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SYNTHESIS OF NOVEL ANALOGUES OF THE DELTA OPIOID LIGAND SNC-80 USING REM RESIN

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Abstract: Focused libraries of delta opioid ligands were synthesised using REM resin methodology. Several high affinity compounds were identified with good selectivity over the μ opioid receptor. Automated REM resin recycling was used to synthesise larger amounts of ligand for *in vivo* studies.

Introduction

Most currently used μ-opioid analgesic drugs, such as fentanyl and its analogues, exhibit a range of serious side effects including respiratory depression, dependence and muscle rigidity. Extensive studies over the past decade have shown that δ-opioid receptor (DOR) agonists produce antinociception in animal models of pain whilst appearing to have a different side effect profile from that of μ opioid receptor (MOR) agonists.[1] For this reason the DOR is an attractive target for the design of novel opioid analgesics. One of the first non-peptide agonists to show good affinity and selectivity at the DOR, SNC-80 **1**, was reported by chemists at Burroughs-Wellcome (Table 1).[2] REM resin methodology[3] is a traceless linker methodology developed in our laboratories, which allows the synthesis of tertiary amines under mild reaction conditions in good yield and purity. We would now like to report the first application of this methodology to a drug discovery project, highlighting its utility for rapidly synthesising libraries of δ opioid ligands.

In our initial SAR study around SNC-80 (IC_{50} 2.2nM), we found that neither the methoxy group nor the two methyl groups on the piperazine were essential for high affinity at the DOR or selectivity over the MOR. Compound **2**, lacking the methoxy group, was found to be slightly more active (IC_{50} 1.1nM) than the parent structure. This observation is consistent with a recent report by Calderon *et al*, in which the des-methoxy compound was also found to be more active than SNC-80.[4] The compound lacking the two methyl groups **3** was about three fold less active (IC_{50} 7.4nM) than SNC-80, whereas compound **4**, lacking both methoxy and methyl groups was two fold less active (IC_{50} 4.1nM). The study also showed that, in terms of affinity at the DOR, the diethylamide group is a key structural feature. Compound **5**, lacking the amide and methoxy groups, was more than 400 fold less active (IC_{50} 420nM) than **2**. Although essential, the precise role of the amide group in the binding of SNC-80 to the DOR is unclear. It appears that the carbonyl oxygen is probably not acting as a hydrogen bond acceptor, given the high affinity (IC_{50} 5.3nM) of the thioamide analogue **6**. Both ester and carboxylic acid-containing analogues, **7** (IC_{50} 240nM) and **8** (IC_{50} 450nM) respectively, were much less active. In contrast, the 2-pyridyl analogue **9** shows better affinity (IC_{50} 22nM) suggesting that the amide could be mimicked by a heterocyclic ring.

Chemistry

In the light of the critical importance of substituent R^1, we decided to synthesise two optimisation libraries using REM resin methodology, containing diversity at this position. The first library contained a

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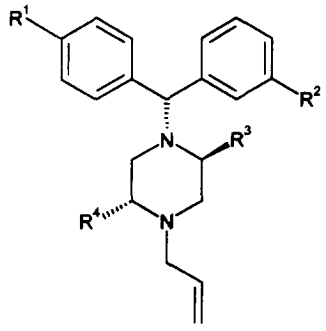
broad range of amides (110) and a small number of esters (10) at R^1. In view of the reasonable affinity of **9**, a second library containing a number of heterocyclic ring systems at R^1, was prepared. Since the methoxy and methyl groups do not contribute significantly towards affinity, these functionalities were excluded from the library compounds.



1 : $R^1 = CONEt_2$; R^2 = OMe, R^3 = Me, R^4 = Me
2 : $R^1 = CONEt_2$; R^2 = H, R^3 = Me, R^4 = Me
3 : $R^1 = CONEt_2$; R^2 = OMe, R^3 = H, R^4 = H
4 : $R^1 = CONEt_2$; R^2 = H, R^3 = H, R^4 = H
5 : R^1 = H; R^2 = H, R^3 = Me, R^4 = Me
6 : $R^1 = CSNEt_2$; R^2 = OMe, R^3 = Me, R^4 = Me
7 : $R^1 = CO_2Me$; R^2 = OMe, R^3 = Me, R^4 = Me
8 : $R^1 = CO_2H$; R^2 = OMe, R^3 = Me, R^4 = Me
9 : R^1 = 2-pyridyl; R^2 = OMe, R^3 = Me, R^4 = Me

Table 1 Opioid binding affinities[5] for SNC-80 analogues

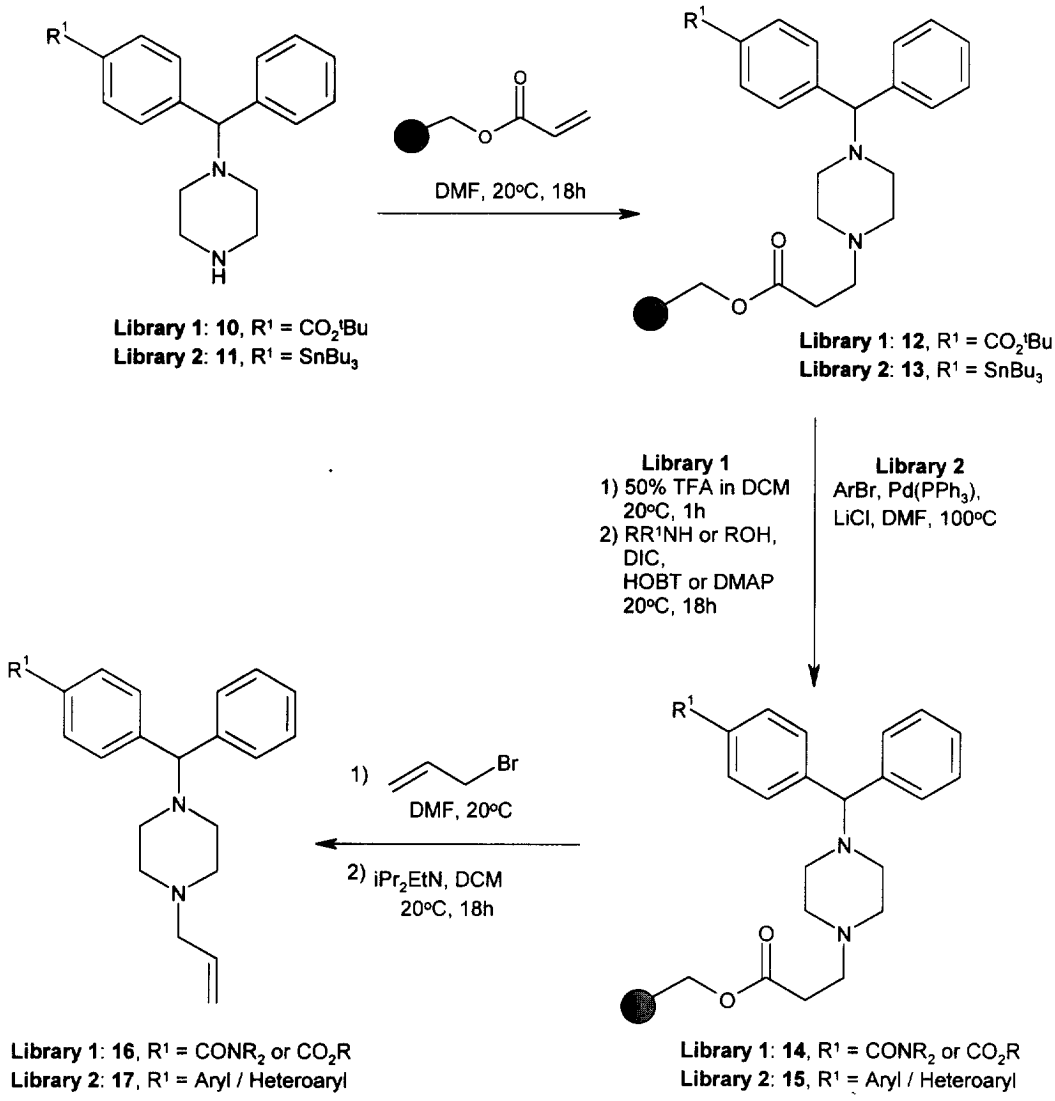
Compound	IC_{50} δ (nM)	IC_{50} μ (nM)	IC_{50} κ (nM)
1 (SNC-80)	2.2	4800	4100
2	1.1	6500	>10000
3[a]	7.4	>10000	>10000
4[a]	4.1	>10000	>10000
5	420	380	nd
6	5.3	6300	8600
7	240	3600	nd
8	450	>10000	nd
9	22	6400	2100

[a] racemic mixture

Thus the t-butyl ester **10** was coupled to REM resin to give the diester **12** (Scheme 1).[6,7] Selective deprotection of the t-butyl ester using TFA / DCM (1/1) produced the carboxylic acid, which was converted to either the amide or the ester **14**. After quaternisation using allyl bromide, the Hofmann elimination was promoted using Hunig's base. Purification using an Isolute™ silica SPE column, with added powdered K_2CO_3, was found to be effective and reasonably quick.[8] Using this route, the diethylamide **4** (**16{1}**) was obtained in 58% overall yield and >95% purity after SPE, as determined by ^1H NMR. Since no major difference in DOR affinity was observed between SPE-purified (IC_{50}= 8nM) and unpurified samples (IC_{50}= 5nM) of **4**, it was decided that no effort would be made to remove small amounts of residual Hunig's base from the library compounds prior to the binding assay. For library 2, the tributylstannane intermediate **11** was loaded onto REM resin, followed by Stille coupling[9,10] of ten aryl and heteroaryl bromides. Quaternization using allyl bromide followed by the Hofmann elimination promoted by Hunig's base afforded crude products **17** containing a small amount of Hunig's base and an impurity resulting from a hydrogen capture side reaction. Purification using Isolute™ SPE columns with added anhydrous K_2CO_3 afforded products in 12-60% yield (>90% purity).

In view of the interesting *in vitro* activity of **4**, we required a larger amount of high purity material for testing *in vivo*. REM resin methodology offers good scope for an effective automated recycling strategy capable of producing multigram amounts of pure tertiary amines.[8] The synthesis was performed using high

loading REM resin and an ACT-496 automated synthesiser (1.8 mmol/g; 100mg for each of 96 reactors) (Scheme 2).[11] This automated synthesis provided enough material (0.66g) of sufficient purity (97%) for all our *in vitro* and *in vivo* experiments. The results of these experiments will be reported separately.

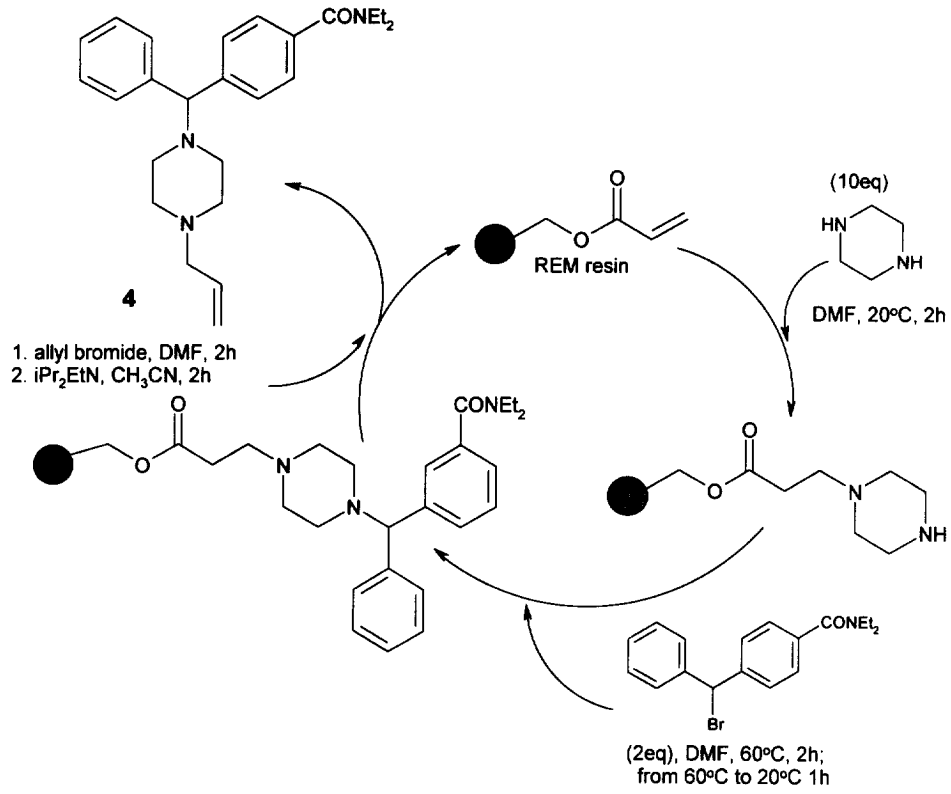


Scheme 1

Discussion

The opioid binding affinities for selected compounds from libraries 1 and 2 are shown in table 2. These confirm that affinity for the DOR is dramatically influenced by the type of substitution at the amide group. A monoethyl analogue of **4**, **16{7}**, showed a significant drop in activity (360nM). It appears that the tertiary amide functionality is crucial for high affinity[12]. The N,N-dimethyl analogue **16{5}** has an affinity (IC_{50}

44nM) higher than any of the secondary amides in Library 1, though it is less active than **4**. Larger groups than the diethylamide are well tolerated in this region. For example, the N,N-dipropyl amide **16{4}** has a similar affinity (IC_{50} 7.2nM) to **4**, as did the bulkier analogues, **16{2}** and **16{3}**. Interestingly, cyclic tertiary amides such as **16{6}** showed significantly lower affinity (IC_{50} 82nM). The most active tertiary amides in the DOR binding assay are also highly selective over the MOR (μ/δ >20000). Ester derivatives exhibited weak affinity, the most active being the methyl ester **16{8}** with an IC_{50} of 607nM. The acid analogue **16{11}** is essentially inactive (IC_{50} 8097nM). The 2-pyridine analogue **17{1}** was the most active of the heterocyclic replacements, with an affinity of 250nM. Interestingly this is substantially lower than for **9**, indicating that the methyl groups on the piperazine ring are more important for affinity in this case. The pyridine nitrogen seems to be important, given the substantially lower activity of the phenyl and 2-thienyl analogues, **17{3}** and **17{4}**.

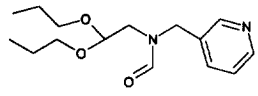
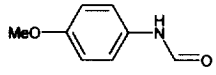


Scheme 2

Summary

Two focused libraries around **4** were synthesised using REM resin methodology. From these libraries, several compounds were identified with DOR binding affinities similar to compound **4** (IC_{50} 4nM). Automated REM resin recycling was used to synthesise 0.66g of **4**. (47% yield, 97% HPLC purity).

Table 2 Opioid binding affinities[5] for selected library compounds

Compound	R^1	IC_{50} δ (nM)	IC_{50} μ (nM)
16{1} (4)	$CONEt_2$ (SPE-purified)	8	>10000
16{1} (4)	$CONEt_2$ (unpurified)	5	>10000
16{2}	$CON(CpCH_2)(CH_2CH_2CH_3)$	3	>10000
16{3}		4	>10000
16{4}	$CON(CH_3CH_2CH_2)_2$	7.2	41600
16{5}	$CONMe_2$	44.3	>50000
16{6}	CO(1-piperidinyl)	82.4	>21600
16{7}	CONHEt	360	nd
16{8}	CO_2Me	607	nd
16{9}	CO_2Et	1394	nd
16{10}		1904	nd
16{11}	CO_2H	8097	nd
17{1}	2-pyridyl	250	nd
17{2}	3-pyridyl	500	nd
17{3}	phenyl	1724	nd
17{4}	2-thienyl	2600	nd

Library compounds **16{2-11}** were not purified and contain small (<20%) amounts of iPr_2EtN. **17{1-4}** were purified to >97% purity by SPE. nd = not determined; Cp = cyclopropyl.

Acknowledgement

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5. The binding affinities of the compounds described for the δ opioid receptor were determined by inhibition of binding of [^{3}H]-naltrindole (0.15nM) to membranes from CHO cells expressing the human DOR. μ opioid binding affinity was determined by the ability of test compounds to displace binding of [^{3}H]-DAMGO (1.5nM) to rat brain membranes. κ opioid binding affinity was determined by the ability of test compounds to displace binding of [^{3}H]-U69593 (1.5nM) to guinea pig brain membranes.
6. Compound **10** was prepared in 4 steps from 4-benzoyl-benzoic acid: ketone reduction with $NaBH_4$ in EtOH, bromination with PBr_3 in dichloromethane, esterification with isobutene in DCM, followed by amination with piperazine in DMF. Compound **11** was prepared in 4 steps from N-Boc piperazine: formation of a masked iminium ion by reflux with 4-bromobenzaldehyde and benzotriazole in toluene, reaction with PhMgBr in THF, stannylation using Bu_6Sn_2 and catalytic $Pd(PPh_3)_4$ in refluxing toluene followed by Boc deprotection with KOH in boiling MeOH.
7. REM resin (262mg; 0.15mmol) was swollen with a solution of the amine **10** (458mg; 1.32mmol) in DMF (3mL) in a polypropylene Multiblock tube. After agitating on the shaker for 18 hours at 20°C, the resin was washed using the cleavage station with DMF (3x2mL), DCM (3x2mL) and methanol (2x2mL) and dried in vacuo. The resin (168mg; 0.08mmol) was resuspended in a mixture of trifluoroacetic acid (1.5mL) and dichloromethane (1.5mL) and agitated by shaking for 1 hour, then was washed using the cleavage station with DCM (3x2mL) and methanol (2x2mL). After drying in vacuo, the resin was re-suspended in a solution of diethylamine (67μl; 0.65mmol) and N-hydroxybenzotriazole (88mg; 0.65mmol) in DMF (2mL). Diisopropylcarbodiimide (102μl; 0.65mmol) was added and the suspension was agitated at 20°C for 18 hours. [To produce resin bound esters **14**, a solution of the alcohol (10eq.), DIC (5eq.) and DMAP (2eq.) in DCM (2mL) was used.]. The resin was washed with DMF (3x2mL), DCM (3x2mL) and methanol (2x2mL) and dried in vacuo. The resin was quaternised using a solution of allyl bromide (57μl; 0.65mmol) in DMF (2mL) and was agitated on the shaker for 18 hours at 20°C. The resin was washed using the VacMaster station with DMF (3x2mL), DCM (3x2mL) and methanol (2x2mL) then dried in vacuo. A suspension of the resin in DCM (3mL) containing DIEA (46μl; 0.26mmol) was agitated on the rotator for 5 hours at 20°C. The resin was drained and washed using the cleavage station with DCM (3x3mL). The filtrate was collected and evaporated. The hydrobromide salt of DIEA and a trace amount of aliphatic impurity was removed using an ISOLUTE™-XL solid phase extraction column, containing 500 mg silica and 50mg of dried, powdered K_2CO_3. The crude material was loaded in Et_2O (0.7mL), then eluted with heptane (2mL: eluted aliphatic impurity) then ethyl acetate (4mL: elutes product). Evaporation of the EtOAc provided the product as a colourless gum (18.4mg; 58%).
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9. $Pd(PPh_3)_4$ (12 mg; 20 mol%) was added under argon atmosphere into a degassed DMF (2 mL) suspension of resin **13** (100mg; 0.052 mmol), LiCl (11 mg; 0.26 mmol) and arylbromide (0.26 mmol). After the reaction suspension was heated at 100°C for 18h, the resin was washed (3 x DMF, 3 x DCM, 3x MeOH) and dried at 45°C *in vacuo*.
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11. Following each of the first three steps in the synthesis, the resin was drained and washed (3xDMF, 3xDCM, 3xMeOH) and the waste was collected in the waste reservoir. After the cleavage step, the filtrate from the reactors was redirected to a second waste reservoir where the product was collected. The crude product, collected after two cycles, was partitioned between DCM and 5% aqueous K_2CO_3, separated, and the solvent evaporated to give 0.66g of **4**, in 47% overall yield and 97% HPLC purity.
12. During the course of this work, a report was published exploring the SAR around the diethylamide in which similar trends were described: Katsura, Y.; Zhang, X.; Homma, K.; Rice, K.C.; Calderon, S.N.; Rothman, R.B.; Yamamura, H.I.; Davis, P.; Flippen-Anderson, J.L.; Xu, H.; Becketts, K.; Foltz, E.J.; Porreca, F. *J. Med. Chem.*, **1997**, *40*, 2936.