compounds (**27–31**) showed slightly reduced NOP affinity (K_i 9–19 nM), and were selected for further hPXR evaluation. A dramatic loss of NOP affinity was observed while carboxylic acid was introduced to the 4-position of the pyridine ring (**32**). In general the polar or large substitutions were not preferred at the 4- or 5-position of the 2-pyridine ring (Table 2).

To further explore the C-3 axial substitution, the C-3 nitrogen directly-attached analogs were prepared. The binding affinity data of these analogs are listed in Table 3. The C-3 unsubstituted amino analogs **33** and **34** exhibited potent NOP affinity (K_i 10 nM; 7 nM, respectively) and decent selectivity over MOP (32-fold). The acetyl analogs (**35** and **36**) of **33** and **34** retained NOP affinity (K_i 6 and 10 nM) and increased selectivity over MOP to 52- and 60-fold, respectively. The methyl carbamates (**37–39**) displayed similar NOP affinity (K_i 10–12 nM) and improved selectivity (74–80-fold) over MOP. However, the *N,N*-di-methyl substitution resulted in

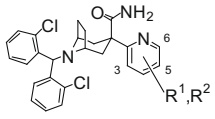
Table 1
SAR of the C-3 substituted 2-pyridyl analogs



Compds	R	NOP K_i (nM)	MOP K_i (nM)
14	H	4	52
15	F	7	123
16	Cl	15	143
17	Br	29	153
18	Me	6	26
19	OMe	22	133
20	NH_2	31	205
21	OH	72	nd
22	CN	75	481
23	CF_3	122	nd
24	$CONH_2$	401	1315
25	CO_2Me	>1000	nd

nd: not determined.

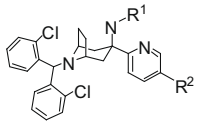
Table 2
SAR of the C-3 substituted 2-pyridyl analogs (continued)



Compds	R^1, R^2	NOP K_i (nM)	MOP K_i (nM)
26	6-F	10	348
27	4-F, 6-F	15	468
28	3-F, 6-F	16	nd
29	3-F, 5-F	47	nd
30	4-F, 5-F	9	74
31	4-CH_2F, 5-F	19	367
32	4-CO_2H, 5-F	>1000	nd

nd: not determined.

Table 3
SAR of the C-3 2-pyridyl C-3 amino analogs

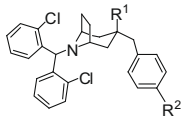


Compds	R^1	R^2	NOP K_i (nM)	MOP K_i (nM)
33	H	H	10	343
34	H	F	7	209
35	Ac	H	6	312
36	Ac	F	10	601
37	CO_2Me	H	10	741
38	CO_2Me	F	12	887
39	CO_2Me	Cl	12	961
40	Me, Me	H	42	840

the diminished NOP affinity (**40**, 42 nM). Overall, the amino, *N*–Ac and *N*–CO_2Me groups were all tolerated at the C-3 axial position for NOP affinity with improved selectivity over MOP, compared to the carboxylamide series (Table 1).

The C-3 benzyl analogs were synthesized to compare to the pyridyl series. The SAR data of the C-3 benzyl analogs are listed in Table 4. The *para*-flouro (**41**) or *para*-chloro (**42**) benzyl substitution at the C-3 equatorial position was tolerated for NOP affinity (K_i 3 and 10 nM, respectively) and led to improved selectivity over MOP (~28-fold), compared to the pyridyl analogs **15** and **16**. Compound **43** with an additional carbon extension on the C-3 carboxylamide retained potent NOP affinity (K_i 3 nM) and enhanced selectivity over MOP (~63-fold), compared to **41**. Replacement of the fluoro with a polar hydroxyl group (**44**) at the *para* position resulted in a sixfold decrease in the NOP binding affinity. Replacement of the C-3 carboxylamide with an amino (**45**) or methyl carbamate (**46**) group led to the diminished NOP binding affinity (K_i 36 and 56 nM, respectively).

Table 4
SAR of the C-3 benzyl substituted analogs



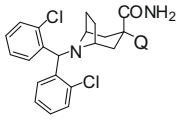
Compds	R^1	R^2	NOP K_i (nM)	MOP K_i (nM)
41	$CONH_2$	F	3	85
42	$CONH_2$	Cl	10	284
43	CH_2CONH_2	F	3	190
44	CH_2CONH_2	OH	17	nd
45	NH_2	Cl	36	447
46	$NHCO_2Me$	Cl	56	nd

nd: not determined.

The 3- and 4-pyridyl analogs (**47**–**49**) were evaluated for NOP and MOP affinities. The 3-pyridyl analogs (**47** and **49**) retained NOP affinity (Table 5) and selectivity over MOP, whereas the 4-pyridyl analog (**48**) showed a 20-fold decrease in NOP affinity, compared to 2-pyridyl **14**. The racemic C-3 piperidinyl carboxylamide (**50**), prepared from reduction of **14** with PtO_2 and H_2, showed a fourfold decrease in NOP affinity, when compared to **14**.

The functional activity (EC_{50}) was evaluated for selected compounds using the GTPγS assay. The data listed in Table 6 showed the NOP and MOP functional activities (EC_{50}) and percent stimulation at certain concentration of compounds **15–17**, **35**, **37**, **41**, and **46**. The data demonstrated their full NOP agonist activity with the EC_{50s} ranging from 11 to 87 nM and >75% stimulation at the high concentration in the NOP GTPγS assay. Most of the compounds (**15**, **35**, **37**, **41**, and **46**) showed medium to high selectivity over MOP, except compounds **16** and **17** with selectivity equal or less than 10-fold over MOP.

Table 5
SAR of the additional C-3 heterocyclic analogs



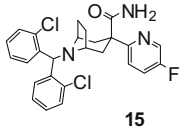
Compds	Q	NOP K_i (nM)	MOP K_i (nM)
47		3	71
48		79	265
49		13	46
50		17	474

Table 6
The functional activity of selected compounds

Compds	GTPγS NOP, EC_{50} (nM)	GTPγS % Stim (μM)	GTPγS MOP EC_{50} (nM)	GTPγS % Stim (μM)
15	20	95 (10)	733	89 (5)
16	44	145 (10)	368	97 (10)
17	63	75 (10)	421	89 (10)
41	11	108 (10)	540	107 (10)
46	14	85 (5.5)	1563	59 (5.5)
35	19	84 (10)	1933	68 (10)
37	87	92 (10)	3777	52 (10)

Selected compounds were evaluated for the potential of Cyp3A4 induction using the hPXR reporter-gene assay.[12] The induction level was compared to the positive control rifampicin (RIF), and the ratio of 0.4 was set to be the screening cut-off to minimize the possibility of false-positive results. Compound **1**, identified previously, showed a high hPXR ratio (0.94) to RIF at 1 μM (Table 7). From this study it was found that substitution on the 5-pyridyl position (**15**–**19**) reduced the hPXR liability, compared to the unsubstituted pyridyl analog (**14**). In the benzyl series, most tested compounds in Table 4 displayed some liability for hPXR, except **43** (ratio to RIF 0.35), indicating the α-benzyl group (e.g., **41**, **42**) could lead to higher hPXR ratio to RIF, compared to the pyridyl analogs (**15**, **16**). In the C-3 nitrogen directly-attached series, compounds **35**–**39** and **46** showed high hPXR ratio (0.98–1.97) to RIF. Therefore the terminal acetamide and methyl carbamate groups were identified as strong inducers for Cyp3A4 through the hPXR reporter gene pathway in the pyridyl series.

Selected compounds which met the above criteria were investigated in vivo.[4,8] The GPPV model was used as a screening tool. Compounds **15** and **16** displayed an oral anxiolytic-like activity with ED_{50s} < 3 mg/kg in the GPPV screening assay. Further dose-response studies determined both of their ED_{50} values as 0.3 mg/kg, compared to the reference compound chlordiazepoxide (CDP) (ED_{50} 3.2 mg/kg). In the rat CLS model, **15** and **16** exhibited the anxiolytic-like activity with ED_{50} values of 1.7 and 2.4 mg/kg, respectively, compared to the reference compound CDP (ED_{50} 8.8 mg/kg). Compound **15** also displayed potent oral antitussive activity in the capsaicin-induced guinea pig model with an ED_{50} of 0.02 mg/kg, at 2 h, compared to the reference compound codeine (ED_{50} 6.7 mg/kg). The acetamide analog **43** only displayed ~38% cough reduction at 0.3 mg/kg, and the amino analogs **33** and **34** did not show any antitussive activity at the low dose (0.3 mg/kg). Compound **43** did not display anxiolytic-like activity in the GPPV model at a dose of 3 mg/kg.



15

NOP Ki: 7 nM
NOP (GTPγS) EC_{50}: 20 nM
MOP Ki: 123 nM
MOP (GTPγS) EC_{50}: 733 nM
DOP: Ki: 3347 nM
KOP: Ki: 1610 nM
Antitussive activity:
ED_{50}: 0.02 mg/kg at 2h
GPPV: ED_{50}: 0.3 mg/kg
CLS: ED_{50}: 1.7 mg/kg

From these SAR studies and screening efforts, compound **15** was found to possess potent dual antitussive and anxiolytic-like activities with the best overall profile. It displayed high binding selectivity for the NOP receptor versus DOP and KOP, and reasonable selectivity over MOP in the functional assay (~37-fold). Compound **15** also exhibited improved hPXR data (ratio 0.29 to RIF) compared to the previously identified lead compound **1** (ratio 0.94 to RIF). Compound **15** displayed stronger efficacy in the antitussive assay (ED_{50} 0.02 mg/kg) than that in the anxiolytic-like GPPV assay (ED_{50} 0.3 mg/kg). The antitussive/anxiolytic-like efficacy ratio was ~15.

In summary the C-3 2-pyridyl carboxylamide (**14**) displayed potent NOP affinity. However, compound **14** showed high hPXR ratio to RIF. Substitution at the 5-position of the pyridine reduced the hPXR liability and led to the discovery of **15**. The C-3 amine, acetamide, and methyl carbamate analogs in the pyridyl series (**33**–**39**) showed good binding and selectivity profiles, whereas they suffered from the hPXR-induction liability. The benzyl series with the C-3 carboxylamide (**41**, **42**) or acetamide (**43**) displayed good binding profile, but again showed positive response in the hPXR assay. The 3-pyridyl analog **47** displayed good affinity profile, however, the hPXR ratio (0.40) was close to the cut-off value, and this agonist was not further evaluated. In conclusion, among those potent NOP agonists identified, compound **15** demonstrated the best overall profile and superior in vivo activity. It exhibited potent oral antitussive and anxiolytic-like activities in the animal models, superior to the standard compounds codeine and chlodiazepoxide, without having the hPXR liability.

Table 7
The hPXR data for selected compounds

Compd	hPXR ratio[a] (1 μM)
1	0.94
14	0.79
15	**0.29**
16	0.27
17	**0.09**
18	0.40
19	**0.19**
26	1.06
27	0.55
28	0.83
30	**0.18**
31	0.77
33	0.47
34	**0.23**
35	1.97
36	1.47
37	0.98
38	1.38
39	1.06
41	0.48
42	0.67
43	**0.35**
44	0.51
46	1.10
47	0.40

[a] Fold of induction relative to rifampicin at 1 μM; the data which met criteria was bold-faced.

Acknowledgments

The authors wish to acknowledge Dr. Jianshe Kong, Dr. Jesse Wong, and Mr. Meng Tao for preparation of intermediates; Dr. Tze-Ming Chan and his group for structure confirmation of some analogs and determination of the C-3 stereochemistry; Drs. Xiaoying Xu, Xiaoming Cui, and DMPK group for acquiring hPXR and pharmacokinetic data.

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11. *Compound* **15**: ^{1}H NMR ($CDCl_3$): δ 1.90 (dd, J = 14.4, 7.0 Hz, 2H), 2.09 (br s, 2H), 2.19 (dd, J = 13.7, 2.7 Hz, 2H), 2.77 (br d, J = 12.3 Hz, 2H), 3.08 (br s, 2H), 5.37 (br s, 1H, –N*H*), 5.42 (s, 1H), 6.15 (br s, 1H, –N*H*), 7.07 (td, J = 7.8, 1.8 Hz, 2H), 7.15 (t, J = 7.6 Hz, 2H), 7.26 (m, 4H), 7.69 (d, J = 7.7 Hz, 2H), 8.31 (d, J = 2.6 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 25.9, 40.3, 48.8, 57.2, 59.4, 121.7 (J = 3.8 Hz), 123.3 (J = 18.2 Hz), 126.6, 127.9, 129.4, 130.1, 134.3, 136.2 (J = 22.8 Hz), 139.6, 158.0 (J = 253 Hz), 160.9, and 176.7. HRMS (ESI) calcd for $C_{26}H_{25}Cl_2N_3OF$, 484.1359 ($M + H^+$); found, 484.1370.
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