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Synthesis and evaluation of N-3 substituted phenoxypropyl piperidine benzimidazol-2-one analogues as NOP receptor agonists with analgesic and sedative properties

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Abstract—A series of 3-phenoxypropyl piperidine benzimidazol-2-one analogues have been discovered as novel NOP receptor agonists. Structure–activity relationships have been explored via N-3 substitution of the benzimidazol-2-one with a range of functionality. The *N*-methyl acetamide derivative (+)-**7f** was found to be a high-affinity, potent NOP agonist with greater than 100-fold selectivity over the MOP receptor. Furthermore (+)-**7f** was shown to be both antinociceptive and sedative when administered iv to rodents.

1. Introduction

Classical opioid receptors (MOP, DOP, and KOP) have important physiological and pharmacological roles, especially in pain. Only MOP agonists, such as morphine, are effective in the treatment of patients with severe pain.[1,2] However, physicians do know that MOP agonist treatment will also produce significant side-effects such as respiratory depression, nausea, constipation, and addiction. A fourth member of the opioid receptor family, opioid receptor like 1 (NOP), was identified in 1994.[3] The receptor mediates the inhibition of adenylate cyclase and shares a high degree of sequence homology with the other opioid receptors. However, despite this similarity none of the native opioid peptides (such as dynorphin A) bind to the new NOP receptor with appreciable affinity.[3] The endogenous agonist for the NOP receptor, named both nociceptin (NC) and orphanin FQ (OFQ), is a 17-amino acid neuropeptide and was identified a year after the receptor was cloned.

NOP receptors are widely distributed in the central nervous system and periphery in several species including human. Isolation of NC has led to considerable research on the physiological role of the NC/NOP system. The NOP receptor and NC have been implicated in several physiological pathways including cognition, pain, locomotion, anxiety, neuroendocrine control, and modulation of cardiovascular and respiratory function.[4] The function of the NC/NOP system in modulating sensory processing has been studied extensively. Analgesia and hyperalgesia have been observed after administration of NC in rodents; the effect appears to be dependent on the dose of NC and route of administration as well as the noxious stimulus used and the stress level of the animals. The use of peptide ligands has plagued the interpretation of these complex results because of the inherent limitations with respect to metabolic stability. Therefore, it is believed that the development of new ligands with high selectivity and potency for NOP would elucidate the physiological role of the NOP receptor and potentially provide a treatment for pain without the life-threatening side-effects observed with MOP agonists.

Recently, several publications have reviewed the efforts in the search for small molecule NOP ligands.[5] Several of these ligands have high selectivity and potency for the NOP receptor vs the other opioid receptors. The NOP antagonist, J-113397 (Fig. 1), based on the benzimidazolone heterocycle has been well-described[6] and

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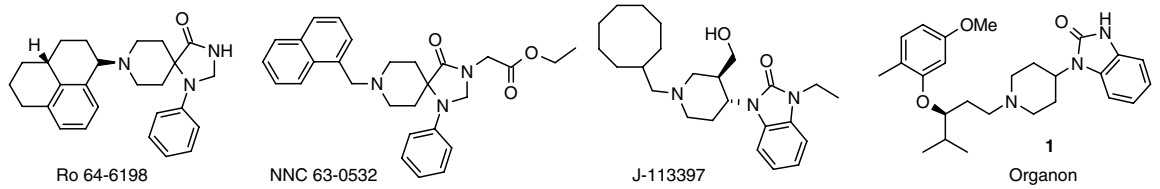


Figure 1. Structures of NOP ligands.

pharmacologically characterised.[7] The use of the triazaspirodecanone heterocyclic moiety has been described by Roche[8] (e.g., Ro 64-6198) and Novo Nordisk[9] (NNC 63-0532). Ro 64-6198 shows >100-fold selectivity for the NOP receptor and displays anxiolytic-like activity without causing tolerance in vivo.[10] Although this compound may mediate its effects through the NOP receptor, it shows moderate affinity towards MOP and KOP receptors as well as towards other receptor sites.[8]

We have recently disclosed our efforts towards developing potent and selective NOP agonists based on the benzimidazol-2-one heterocycle.[11] Compound **1** was identified with an NOP K_i of 4 nM and modest selectivity over MOP (K_i = 59 nM, NOP/MOP = 15). In a functional cAMP assay **1** behaved as a full agonist with modest potency and good efficacy with respect to the NC response (IC_{50} = 38 nM, 100% NC response). Compound **1** was also shown to exert an antinociceptive effect in vivo which could be reversed with an in-house selective NOP antagonist.[12]

Despite the extensive research into agonists it is still unclear what pharmacophoric features of the heterocycle confer selectivity and functional activity. It had been demonstrated in the triazaspirodecanone series[13] that substitution on the amide nitrogen with both polar (ethyl alcohol) and lipophilic (allyl) groups has little effect on NOP affinity but does confer greater affinity for MOP. Due to the similarities of the triazaspirodecanone and the benzimidazol-2-one it was postulated that the benzimidazol-2-one N-3 amide proton does not participate in any type of H bonding interaction with the NOP receptor and that this part of the molecule is also exposed to the water layer. On this premise our aim was to study N-3 substitution on the benzimidazol-2-one of **1** using functionality that would improve water solubility suitable for intravenous administration whilst retaining affinity for NOP. Here, we report the synthesis and SAR of a series of N-3 substituted 3-phenoxypropyl piperidine benzimidazol-2-ones as potential water-soluble intravenous analgesics.

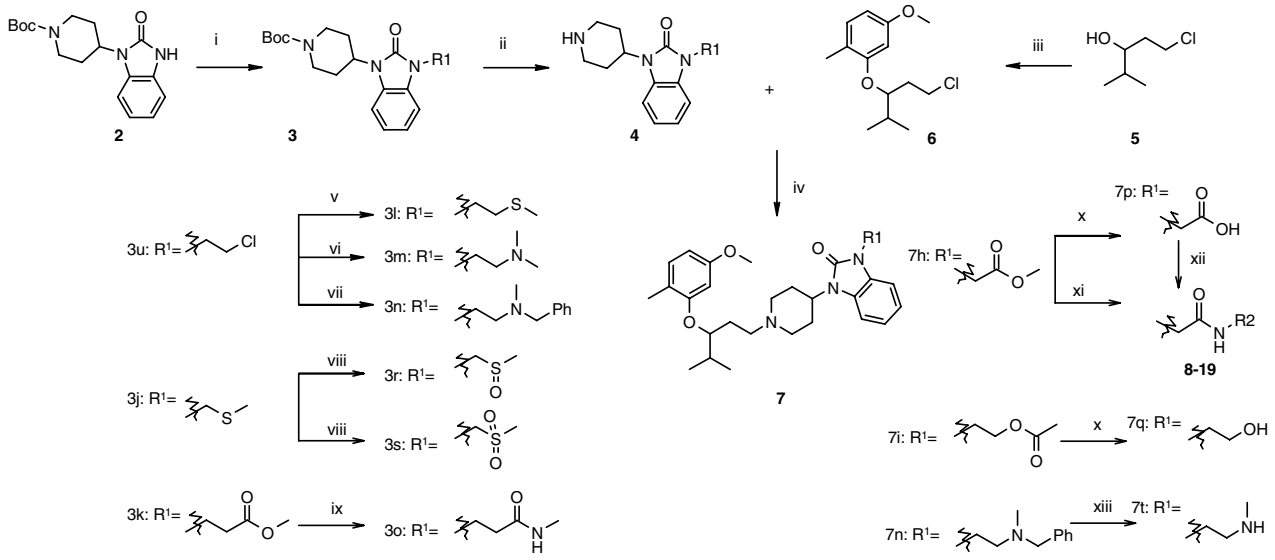
2. Results and discussion

2.1. Chemistry

The synthesis of N-3 substituted benzimidazol-2-ones is outlined in Scheme 1. N-Boc benzimidazol-2-one was prepared according to literature methods,[14] treating the commercially available 1-piperidin-4-yl-1,3-dihydro-benzimidazol-2-one with di-*tert*-butyl dicarbonate in tetrahydrofuran. With **2** in hand a range of functionality at the N-3 position was accessed by deprotonation of the N-3 proton with sodium hydride and subsequent reaction with the appropriate alkylating agent to afford compounds of type **3**. Alkylation with 1-bromo-2-chloroethane resulted in a pendant halide **3u** that was displaced with the appropriate nucleophile to give the thioether **3l**, dimethylamine **3m** and *N*-methylbenzylamine **3n**. Transformation of the thioether **3j** by oxidation to sulfoxide **3r** and sulfone **3s** was effected using sodium perborate.[15] Moderate selectivity in the formation of sulfoxide was achieved by adjusting the number of equivalents of sodium perborate. Sulfoxide is the major product (60–70%) with 1 equiv of sodium perborate whilst the sulfone was obtained in 80–95% with 3 equivalent of sodium perborate. Separation and purification of either was easily achieved, employing flash silica-gel column chromatography. The amide **3o** was prepared by alkylating the N-3 position with methyl acrylate to form the ester (**3k**) and subsequently heating **3k** with methylamine. All intermediates were treated with TFA in dichloromethane to remove the N-Boc-protecting group, and subsequently coupled with intermediate phenoxypropyl chloride **6** using triethylamine in *N*,*N*-dimethylformamide to provide the target molecules.

The intermediate **6** was prepared by a Mitsunobu reaction[16] of the secondary alcohol **5** and 2-methyl-5-methoxy phenol.[17] The yield for this reaction was somewhat disappointing (20–30%), presumably resulting from elimination to form the volatile 4-methyl-1,3-pentadiene before the encumbered phenol can undergo nucleophilic substitution. Alternative reagents, such as DIAD, were investigated but the yields remained poor.[18] Ester **7h** and acetate **7i** underwent hydrolysis to afford the acid **7p** and alcohol **7q**, respectively. The secondary amine **7t** was derived from **7n** using hydrogenolysis of the benzyl group with Pearlman's catalyst.[19]

In a more expedient route to a wider range of amides (**8–19**), the ester **7h** was treated with primary amines directly under forcing conditions to afford the target compounds or from the acid **7p** employing standard acid chloride chemistry. The more interesting compounds **7a**, **7b**, and **7f** were separated into their enantiomers using chiral HPLC methodology to obtain enough material for further profiling. Triazaspirodecanone analogues **20** and **21** (Table 3) were prepared in a similar fashion to **7f** starting with the commercially available 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one. All compounds were prepared as either the hydrochloride or methanesulfonate salt to aid solubility in water.



Scheme 1. Reagents and conditions: (i) 60% NaH, DMF, 1 h, then R^1CH_2Cl or K_2CO_3, R^1CH_2Cl, DMF, 50 °C; (ii) TFA, DCM, 24 h, rt; (iii) DEAD, 2-methyl-5-methoxyphenol, PPh_3, THF, 18 h, rt; (iv) Et_3N, NaI, DMF, 3 days, 80 °C; (v) NaSMe, MeOH, 12 h, reflux; (vi) 33% dimethylamine in EtOH, 12 h, 85 °C; (vii) *N*-benzylamine, Et_3N, NaI, CH_3CN, 4 days, reflux; (viii) AcOH, sodium perborate tetrahydrate, 3 h, rt; (ix) 2 M methylamine in MeOH, 24 h, 85 °C; (x) 4 M NaOH, EtOH, 4 h, 60 °C; (xi) R^2NH_2, MeOH, 65 °C, 48 h; (xii) $(COCl)_2$, DiPEA, R^2NH_2, 1 h, rt; (xiii) H_2, MeOH, $Pd(OH)_2$, 10 h, rt. Compounds **7a**, **7b**, and **7f** were separated using chiral chromatography on a Chiralpak AD column.

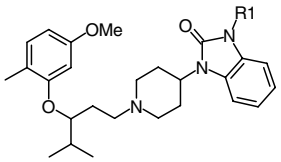
2.2. Affinity and functional data

Table 1 shows the effect of incorporating a series of heteroatom-containing substituents at the benzimidazol-2-one N-3 position. The methoxymethyl derivative **7a** shows similar NOP and MOP affinities as the parent **1**, but a 2-fold decrease in functional potency was observed. Increasing the chain length to the methoxyethyl **7b** maintained NOP affinity, gave a 3-fold increased selectivity over MOP but also showed a modest decrease in functional potency relative to the unsubstituted analogue **1**. Surprisingly, switching the position of the ether oxygen of **7b** to give the ethoxymethyl **7c** resulted in complete loss of selectivity over MOP, highlighting the sensitivity of this region to small structural changes. The more lipophilic thioethers **7j** and **7l** showed high NOP-affinity but unfortunately no improvement in selectivity over MOP was observed relative to the ether analogues **7a** and **7b**.

Replacement of the ether oxygen of **7c** by a carbonyl group as in **7d** restored selectivity over MOP resulting in a 2.5-fold increase in selectivity and similar functional potency relative to the unsubstituted parent **1**. This suggests that the increased hydrogen bond acceptor ability of the carbonyl may confer the increased selectivity. This is further strengthened by esters **7h** and **7k** which both show high NOP-affinity and good selectivity over MOP of 68- and 54-fold, respectively. However, in both cases functional potency in the cAMP assay decreased relative to ketone **7d** and the unsubstituted parent **1**. The reversed ester (acetate) **7i** maintains high NOP-affinity but an increase in MOP affinity results in diminished selectivity over MOP. Ethyl alcohol derivative **7q** exhibits a 2.5-fold decrease in NOP affinity relative to **1**, yielding a modest 6-fold selectivity over MOP, whereas binding affinity for both NOP and MOP was effectively abolished for carboxylic acid **7p**. Sulfoxide **7r** and sulfone **7s** showed similar profiles with high NOP affinity, modest selectivity over MOP but excellent functional potency with a 3- to 5-fold increase compared to **1**.

The effect of introducing a hydrogen bond donor functionality into the substituent was further probed via amides **7e–g**. Primary amide **7e** exhibited high-NOP affinity and improved selectivity over MOP compared to the unsubstituted parent **1**, but unlike esters **7h** and **7k** that showed poor functional potency in the cAMP assay, amide **7e** was effectively equipotent to **1**. Most notable was the secondary amide **7f** showing excellent NOP affinity and 164-fold selectivity over MOP. Furthermore **7f** was highly potent in the functional assay, with an almost 4-fold increase in potency upon comparison with **1**. A significant decrease in NOP affinity and selectivity was observed for the dimethyl amide analogue **7g** relative to **7f** suggesting that the presence of a hydrogen bond donor moiety may afford selectivity over MOP. Replacement of the carbonyl of **7f** by a methylene moiety as in secondary amine **7t** maintained high NOP-affinity but a 7-fold decrease in selectivity over MOP was observed upon comparison with the amide equivalent **7f**. Functional potency was also decreased by an order of magnitude. Tertiary amine **7m** shows a similar binding affinity profile to the secondary amine **7t** but has inferior functional potency. Bulky amine **7n** and the extended amide **7o** both show reduced NOP affinity compared to **7f**. The SAR therefore suggests that the combination of the hydrogen bond donor and acceptor properties offered by the secondary amide appears to be highly favourable for NOP affinity, selectivity over MOP and NOP agonism. It is noted that all compounds assayed for DOP receptor affinity showed high selectivity and only the unsubstituted analogue **1**,

Table 1. Opioid receptor binding affinity of N-3 substituted benzimidazol-2-one analogues



Compound	R1	NOP K_i (nM)	MOP K_i (nM)	NOP/MOP	DOP K_i (nM)	KOP K_i (nM)	cAMP	
							IC_{50} (nM)	% NC resp.
1	H	4	59	15	764	100	38	100
1(+)	H	2	34	17	368	73	43	102
1(−)	H	47	109	2	2135	137	677	93
7a	CH_2OCH_3	4	57	14	1986	155	81	118
(+)7a	CH_2OCH_3	3	70	23	1251	94	27	117
(−)7a	CH_2OCH_3	153	195	—	NT	NT	NT	NT
7b	$CH_2CH_2OCH_3$	4	184	46	2159	501	60	109
(+)7b	$CH_2CH_2OCH_3$	2	60	30	2123	394	24	109
(−)7b	$CH_2CH_2OCH_3$	375	273	—	NT	NT	7726	61
7c	$CH_2OCH_2CH_3$	5	9	2	NT	NT	92	106
7d	$CH_2C(O)CH_2CH_3$	3	113	38	NT	1547	44	102
7e	CH_2CONH_2	3	138	46	2020	503	32	110
7f	$CH_2CONHCH_3$	1	164	164	3654	477	10	113
(+)7f	$CH_2CONHCH_3$	0.5	54	108	3411	534	4	120
(−)7f	$CH_2CONHCH_3$	101	449	4	4531	4772	712	110
7g	$CH_2CON(CH_3)_2$	22	188	9	NT	NT	358	104
7h	$CH_2CO_2CH_3$	4	271	68	3084	671	123	114
7i	$CH_2CH_2OC(O)CH_3$	6	103	17	NT	NT	104	99
7j	CH_2SCH_3	1	23	23	608	99	33	104
7k	$CH_2CH_2CO_2CH_3$	5	271	54	3084	671	123	114
7l	$CH_2CH_2SCH_3$	1	27	27	1631	166	42	110
7m	$CH_2CH_2N(CH_3)_2$	4	106	27	NT	4275	810	93
7n	$CH_2CH_2NH(CH_3)CH_2Ph$	46	172	4	NT	NT	NT	NT
7o	$CH_2CH_2CONHCH_3$	12	122	10	NT	NT	158	106
7p	CH_2CO_2H	1595	1701	—	NT	NT	NT	NT
7q	CH_2CH_2OH	10	62	6	NT	NT	77	110
7r	$CH_2S(O)CH_3$	4	125	31	1471	627	8	103
7s	$CH_2S(O)_2CH_3$	3	70	23	1175	374	13	98
7t	$CH_2CH_2NHCH_3$	2	46	23	NT	4209	99	90

NT, not tested.

dimethylether **7a**, and thioethers **7j** and **7l** exhibited appreciable KOP affinity. All compounds showing high NOP affinity were also found to be full agonists in the cAMP assay.

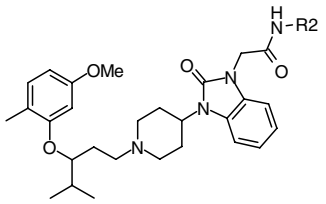
To further probe the SAR surrounding the methyl amide **7f**, a series of secondary amides **8–19** were prepared with binding and functional data detailed in Table 2. Only the ethyl amide **8** and the cyclopropyl amide **18** maintained high NOP affinity. Both showed a decreased selectivity over MOP in comparison with the methyl amide **7f**. Additionally compounds **8** and **18** showed decreased functional potencies of 9- and 4-fold, respectively, compared to **7f**. It is clear that increasing the bulk around the amide leads to a significant reduction in NOP affinity suggesting strict steric requirements in this region.

Separation of **1**, **7a**, **7b**, and **7f** into their enantiomeric pairs consistently found that the (+) enantiomer was the eutomer. Compound (+)-**7f** demonstrated subnanomolar binding affinity for NOP (K_i = 0.5 nM) with a eudismic ratio [(+)/(−) activity ratio] of >200. This compound also showed an exceptional selectivity of greater than 100-fold over MOP, DOP, and KOP and furthermore exhibited excellent functional activity (IC_{50} = 4 nM) in the cAMP assay. As the methanesulfonate salt the compound had a water solubility >200 mg/mL.

Compound (+)-**7f** was screened against a panel of 55 unrelated molecular targets.[20] At 10^{-7} M no activity was observed against any of the targets except for the non-selective opiate assays, as expected. These data indicate that there is a greater than 100-fold selectivity between binding of (+)-**7f** at the NOP receptor and 55 other molecular targets.

Since (+)-**7f** also has some affinity for the other classical opioid receptors, it might also be able to act as an opioid receptor agonist. Therefore (+)-**7f** was evaluated in a mouse vas deferens (MVD) preparation (Fig. 2). Compound (+)-**7f** causes inhibition of MVD contractions, however, pretreatment with 1 μM of the MOP antagonist naloxone did not cause a significant change in the pIC_{50} value for (+)-**7f**, suggesting that this compound

Table 2. NOP and MOP receptor binding affinity of N-substituted benzimidazol-2-one amides



Compound	R2	NOP K_i (nM)	MOP K_i (nM)	NOP/MOP	cAMP	
					IC_{50} (nM)	%NC resp.
8	CH_2CH_3	5	289	57	90	111
9	$CH_2CH_2CH_3$	19	272	14	1219	107
10	$CH_2CH_2CH_2CH_3$	29	161	6	NT	NT
11	$CH(CH_3)_2$	20	238	12	1028	92
12	$C(CH_3)_3$	155	245	2	NT	NT
13	CH_2CH_2OH	15	75	5	103	107
14	$CH_2CH_2OCH_3$	20	180	9	436	103
15	CH_2CHCH_2	12	199	17	166	102
16	CH_2Ph	29	432	15	569	102
17	CH_2cyclopropyl	12	100	8	2034	107
18	Cyclopropyl	3	119	40	40	101
19	Cyclobutyl	23	108	5	328	106

NT, not tested.

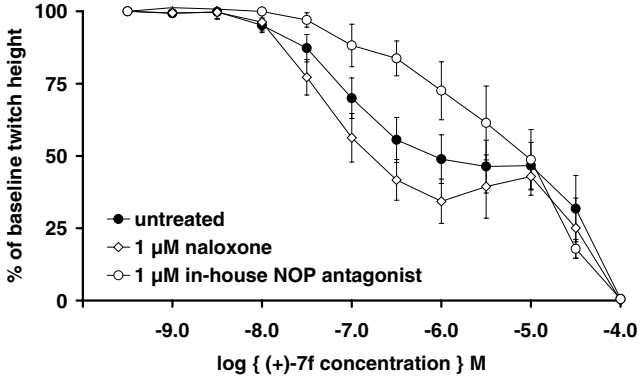


Figure 2. Effect of cumulative administration of (+)-**7f** on mouse vas deferens in the absence of any opioid antagonist (•), in the presence of 1 μM naloxone (◊) and in the presence of an in-house NOP antagonist (○). Data are plotted as means ± SEM; $n = 9$ (•), $n = 5$ (◊), and $n = 4$ (○).

does not act as a classical opioid agonist. The cumulative concentration–response curve (CCRC) was biphasic (pIC_{50}'s for the two sites ~7 and ~4.3), suggesting that more than one mechanism might be involved in the inhibitory response (Fig. 2). The first component is likely to be NOP-mediated since this can be blocked by 1 μM of an in-house NOP antagonist[12], but the second component is probably mediated by another mechanism, which is insensitive to naloxone (Fig. 2). The significance of the lower affinity component is unknown but is not considered an issue due to the >100-fold separation over the NOP-mediated mechanism.

2.3. Pharmacophore analysis

The ability to modulate NOP affinity and selectivity over MOP via N-3 substitution of the benzimidazol-2-one core was unexpected. Previous studies have suggested that the 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one moiety, common to many NOP agonists (Fig. 1), is pharmacophorically equivalent to the benzimidazol-2-one piperidine with respect to opioid receptor affinity.[13] N-3 substitution of 1,3,8-triazaspiro[4.5]decan-4-one-containing NOP agonists with a limited number of polar and small lipophilic substituents was shown to have little effect on NOP affinity and modest increases in MOP, DOP, and KOP affinities over the parent leading to the proposal that this region may be interacting with solvent.[21] Further published studies describing an *N*-methylacetamide substituted 1,3,8-triazaspiro[4.5]decan-4-one opioid receptor ligand demonstrated a 5-fold increase in NOP affinity, 2-fold increase in MOP and KOP affinities and a 12-fold increase in DOP affinity over the unsubstituted parent.[22] Therefore, the combination of the 3-fold increase in NOP affinity and almost 3-fold decrease in MOP affinity of the *N*-methylacetamide benzimidazol-2-one analogue **7f** over the parent **1** leading to greater than 100-fold selectivity for NOP over MOP was surprising.

In an effort to explore these differences a superposition model of the *N*-methylacetamide substituted benzimidazol-2-one piperidine and 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one moieties was developed. Molecular mechanics energy-minimised conformations of both fragments were found to be consistent with experimental conformations observed in the Cambridge Structural Database.[23] Three key pharmacophoric features consisting of the aromatic ring centroids, the ionisable amines and their attached protons were used to superimpose the fragments by RMS fitting. The hydrophobic aryl rings and the ionisable amines in combination with a general hydrophobic group attached to the ionisable amine have been proposed to form the NOP receptor pharmacophore.[24,25] The superposition model (Fig. 3) clearly

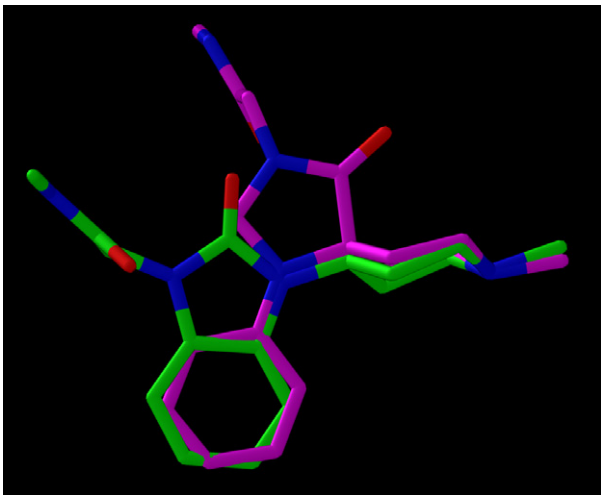


Figure 3. Superposition of the *N*-methylacetamide substituted benzimidazol-2-one piperidine (green, **7f**) and 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (magenta, **21**) highlighting the differing regions occupied by the *N*-methylacetamide substituents.

shows the superposition of the aryl hydrophobes and ionisable amines allowing the general hydrophobes (not shown) to occupy the same region. Furthermore it is clear that to maintain this alignment of the pharmacophoric features the N-3 substituents of the benzimidazol-2-one and triazaspiro[4.5]decan-4-one moieties must occupy different regions in space. It could be reasoned that the differences in the general hydrophobes attached to the ionised amines between the published data[13,21,22] and present data may also lead to the differences in affinities between the series. However N-3 *N*-methylacetamide substitution of the 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one core with the phenoxypropyl general hydrophobe (**21**) shows similar NOP affinity and a modest 2-fold increase in MOP affinity in comparison with the unsubstituted analogue **20** (Table 3). These results are generally consistent with the previously published data for this series. Therefore, it can be concluded that the differences in affinity changes between N-3 substitution of the benzimidazol-2-one piperidine series and the published 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one series are independent of the hydrophobe attached to the ionised amine and can be attributed to the N-3 substituents occupying different regions in space. Furthermore the introduction of a hydrogen bond donor/acceptor function of the *N*-methylacetamide into the current series and the consequent increase in selectivity suggests that the secondary amide functionality forms specific interactions with the NOP receptor but is less favourable for the MOP receptor.

2.4. Behavioural effects of compound (+)-7f

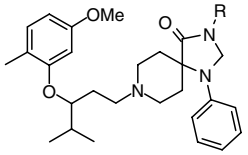
The antinociceptive properties of compound (+)-**7f** were evaluated in the mouse formalin paw test[26] (FPT), a model that assesses behavioural responses to continuous, noxious stimulation generated by injection of a dilute solution of formalin into one hindpaw. Compound (+)-**7f** was administered iv as a bolus (0.03–3.0 μmol/kg) and dose dependently reduced the amount of time spent licking the formalin-injected paw, during both phases of licking (Fig. 4). This effect was significant after administration of the 3 μmol/kg dose for both phases of licking (Dunn's vehicle versus 3 μmol/kg, $p < 0.01$ and $p < 0.05$ for phase 1 and 2, respectively). The calculated ED_{50} for the inhibition of licking during the first phase was 1.96 μmol/kg (95% CI 0.61–3.32 μmol/kg) and the second phase was 1.03 μmol/kg (95% CI 0.27–1.81 μmol/kg). In a second experiment the antinociception produced by (+)-**7f** (3 μmol/kg) in this test was antagonised by pre-treatment with our in-house NOP antagonist[12] (10 μmol/kg sc). In this experiment an 86% block of the inhibition of phase 2 licking was observed; 3μmol/kg of (+)-**7f** produced a 67 ± 14% inhibition of licking during phase 2, whereas in mice pre-treated with the antagonist[12] only a 9 ± 15% inhibition of licking was observed.

In addition to the antinociceptive effect of the NOP agonist (+)-**7f**, we also evaluated the sedative/anaesthetic effect in the loss of righting reflex (LRR) assay in mice (Table 4). After administration of 8 μmol/kg of (+)-**7f**, all animals lost their righting reflex with a mean onset time of 1.8 ± 0.2 min. and a mean sleep time of 38.6 ± 3.8 min. The calculated HD_{50} value was 4.7 μmol/kg (95% confidence limits could not be calculated for (+)-**7f** as only three data points were obtained due to a steep dose–response).

3. Conclusion

In summary, a series of 3-phenoxypropyl piperidine benzimidazol-2-one analogues was synthesised varying the substituent at the N-3 position. SAR indicates that polar

Table 3. Opioid receptor binding affinity of N-substituted triazaspirodecanone analogues



Compound	R	NOP K_i (nM)	MOP K_i (nM)	NOP/MOP	DOP K_i (nM)	KOP K_i (nM)	cAMP	
							IC_{50} (nM)	%NC resp.
20	H	2	34	17	NT	47	56	108
21	$CH_2C(O)NHCH_3$	2	16	8	320	44	17	106

NT, not tested.

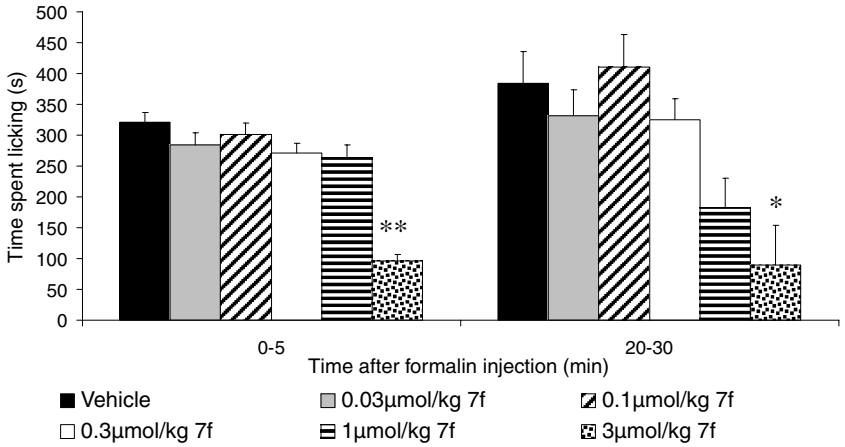


Figure 4. The effect of (+)-**7f** on the time spent licking the formalin-injected paw. $N = 6$ per dose group. $^{*}P < 0.05$ $^{**}P < 0.001$ compared to vehicle, Kruskal–Wallis one was ANOVA followed by Dunn's post hoc test.

Table 4. Compound (+)-**7f** dosed iv shows the percent of animals that lost their righting reflex, onset time and sleep time

Dose (μmol/kg)	% LRR	Onset time (min)	Sleep time (min)
4.0	0	—	—
4.7	37.5	5.7 ± 0.9	36.7 ± 5.4
5.6	100	4.6 ± 0.6	29.4 ± 4.0
8.0	100	1.8 ± 0.2	38.6 ± 3.8

Each treatment group contained eight animals.

substituents are not only tolerated but afforded compounds with increased NOP affinity, selectivity over MOP and functional potency relative to the unsubstituted compound **1**. This indeed was a surprise because previously N-3 substitution of 1,3,8-triazaspiro[4.5]decan-4-one was shown to have a minor effect on NOP affinity and selectivity. In particular the *N*-methyl acetamide substituent of **7f** appears to be optimal with respect to steric requirements. Furthermore, the presence of the hydrogen bond acceptor and donor functions may be responsible for the optimal balance between high NOP affinity and reduced MOP affinity resulting in the increased selectivity over MOP.

Compound (+)-**7f** was found to be selective for NOP over a panel of 55 unrelated molecular targets. The functional assay showed that (+)-**7f** was a full NOP receptor agonist and was confirmed in a native system using the MVD preparation. Furthermore, (+)-**7f** given iv produced antinociceptive effects comparable to morphine in the FPT and showed potent anaesthetic activity in the LRR assay. Both activities were reversed by an in-house NOP receptor antagonist suggesting that the effects are mediated by NOP. These data suggest that (+)-**7f** should be profiled further and may have a role as a water-soluble analgesic for future pain management.

4. Experimental

4.1. Chemistry

4.1.1. General information. All chemicals and reagents were obtained from commercial suppliers and used without further purification. All NMR spectra were recorded at 400 MHz on Bruker DPX400 spectrometer and chemical shifts are reported in ppm relative to TMS. All mass spectrometry was carried out on either a PE SCIEX API 150EX or ESCi micromassZQ machine. Optical rotations were determined on an Optical Activity AA-1000 polarimeter. All compounds passed in-house purity analysis of >90% as determined by NMR and LC.

4.1.2. 4-(2-Oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (2). 1-Piperidin-4-yl-1,3-dihydro-benzimidazol-2-one (30.0 g, 138.0 mmol) was added portionwise at 0 °C to a stirred solution of di-*tert*-butyldicarbonate (30.15 g, 138.0 mmol) in dry tetrahydrofuran (350 mL). Stirring at 0 °C was continued for 30 min, then allowed to warm to room temperature. The solution was treated with dilute aqueous sodium carbonate solution (200 mL) and extracted with diethyl ether (2 × 200 mL). The combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure. The residual pale yellow solid was used without further purification (40.0 g, 90%). ESI-MS $m/z = 318.0$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 10.04 (s, 1H), 7.15–7.10 (m, 2H), 7.09–7.02 (m, 2H), 4.49 (tt, J = 4.2, 12.5 Hz, 1H), 4.32 (br s, 2H), 2.86 (br t, J = 12.5 Hz, 2H), 2.35 (ddd, J = 4.5, 12.7, 12.7 Hz, 2H), 1.88–1.79 (m, 2H), 1.51 (s, 9H).

4.1.3. 4-(3-Methylcarbamoylmethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (3f). A magnetically stirred solution of **2** (4.99 g, 15.7 mmol) in anhydrous *N*,*N*-dimethylformamide (50 mL) was treated with sodium hydride (60% dispersion in mineral oil, 0.63 g, 15.7 mmol) added portionwise under a nitrogen atmosphere. The reaction mixture was stirred for 1 h to give a colourless solution. 2-Chloro-*N*-methyl acetamide (1.69 g, 15.7 mmol) in anhydrous *N*,*N*-dimethylformamide (5.0 mL) was added dropwise with cooling to maintain ambient temperature and the resultant mixture stirred overnight. The reaction mixture was quenched with ice, poured into water, and extracted with ethyl acetate. The organic extracts were combined and washed with water followed by brine, dried (Na_2SO_4),

filtered, and concentrated under reduced pressure to low volume. This was then cooled in ice, and the resultant solid filtered, washed with ethyl acetate, and dried to give the title compound as a colourless solid (4.25 g, 70%). The previous aqueous washings were combined and extracted with dichloromethane, the organics dried (Na_2SO_4), and evaporated under reduced pressure. The residue was combined with the previously evaporated filtrate from above, triturated with ethyl acetate, cooled in ice, filtered, washed with ethyl acetate, and dried to give a second crop of the title compound as a colourless solid (1.19 g, 20%). ESI-MS $m/z = 389.2$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.17–7.05 (m, 4H), 6.13 (br s, 1H), 4.51 (s, 2H), 4.51–4.43 (m, 1H), 4.34–4.32 (m, 2H), 2.86 (br t, $J = 12.7$ Hz, 2H), 2.80 (d, $J = 4.9$ Hz, 3H), 2.33 (ddd, $J = 4.6$, 12.7, 12.7 Hz, 2H), 1.85–1.82 (m, 2H), 1.50 (s, 9H).

The following compounds were prepared in the same manner as **3f**, using the appropriate halo-alkyl reagent.

4.1.4. 4-(3-Methoxymethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (3a). Flash chromatography on silica gel (dichloromethane/ethanol; 99:1; as eluant) gave a yellow gum in 79% yield. ^1H NMR (400 MHz, $CDCl_3$): δ 7.19–7.07 (m, 4H), 5.28 (s, 2H), 4.47 (tt, $J = 4.1$, 12.5 Hz, 1H), 4.31 (br s, 2H), 3.36 (s, 3H), 2.86 (t, $J = 12.4$ Hz, 2H), 2.23 (ddd, $J = 4.6$, 12.7, 12.7 Hz, 2H), 1.82 (d, $J = 10.4$ Hz, 2H), 1.50 (s, 9H).

4.1.5. 4-[3-(2-Methoxy-ethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (3b). Flash chromatography on silica gel (dichloromethane/ethanol; 99:1; as eluant) gave a colourless oil in 79% yield which solidified on standing. ^1H NMR (400 MHz, $CDCl_3$): δ 7.12–7.02 (m, 4H), 4.48 (tt, $J = 4.1$, 12.5 Hz, 1H), 4.35–4.25 (br m, 2H), 4.05 (t, $J = 5.6$ Hz, 2H), 3.68 (t, $J = 5.6$ Hz, 2H), 3.34 (s, 3H), 2.86 (t, $J = 12.4$ Hz, 2H), 2.32 (ddd, $J = 4.6$, 12.7, 12.7 Hz, 2H), 1.81 (d, $J = 10.4$ Hz, 2H), 1.50 (s, 9H).

4.1.6. 4-(3-Ethoxymethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (3c). Flash chromatography on silica gel (dichloromethane/ethanol; 99:1; as eluant) gave a colourless oil in 78% yield which solidified on standing. ^1H NMR (400 MHz, $CDCl_3$): δ 7.21–7.18 (m, 1H), 7.14–7.08 (m, 3H), 5.33 (s, 2H), 4.47 (tt, $J = 4.0$, 12.5 Hz, 1H), 4.31 (br s, 2H), 3.58 (q, $J = 7.0$ Hz, 2H), 2.90–2.83 (m, 2H), 2.33 (ddd, $J = 4.6$, 12.6, 12.6 Hz, 2H), 1.81 (d, $J = 12.2$ Hz, 2H), 1.50 (s, 9H), 1.18 (t, $J = 7.0$ Hz, 3H).

4.1.7. 4-[2-Oxo-3-(2-oxo-butyl)-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (3d). Flash chromatography on silica gel (dichloromethane/ethanol; 99:1; as eluant) gave a white solid in 65% yield. ESI-MS $m/z = 388.1$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.16–7.12 (m, 1H), 7.08–7.03 (m, 2H), 6.81–6.77 (m, 1H), 4.60 (s, 2H), 4.46 (tt, $J = 4.2$, 12.5 Hz, 1H), 4.31 (d, $J = 11.6$ Hz, 2H), 2.86 (t, $J = 12.5$ Hz, 2H), 2.50 (q, $J = 7.4$ Hz, 2H), 2.34 (ddd, $J = 4.6$, 12.4, 12.4 Hz, 2H), 1.84 (d, $J = 12.2$ Hz, 2H), 1.50 (s, 9H), 1.10 (t, $J = 7.3$ Hz, 3H).

4.1.8. 4-(3-Carbamoylmethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (3e). Crude product was heated in AR methanol, hot-filtered, and the filtrate evaporated to give the title compound in 93% yield. ESI-MS $m/z = 375$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.19–7.05 (m, 4H), 6.14 (br s, 1H), 5.55 (br s, 1H), 4.52 (s, 2H), 4.52–4.41 (m, 1H), 4.40–4.22 (m, 2H), 2.86 (br t, $J = 12.3$ Hz, 2H), 2.33 (ddd, $J = 4.5$, 12.7, 12.7 Hz, 2H), 1.83 (br d, $J = 12.3$ Hz, 2H), 1.50 (s, 9H).

4.1.9. 4-(3-Dimethylcarbamoylmethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (3g). Crude product crystallised from diethyl ether to give the title compound in 100% yield. ESI-MS $m/z = 403.4$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.14–7.00 (m, 4H), 4.67 (s, 2H), 4.46 (tt, $J = 4.1$, 12.4 Hz, 1H), 3.14 (s, 3H), 2.97 (s, 3H), 2.85 (br t, $J = 11.9$ Hz, 2H), 2.33 (ddd, $J = 4.6$, 12.7, 12.7 Hz, 2H), 1.83 (br d, $J = 12.2$ Hz, 2H), 1.50 (s, 9H).

4.1.10. 4-(3-Methoxycarbonylmethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (3h). Flash chromatography on silica gel (dichloromethane/ethanol; 98:2; as eluant) gave the title compound as an off-white solid in 84% yield. ESI-MS $m/z = 390.2$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.15–7.12 (m, 1H), 7.09–7.05 (m, 2H), 6.90–6.88 (m, 1H), 4.63 (s, 2H), 4.47 (tt, $J = 4.1$, 12.5 Hz, 1H), 4.31 (br s, 2H), 3.76 (s, 3H), 2.86 (t, $J = 12.4$ Hz, 2H), 2.33 (ddd, $J = 4.6$, 12.7, 12.7 Hz, 2H), 1.83 (d, $J = 12.3$ Hz, 2H), 1.50 (s, 9H).

4.1.11. 4-(3-Methylsulfanylmethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (3j). Crude material was crystallised from diethyl ether to give the title compound as a colourless solid in 56% yield. ESI-MS $m/z = 378.2$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.16–7.08 (m, 4H), 4.98 (s, 2H), 4.46 (tt, $J = 4.1$, 12.5 Hz, 1H), 4.31 (br s, 2H), 3.36 (s, 3H), 2.86 (t, $J = 11.9$ Hz, 2H), 2.32 (ddd, $J = 4.6$, 12.7, 12.7 Hz, 2H), 2.17 (s, 3H) 1.81 (d, $J = 12.2$ Hz, 2H), 1.50 (s, 9H).

4.1.12. 4-[3-(2-Methoxycarbonyl-ethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (3k). A magnetically stirred mixture of **2** (9.93 g, 3.13 mmol), methyl acrylate (2.69 g, 3.13 mmol) and anhydrous potassium carbonate (5.0 g) in anhydrous *N*,*N*-dimethylformamide (100 mL) was heated at 85 °C, under a nitrogen atmosphere, overnight. The mixture was allowed to cool to room temperature, then poured onto water and extracted with ethyl acetate. The organic extracts were combined, washed with water, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give the title compound as a pale orange oil (12.60 g, 100%). ESI-MS $m/z = 404.4$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.07–6.95 (m, 4H), 4.47 (tt, $J = 4.1$, 12.5 Hz, 1H), 4.24 (br s, 2H), 4.11 (t, $J = 7.1$ Hz, 2H), 3.60 (s, 3H), 2.85–2.70 (m, 2H), 2.72 (t, $J = 7.1$ Hz, 2H), 2.33 (ddd, $J = 4.6$, 12.7, 12.7 Hz, 2H), 1.83 (d, $J = 12.0$ Hz, 2H), 1.55 (s, 9H).

4.1.13. 4-[3-(2-Chloro-ethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (3u). A magnetically stirred suspension of **1** (9.51 g, 30.0 mmol), 1-bromo-2-chloroethane (17.21 g, 120.0 mmol) and anhydrous potassium carbonate (18.22 g, 130.0 mmol) in anhydrous *N*,*N*-dimethylformamide (80 mL) was warmed under nitrogen at 50 °C for 72 h. The reaction mixture was allowed to cool to room temperature and then partitioned between ethyl acetate and water. The organic layer was collected, washed with water, dried (Na_2SO_4), filtered and evaporated to give the title compound as a colourless solid (10.61 g, 93%). ESI-MS m/z = 380.2 $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.16–7.04 (m, 4H), 4.47 (tt, J = 4.1, 12.5 Hz, 1H), 4.39–4.25 (br m, 2H), 4.20 (t, J = 6.5 Hz, 2H), 3.81 (t, J = 6.5 Hz, 2H), 2.86 (br t, J = 12.5 Hz, 2H), 2.32 (ddd, J = 4.6, 12.7, 12.7 Hz, 2H), 1.82 (d, J = 12.3 Hz, 2H), 1.50 (s, 9H).

4.1.14. 4-[3-(2-Methylsulfanyl-ethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (3l). A suspension of **3u** (3.54 g, 9.3 mmol) and sodium thiomethoxide (2.00 g, 28.6 mmol) in methanol (50 mL) was heated under reflux for 12 h, then allowed to cool to room temperature. The reaction mixture was poured onto dilute aqueous sodium carbonate solution and extracted with dichloromethane. The combined extracts were combined, washed with water, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give the title compound as a colourless solid (3.12 g, 73%). ESI-MS m/z = 392.0 $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.15–7.00 (m, 4H), 4.48 (tt, J = 4.1, 12.5 Hz, 1H), 4.35–4.25 (br m, 2H), 4.08 (t, J = 7.3 Hz, 2H), 2.89 (m, 2H), 2.84 (t, J = 7.3 Hz, 2H), 2.32 (ddd, J = 4.6, 12.7, 12.7 Hz, 2H), 2.18 (s, 3H), 1.81 (d, J = 12.1 Hz, 2H), 1.50 (s, 9H).

4.1.15. 4-{3-[2-(Benzyl-methyl-amino)-ethyl]-2-oxo-2,3-dihydro-benzimidazol-1-yl}-piperidine-1-carboxylic acid *tert*-butyl ester (3n). A mixture of **3u** (4.20 g, 11 mmol), *N*-benzyl methylamine (1.34 g, 11 mmol), sodium iodide (catalytic) and triethylamine (2.0 mL, 14 mmol) in acetonitrile (20 mL) was refluxed for 4 days. The reaction mixture was evaporated to dryness, treated with water and extracted with dichloromethane. The organic extracts were combined, washed with water, dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was subjected to flash chromatography on silica gel eluting with ethyl acetate to give the title compound as an oil (4.17 g, 82%). ESI-MS m/z = 465.0 $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.25–7.15 (m, 5H), 7.14–7.10 (m, 1H), 7.06–6.98 (m, 2H), 6.90–6.84 (m, 1H), 4.48 (tt, J = 4.1, 12.5 Hz, 1H), 4.30 (br s, 2H), 3.99 (t, J = 6.9 Hz, 2H), 3.55 (s, 2H), 2.96–2.77 (br m, 2H), 2.71 (t, J = 6.9 Hz, 2H), 2.38–2.28 (m, 2H), 2.35 (s, 3H), 1.81 (d, J = 11.9 Hz, 2H), 1.50 (s, 9H).

4.1.16. 4-[3-(2-Methylcarbamoyl-ethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (3o). A solution of **3k** (1.0 g, 2.5 mmol) in methanol (5.0 mL) was treated with methylamine (2 M solution in methanol, 4.0 mL, 8.0 mmol), and the resultant solution heated in a sealed tube at 85 °C overnight. The solution was evaporated to dryness under reduced pressure and the residue crystallised from ethyl acetate. The colourless solid was collected by filtration, washed with ethyl acetate and dried to give the title compound (0.34 g, 34%). ESI-MS m/z = 425.2 $(M+Na)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.18–7.02 (m, 4H), 6.12 (br s, 1H), 4.44 (tt, J = 4.1, 12.5 Hz, 1H), 4.31 (br s, 2H), 4.12 (t, J = 6.7 Hz, 2H), 2.92–2.80 (m, 2H), 2.76 (d, J = 4.8 Hz, 3H), 2.67 (t, 2H), 2.33 (ddd, J = 4.5, 12.6, 12.6 Hz, 2H), 1.80 (d, J = 12.2 Hz, 2H), 1.50 (s, 9H).

4.1.17. 4-(3-Methanesulfinylmethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (3r). A solution of **3j** (1.3 g, 3.5 mmol) in glacial acetic acid (15 mL) was treated with sodium perborate tetrahydrate (530 mg, 3.4 mmol) with vigorous stirring, and stirred for 2.5 h at ambient temperature. The solution was poured into ice/water and extracted with dichloromethane. The extracts were combined, washed with dilute aqueous sodium carbonate solution followed by brine. The solution was then dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was subjected to flash chromatography on silica gel (dichloromethane/ethanol; 99:1–96:4; gradient elution) to give the title compound as a clear gum (1.2 g, 87%). ESI-MS m/z = 416.1 $(M+Na)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.32–7.28 (m, 1H), 7.15–7.10 (m, 3H), 5.08 (d, J = 13.6 Hz, 1H), 4.86 (d, J = 13.6 Hz, 1H), 4.43 (tt, J = 4.2, 12.4 Hz, 1H), 4.32 (d, J = 11.5 Hz, 2H), 2.83 (t, J = 12.5 Hz, 2H), 2.66 (s, 3H), 2.34 (ddd, J = 4.6, 12.7, 12.7 Hz, 2H), 1.81 (d, J = 12.2 Hz, 2H), 1.50 (s, 9H).

4.1.18. 4-(3-Methanesulfonylmethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (3s). A solution of **3j** (1.1 g, 2.94 mmol) in glacial acetic acid (20 mL) was treated with sodium perborate tetrahydrate (1.36 g, 8.8 mmol) with vigorous stirring, and stirred overnight at ambient temperature. The mixture was poured into ice/water and extracted with dichloromethane. The extracts were combined, washed with dilute aqueous sodium carbonate solution followed by brine. The solution was then dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was crystallised from diethyl ether to give the title compound as a white solid (1.05 g, 87%). ESI-MS m/z = 432.3 $(M+Na)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.29–7.25 (m, 1H), 7.18–7.15 (m, 3H), 5.05 (s, 2H), 4.45–4.31 (m, 3H), 2.97 (m, 3H), 2.84 (t, J = 12.5 Hz, 2H), 2.34 (ddd, J = 4.6, 12.7, 12.7 Hz, 2H), 1.82 (d, J = 12.1 Hz, 2H), 1.50 (s, 9H).

4.1.19. *N*-Methyl-2-(2-oxo-3-piperidin-4-yl-2,3-dihydro-benzimidazol-1-yl)-acetamide (4f). Trifluoroacetic acid (3.8 mL) was added to a magnetically stirred solution of **3f** (3.32 g, 8.5 mmol) in dichloromethane (40 mL). The reaction mixture was left to stir overnight at ambient temperature and then washed with 4 M aqueous sodium hydroxide solution. The layers were separated and the aqueous further extracted with dichloromethane. The organic extracts were combined, dried (Na_2SO_4), and concentrated under reduced pressure to

give the title compound as a colourless solid (2.0 g, 81%). ESI-MS $m/z = 288.9$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.73–7.33 (m, 1H), 7.16–7.05 (m, 3H), 6.18 (s, 1H), 4.50 (s, 2H), 4.42 (tt, $J = 4.2$, 12.4 Hz, 1H), 3.27 (d, $J = 12.2$ Hz, 2H), 2.84–2.74 (m, 5H), 2.33 (ddd, $J = 4.2$, 12.5, 12.5 Hz, 2H), 1.85 (d, $J = 12.0$ Hz, 2H).

The following compounds were prepared according to the same procedure as **4f**.

4.1.20. 1-Methoxymethyl-3-piperidin-4-yl-1,3-dihydro-benzimidazol-2-one (4a). The title compound was obtained from **3a** as a yellow oil without further purification in 94% yield. ESI-MS $m/z = 262.3$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.29–7.26 (m, 1H), 7.17–7.14 (m, 1H), 7.12–7.07 (m, 2H), 5.29 (s, 2H), 4.44 (tt, $J = 4.2$, 12.5 Hz, 1H), 3.36 (s, 3H), 3.26 (d, $J = 12.2$ Hz, 2H), 2.79 (dt, $J = 2.4$, 12.4 Hz, 2H), 2.34 (ddd, $J = 4.3$, 12.6, 12.6 Hz, 2H), 1.89 (br s, 1H), 1.83 (d, $J = 12.0$ Hz, 2H).

4.1.21. 1-(2-Methoxy-ethyl)-3-piperidin-4-yl-1,3-dihydro-benzimidazol-2-one (4b). The title compound was obtained from **3b** as an orange oil without further purification in 100% yield. ESI-MS $m/z = 276.3$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.28–7.26 (m, 1H), 7.17–7.03 (m, 3H), 4.45 (tt, $J = 4.2$, 12.4 Hz, 1H), 4.05 (t, $J = 5.7$ Hz, 2H), 3.68 (t, $J = 5.6$ Hz, 2H), 3.34 (s, 3H), 3.27 (d, $J = 12.4$ Hz, 2H), 2.81 (dt, $J = 4.3$, 12.6, 12.6 Hz, 2H), 2.37 (ddd, $J = 4.2$, 12.6, 12.6 Hz, 2H), 2.00 (br s, 1H), 1.85 (d, $J = 11.8$ Hz, 2H).

4.1.22. 2-(2-Oxo-3-piperidin-4-yl-2,3-dihydro-benzimidazol-1-yl)-acetamide (4e). The title compound was obtained from **3e** as an oil without further purification in 42% yield. ESI-MS $m/z = 275.2$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.33–7.27 (m, 1H), 7.14–7.04 (m, 3H), 6.19 (br s, 1H), 5.54 (br s, 1H), 4.52 (s, 2H), 4.42 (tt, $J = 4.2$, 12.5 Hz, 1H), 3.26 (d, $J = 12.2$ Hz, 2H), 2.79 (dt, $J = 2.3$, 12.4 Hz, 2H), 2.33 (ddd, $J = 4.2$, 12.5, 12.5 Hz, 2H), 1.84 (d, $J = 12.1$ Hz, 2H).

4.1.23. *N*,*N*-Dimethyl-2-(2-oxo-3-piperidin-4-yl-2,3-dihydro-benzimidazol-1-yl)-acetamide (4g). The title compound was obtained from **3g** as an oil without further purification in 91% yield ESI-MS $m/z = 303.2$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.29–7.23 (m, 1H), 7.10–7.00 (m, 3H), 4.67 (s, 2H), 4.42 (tt, $J = 4.2$, 12.4 Hz, 1H), 3.26 (d, $J = 12.3$ Hz, 2H), 3.13 (s, 3H), 2.97 (s, 3H), 2.79 (dt, $J = 2.2$, 12.4 Hz, 2H), 2.33 (ddd, $J = 4.2$, 12.5, 12.5 Hz, 2H), 1.84 (d, $J = 12.1$ Hz, 2H).

4.1.24. 1-Methylsulfanylmethyl-3-piperidin-4-yl-1,3-dihydro-benzimidazol-2-one (4j). The title compound was obtained from **3j** as a colourless oil without further purification in 100% yield ESI-MS $m/z = 277.8$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.33–7.20 (m, 1H), 7.16–7.07 (m, 3H), 4.98 (s, 2H), 4.44 (tt, $J = 4.2$, 12.4 Hz, 1H), 3.29 (d, $J = 12.3$ Hz, 2H), 2.81 (dt, $J = 2.4$, 12.5 Hz, 2H), 2.38 (ddd, $J = 4.1$, 12.3, 12.3 Hz, 2H), 2.17 (s, 3H), 1.83 (d, $J = 12.0$ Hz, 2H).

4.1.25. 3-(2-Oxo-3-piperidin-4-yl-2,3-dihydro-benzimidazol-1-yl)-propionic acid methyl ester (4k). The title compound was obtained from **3k** as an oil without further purification in 90% yield. ESI-MS $m/z = 304.0$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.30–7.25 (m, 1H), 7.12–7.03 (m, 3H), 4.43 (tt, $J = 4.3$, 12.5 Hz, 1H), 4.12 (t, $J = 7.13$ Hz, 2H), 3.67 (s, 3H), 3.27 (d, $J = 12.2$ Hz, 2H), 2.84–2.76 (m, 4H), 2.35 (ddd, $J = 4.3$, 12.5, 12.5 Hz, 2H), 1.83 (d, $J = 12.0$ Hz, 2H).

4.1.26. 1-(2-Methylsulfanyl-ethyl)-3-piperidin-4-yl-1,3-dihydro-benzimidazol-2-one (4l). The title compound was obtained from **3l** as an oil without further purification in 100% yield ESI-MS $m/z = 292.2$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.31–7.27 (m, 1H), 7.12–7.00 (m, 3H), 4.45 (tt, $J = 4.2$, 12.4 Hz, 1H), 4.08 (t, $J = 7.3$ Hz, 2H), 3.28 (d, $J = 12.2$ Hz, 2H), 2.87–2.76 (m, 4H), 2.35 (ddd, $J = 4.2$, 12.6, 12.6 Hz, 2H), 2.19 (s, 3H), 1.84 (d, $J = 12.0$ Hz, 2H).

4.1.27. 1-[2-(Benzyl-methyl-amino)-ethyl]-3-piperidin-4-yl-1,3-dihydro-benzimidazol-2-one (4n). The title compound was obtained from **3n** as an oil without further purification in 90% yield ESI-MS $m/z = 365.2$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.34–7.17 (m, 6H), 7.06–6.96 (m, 2H), 6.88–6.83 (m, 1H), 4.43 (tt, $J = 4.2$, 12.5 Hz, 1H), 3.99 (t, $J = 7.0$ Hz, 2H), 3.55 (s, 2H), 3.25 (d, $J = 12.3$ Hz, 2H), 2.78 (dt, $J = 2.0$, 12.4 Hz, 2H), 2.72 (t, $J = 7.0$ Hz, 2H), 2.40–2.28 (m, 2H), 2.35 (s, 3H), 1.82 (d, $J = 12.1$ Hz, 2H).

4.1.28. *N*-Methyl-3-(2-oxo-3-piperidin-4-yl-2,3-dihydro-benzimidazol-1-yl)-propionamide (4o). The title compound was obtained from **3o** as an oil without further purification in 100 % yield ESI-MS $m/z = 303.2$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.30–7.25 (m, 1H), 7.16–7.04 (m, 3H), 6.26 (br s, 1H), 4.40 (tt, $J = 4.2$, 12.4 Hz, 1H), 4.19 (t, $J = 6.8$ Hz, 2H), 3.26 (d, $J = 12.2$ Hz, 2H), 2.84–2.73 (m, 5 H), 2.67 (t, $J = 6.8$ Hz, 2H), 2.35 (ddd, $J = 4.2$, 12.5, 12.5 Hz, 2H), 1.82 (d, $J = 12.1$ Hz, 2H).

4.1.29. 1-(2-Dimethylamino-ethyl)-3-piperidin-4-yl-1,3-dihydro-benzimidazol-2-one (4m). A solution of **3u** (7.38 g, 19.4 mmol) in ethanol (50 mL) was treated with dimethylamine (50.0 mL, 33% solution in ethanol) and the resultant solution heated at 85 °C for 12 h. The volatiles were removed under reduced pressure and the oily residue partitioned between ethyl acetate and water. The organic layer was collected and extracted with 2 N hydrochloric acid solution. The acid extracts were collected, washed with ethyl acetate then adjusted to pH 14 by addition of 10 M potassium hydroxide solution and extracted with ethyl acetate. The organic extracts were washed with water, dried (Na_2SO_4), filtered, and evaporated to give the title compound as a yellow oil (3.74 g, 67%). ESI-MS $m/z = 289.2$ $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.29–7.24 (m, 1H), 7.10–7.00 (m, 3H), 4.45 (tt, $J = 4.2$, 12.4 Hz, 1H), 3.98 (t, $J = 7.3$ Hz, 2H), 3.26 (d, $J = 12.2$ Hz, 2H), 2.79 (dt, $J = 4.3$, 12.4 Hz, 2H), 2.64 (t, $J = 7.3$ Hz, 2H), 2.39-2.27

(ddd, J = 4.2, 12.6, 12.6 Hz, 2H), 2.33 (s, 6H), 1.83 (d, J = 12.0 Hz, 2H).

4.1.30. 1-Methanesulfinylmethyl-3-piperidin-4-yl-1,3-dihydro-benzimidazol-2-one (4r). A solution of **3r** (1.20 g, 3.0 mmol) in dichloromethane (15 mL) was treated with trifluoroacetic acid (2.5 mL, 30 mmol) and left to stand overnight at ambient temperature. The solution was evaporated to dryness, the residue triturated with diethyl ether, filtered and dried to give the title compound as a colourless oil (1.10 g, 90%). ESI-MS m/z = 294.3 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.79 (br s, 1H, NH), 8.55 (br s, 1H, NH), 7.42–7.36 (m, 2H), 7.15–7.08 (m, 2H), 5.10 (dd, J = 13.5, 13.5 Hz, 2H), 4.63–4.57 (m, 1H), 3.45 (d, J = 11.7 Hz, 2H), 3.16–3.11 (m, 2H), 2.68 (s, 3H), 2.60–2.54 (m, 2H), 1.92 (d, J = 12.5 Hz, 2H).

4.1.31. 1-Methanesulfonylmethyl-3-piperidin-4-yl-1,3-dihydro-benzimidazol-2-one (4s). Prepared in the same manner as **4r**, using **3s**. Title compound isolated as a white solid in 100% yield. ESI-MS m/z = 310.1 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.77 (br s, 1H, NH), 8.51 (br s, 1H, NH), 7.42 (d, J = 7.1 Hz, 1H), 7.38 (d, J = 7.1 Hz, 1H), 7.18–7.11 (m, 2H), 5.39 (s, 2H), 4.64–4.56 (m, 1H), 3.45 (d, J = 12.3 Hz, 2H), 3.16–3.00 (m, 2H), 3.05 (s, 3H), 2.62-2.55 (m, 2H), 1.94 (d, J = 12.1 Hz, 2H).

4.1.32. 1-Chloro-4-methyl-pentan-3-ol (5). A magnetically stirred mixture of 1-chloro-4-methyl-pentan-3-one[27] (12.63 g, 93.9 mmol) in tetrahydrofuran (100 mL) and ethanol (10 mL) was cooled in an ice/salt bath. Sodium borohydride (3.93 g, 103.3 mmol) was added portionwise at 0 °C. On completion, the cooling bath was removed and the mixture was stirred at room temperature for 24 h. The reaction was quenched with ice-cold saturated aqueous ammonium chloride solution and extracted with ether. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give the title compound as a dark oil (6.70 g, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.67–3.69 (m, 2H), 3.62–3.58 (m, 1H), 1.95–1.78 (m, 2H), 1.72–1.62 (m, 1H), 1.52 (m, 1H), 0.94 (dd, J = 6.9 and 1.7 Hz, 6H).

4.1.33. 2-[1-(2-Chloro-ethyl)-2-methyl-propoxy]-4-methoxy-1-methyl-benzene (6). Diethyl azodicarboxylate (0.475 mL, 3.00 mmol) was added to a magnetically stirred solution of 5-methoxy-2-methylphenol (0.413 g, 3.00 mmol), **5** (0.342 g, 2.50 mmol) and triphenylphosphine (0.785 g, 3.00 mmol) in tetrahydrofuran (20 mL). After 18 h, the solution was concentrated under reduced pressure and the residue was subjected to flash chromatography on silica gel (dichloromethane/diethyl ether; 100:0–80:20; gradient elution) to give the title compound as a colourless oil (0.172 g, 27%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.94 (d, J = 8.3 Hz, 1H), 6.44 (d, J = 2.4 Hz, 1H), 6.31 (dd, J = 8.2 and 2.4 Hz, 1H), 4.31–4.26 (m, 1H), 3.70 (s, 3H), 3.63–3.51 (m, 2H), 2.12–2.04 (m, 1H), 2.06 (s, 3H), 2.01–1.89 (m, 2H), 0.88 (dd, J = 10.1 and 6.9 Hz, 6H).

4.1.34. 2-(3-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-*N*-methyl-acetamide (7f). A magnetically stirred suspension of **6** (4.13 g, 16.1 mmol), **4f** (3.57 g, 12.4 mmol), triethylamine (3.0 mL, 40.0 mmol) and sodium iodide (catalytic) in anhydrous *N*,*N*-dimethylformamide (40 mL) was heated at 80 °C for 3 days. The reaction mixture was allowed to cool and then partitioned between ethyl acetate and water. The organic layer was collected and the aqueous layer was again extracted with ethyl acetate. The organic layers were combined, washed with water, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was subjected to flash chromatography on silica (ethyl acetate/methanol; 100:0–9:1; gradient elution) to give the title compound as a pale yellow oil (3.80 g, 40%). ESI-MS m/z = 509.4 $(M+H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39–7.35(m, 1H), 7.14–7.09 (m, 2H), 7.08–7.04 (m, 1H), 7.03 (d, J = 8.2 Hz, 1H), 6.63 (d, J = 2.3 Hz, 1H), 6.37 (dd, J = 2.4, 8.2 Hz, 1H), 6.15 (d, J = 4.1 Hz, 1H), 4.51 (s, 2H), 4.40 (tt, J = 4.2, 12.5 Hz, 1H), 4.27–4.21 (m, 1H), 3.76 (s, 3H), 3.11 (d, J = 11.7 Hz, 1H), 3.00 (d, J = 11.8 Hz, 1H), 2.79 (d, J = 4.9 Hz, 3H), 2.55–2.38 (m, 4H), 2.16 (s, 3H), 2.17–1.96 (m, 3H), 1.89–1.75 (m, 4H), 0.98 (dd, J = 6.8, 14.9 Hz, 6H).

4.1.35. (+)-2-(3-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-*N*-methyl-acetamide methanesulfonate [(+)-7f] and (−)-2-(3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-*N*-methyl-acetamide methanesulfonate [(−)-7f]. Racemic **7f** was subjected to chiral chromatography on a Chiralpak® AD column (2 × 25 cm), eluting with isohexane/isopropanol/diethylamine (85:15:0.1; v/v) at 15 mL/min. The purified enantiomers were converted to the methanesulfonate salts with 1 equiv of methanesulfonic acid in dichloromethane and then precipitated twice from a concentrated acetone solution by flooding with diethyl ether to give the title compounds. Compound (+)-**7f** HPLC purity 98.3%, enantiomeric ratio 100:0.0, $[\alpha]_D$ = +7.3° (3.54 mg/mL in $CHCl_3$ at 20 °C), ESI-MS m/z = 509.4 $(M+H)^+$. Compound (−)-**7f**; HPLC purity 98.0%, enantiomeric ratio 1.8:98.2, $[\alpha]_D$ = −5.7° (3.19 mg/mL in $CHCl_3$ at 20 °C), ESI-MS m/z = 509.4 $(M+H)^+$.

The following compounds were prepared by the same procedure as for **7f**.

4.1.36. 1-Methoxymethyl-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one hydrochloride (7a). Flash chromatography on silica gel (dichloromethane/methanol; 99:1 to 98:2; gradient elution) gave the title compound as the free base. The compound was dissolved in dichloromethane and 2.0 M hydrochloric acid in diethyl ether added until a precipitate formed. This was filtered and dried to give the hydrochloride salt as a white solid in 19% yield. ESI-MS m/z = 482.5 $(M+H)^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 7.34 (d, J = 7.0 Hz, 1H), 7.27-7.24 (m, 1H), 7.19–7.13 (m, 2H), 7.04 (d, J = 8.3 Hz, 1H), 6.52 (d, J = 2.3 Hz, 1H), 6.43 (dd, J = 2.3, 8.3 Hz,

1H), 5.28 (s, 2 H), 4.58 (tt, J = 4.1, 12.2 Hz, 1H), 4.36 (dd, J = 5.6, 10.9 Hz, 1H), 3.76 (s, 3H), 3.76–3.71 (m, 2H), 3.33 (s, 3H), 3.34–3.15 (m, 4H), 2.84 (ddd, J = 3.9, 12.8, 14.0 Hz, 2H), 2.16 (s, 3H), 2.20–2.04 (m, 5H), 1.03 (dd, J = 6.8, 14.9 Hz, 6H).

4.1.37. (+)-1-Methoxymethyl-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one methanesulfonate [(+)-7a] and (−)-1-Methoxymethyl-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one methanesulfonate [(−)-7a]. Racemic **7a** was subjected to chiral chromatography on a Chiralpak® AD column (2 × 25 cm), eluting with isohexane/isopropanol (90:10; v/v) at 15 mL/min. The purified enantiomers were converted to the methanesulfonate salts with 1 equiv of methanesulfonic acid in dichloromethane and then precipitated twice from a concentrated acetone solution by flooding with diethyl ether to give the title compounds. Compound (+)-**7a** HPLC purity 99.2%, enantiomeric ratio 99.6:0.4, $[\alpha]_D$ = +26.6° (5.52 mg/mL in MeOHat 20 °C), ESI-MS m/z = 482.4 $(M+H)^+$. Compound (-)-**7a** HPLC purity 99.4%, enantiomeric ratio 0.6:99.4, $[\alpha]_D$ = −26.2° (5.62 mg/mL in MeOH at 20 °C), ESI-MS m/z = 482.4 $(M+H)^+$.

4.1.38. 1-(2-Methoxy-ethyl)-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one hydrochloride (7b). Flash chromatography on silica gel (dichloromethane/methanol; 19:1; as eluant) gave the title compound as the free base. The compound was dissolved in dichloromethane and 2.0 M hydrochloric acid in diethyl ether added until a precipitate formed. The solid was collected by filtration and crystallised from acetone and dried to give the hydrochloride salt as a colourless solid in 70% yield. ESI-MS m/z = 496.2 $(M+H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32–7.30 (m, 1H), 7.12–7.05 (m, 3H), 7.01 (d, J = 8.0 Hz, 1H), 6.61 (d, J = 2.3 Hz, 1H), 6.36 (dd, J = 2.4, 8.2 Hz, 1H), 4.44–4.35 (m, 1H), 4.23 (dd, J = 5.0, 11.3 Hz, 1H), 4.05 (t, J = 5.7 Hz, 2H), 3.76 (s, 3H), 3.67 (t, J = 5.6 Hz, 2H), 3.34 (s, 3H), 3.09 (d, J = 10.4 Hz, 1H), 2.98 (d, J = 10.9 Hz, 1H), 2.49–2.39 (m, 4H), 2.15 (s, 3H), 2.14–1.98 (m, 3H), 1.88–1.76 (m, 4H), 0.98 (dd, J = 6.8, 9.0 Hz, 6H).

4.1.39. (+)-1-(2-Methoxy-ethyl)-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one methanesulfonate [(+)-7b] and (−)-1-(2-methoxy-ethyl)-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one methanesulfonate [(−)-7b]. Racemic **7b** was subjected to chiral chromatography as above for **7a** to give the title compounds. Compound [(+)-**7b**] HPLC purity 99.0%, enantiomeric ratio 100:0.0, $[\alpha]_D$ = +6.4°(4.43 mg/mL in $CHCl_3$ at 20 °C), ESI-MS m/z = 496.2 $(M+H)^+$. Compound [(−)-**7b**] HPLC purity 98.1%, enantiomeric ratio 0.3:99.7, $[\alpha]_D$ = −4.8°(5.17 mg/mL in $CHCl_3$ at 20 °C), ESI-MS m/z = 496.2 $(M+H)^+$.

4.1.40. 2-(3-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetamide (7e). Crude material was crystallised from ethyl acetate to give the title compound in 2 crops as a white solid in 55% yield. ESI-MS m/z = 495.2 $(M+H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): 7.40–6.95 (m, 5H), 6.70–6.54 (m, 1H), 6.44–6.28 (m, 1H), 6.16 (br s, 1H), 5.45 (br s, 1H), 4.58–4.44 (m, 2H), 4.43–4.29 (m, 1H), 4.27–4.18 (m, 1H), 4.16–04.04 (m, 1H), 3.82–3.68 (m, 3H), 3.14–2.90 (2d, J = 9.8 Hz, rotamer 1 + rotamer 2, 2H), 2.56–2.32 (m, 4H), 2.21–1.96 (m, 8H), 1.86–1.72 (m, 4H), 1.71–1.59 (m, 2H).

4.1.41. 2-(3-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-*N*,*N*-dimethyl-acetamide (7g). Flash chromatography on silica gel (ethyl acetate/triethylamine; 100:0–9:1; gradient elution) gave the title compound as an oil (80%). ESI-MS m/z = 523.0 $(M+H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.28 (m, 1H), 7.08–7.00 (m, 4H), 6.62 (d, J = 2.3 Hz, 1H), 6.36 (dd, J = 2.4, 8.2 Hz, 1H), 4.67 (s, 2H), 4.37 (tt, J = 4.2, 12.5 Hz, 1H), 4.24 (dd, J = 4.9, 11.4 Hz, 1H), 3.76 (s, 3H), 3.16–3.04 (m, 1H), 3.13 (s, 3H), 3.02–2.92 (m, 1H), 2.97 (s, 3H), 2.56–2.37 (m, 4H), 2.18–1.95 (m, 3H), 2.16 (s, 3H), 1.88–1.64 (m, 4H), 1.00 (dd, J = 6.8, 8.9 Hz, 6H).

4.1.42. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-methylsulfanylmethyl-1,3-dihydro-benzimidazol-2-one (7j). Flash chromatography on silica (ethyl acetate/40–60 petrol; 1:1) gave the title compound as an oil in 47% yield. ESI-MS m/z = 497.8 $(M+H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.30 (m, 1H), 7.14–7.06 (m, 3H), 7.02 (d, J = 8.1 Hz, 1H), 6.62 (d, J = 2.4 Hz, 1H), 6.36 (dd, J = 2.4, 8.2 Hz, 1H), 4.98, (s, 2H), 4.37 (tt, J = 4.2, 12.5 Hz, 1H), 4.27–4.21 (m, 1H), 3.76 (s, 3H), 3.09 (d, J = 11.8 Hz, 1H), 2.97 (d, J = 11.7 Hz, 1H), 2.54-2.37 (m, 4H), 2.17 (s, 3H), 2.16 (s, 3H), 2.12–1.97 (m, 4H), 1.87–1.72 (m, 3H), 0.98 (dd, J = 6.9, 9.0 Hz, 6H).

4.1.43. 3-(3-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-propionic acid methyl ester (7k). Flash chromatography on silica gel (ethyl acetate) gave the title compound as a pale yellow oil in 51% yield. ESI-MS m/z = 524.4 $(M+H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.28 (m, 1H), 7.11–7.03 (m, 3H), 7.02 (d, J = 8.0 Hz, 1H), 6.62 (d, J = 2.4 Hz, 1H), 6.36 (dd, J = 2.4, 8.2 Hz, 1H), 4.37 (tt, J = 4.2, 12.5 Hz, 1H), 4.24 (dd, J = 4.7, 11.5 Hz, 1H), 4.18 (t, J = 7.1 Hz, 2H), 3.76 (s, 3H), 3.67 (s, 3H), 3.08 (d, J = 11.8 Hz, 1H), 2.97 (d, J = 11.8 Hz, 1H), 2.79 (t, J = 7.2 Hz, 2H), 2.52–2.34 (m, 4H), 2.18–1.96 (m, 3H), 2.16 (s, 3H), 1.88–1.72 (m, 4H), 0.98 (dd, J = 6.8, 9.0 Hz, 6H).

4.1.44. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-(2-methylsulfanyl-ethyl)-1,3-dihydro-benzimidazol-2-one (7l). Flash chromatography on silica gel (ethyl acetate/methanol; 100:0–9:1; gradient elution) gave the title compound as a clear oil in 58% yield. ESI-MS m/z = 512.4 $(M+H)^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 7.3–7.28 (m, 1H), 7.23–7.18 (m, 1H), 7.18-7.12 (m, 2H), 7.02 (d, J = 8.2 Hz, 1H), 6.52 (m, 1H), 6.43 (dd, J = 2.4, 8.3 Hz, 1H), 4.56 (tt,

J = 3.85, 12.05 Hz, 1H), 4.36 (dd, J = 5.3, 10.6 Hz, 1H), 4.11 (t, J = 6.8 Hz, 2H), 3.76 (s, 3H), 3.77–3.69 (m, 2H), 3.35-3.16 (m, 5H), 2.87–2.75 (m, 2H), 2.85 (t, J = 6.8 Hz, 2H), 2.20–2.04 (m, 4H), 2.16 (s, 3H), 2.12 (s, 3H), 1.03 (dd, J = 6.8, 14.5 Hz, 6H).

4.1.45. 1-(2-Dimethylamino-ethyl)-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one (7m). Flash chromatography on silica gel (ethyl acetate/triethylamine; 9:1; as eluant) gave the product as an oil in 62 % yield. ESI-MS m/z = 509.4 $(M+H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.28 (m, 1H), 7.10–7.00 (m, 4H), 6.62 (d, J = 2.4 Hz, 1H), 6.36 (dd, J = 2.4, 8.2 Hz, 1H), 4.38 (tt, J = 4.2, 12.5 Hz, 1H), 4.24 (dd, J = 4.8, 11.5 Hz, 1H), 3.98 (t, J = 7.3 Hz, 2H), 3.76 (s, 3H), 3.67 (s, 3H), 3.08 (d, J = 11.8 Hz, 1H), 2.97 (d, J = 12.5 Hz, 1H), 2.63 (t, J = 7.3 Hz, 2H), 2.52–2.34 (m, 4H), 2.33 (s, 6H), 2.18–1.96 (m, 3H), 2.16 (s, 3H), 1.88–1.72 (m, 4H), 0.98 (dd, J = 6.8, 9.0 Hz, 6H).

4.1.46. 1-[2-(Benzyl-methyl-amino)-ethyl]-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one (7n). Flash chromatography on silica gel (dichloromethane/methanol; 98:2; as eluant) gave the product as an oil in 46% yield. ESI-MS m/z = 585.2 $(M+H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 (d, J = 7.3 Hz, 1H), 7.24–7.15 (m, 5H), 7.06–6.96 (m, 3H), 6.87–6.83 (m, 1H), 6.62 (d, J = 2.4 Hz, 1H), 6.36 (dd, J = 2.4, 8.2 Hz, 1H), 4.38 (tt, J = 4.2, 12.5 Hz, 1H), 4.24 (dd, J = 4.8, 11.5 Hz, 1H), 3.98 (t, J = 7.0 Hz, 2H), 3.76 (s, 3H), 3.55 (s, 2H), 3.08 (d, J = 11.7 Hz, 1H), 2.97 (d, J = 11.7 Hz, 1H), 2.71 (t, J = 7.0 Hz, 2H), 2.52–2.36 (m, 4H), 2.35 (s, 3H), 2.18–1.96 (m, 3H), 2.16 (s, 3H), 1.88–1.72 (m, 4H), 0.98 (dd, J = 6.8, 9.0 Hz, 6H).

4.1.47. 3-(3-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-*N*-methyl-propionamide (7o). Flash chromatography on silica gel (dichloromethane/methanol; 98:2; as eluant) gave the product as an oil in 32% yield. ESI-MS m/z = 523.0 $(M+H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (d, J = 6.9 Hz, 1H), 7.16–7.04 (m, 3H), 7.02 (d, J = 8.7 Hz, 1H), 6.61 (d, J = 2.4 Hz, 1 H), 6.37 (dd, J = 2.4, 8.2 Hz, 1H), 6.26 (br d, J = 3.9 Hz, 1H), 4.36 (tt, J = 4.2, 12.5 Hz, 1H), 4.23 (dd, J = 4.6, 11.6 Hz, 1H), 4.19 (t, J = 6.7 Hz, 2H), 3.76 (s, 3 H), 3.09 (d, J = 11.7 Hz, 1H), 2.99 (d, J = 11.5 Hz, 1H), 2.76 (d, J = 4.7 Hz, 3H), 2.67 (t, J = 6.7 Hz, 2H), 2.54–2.36 (m, 4H), 2.18–1.96 (m, 3H), 2.16 (s, 3H), 1.88–1.68 (m, 4H), 0.98 (dd, J = 6.8, 9.0 Hz, 6H).

4.1.48. 1-Methanesulfinylmethyl-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one methanesulfonate (7r). Flash chromatography on silica gel (dichloromethane/methanol; 98:2; as eluant) gave the title compound as the free base. Dissolving the compound in dichloromethane and adding one equivalent of methanesulfonic acid prepared the methane sulfonate salt. The solution was then evaporated and acetone/diethyl ether added to form a precipitate, which was collected by filtration and dried to give the title compound as a white solid in 69% yield. ESI-MS m/z = 514.3 $(M+H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34-7.28 (m, 2H), 7.13–7.11 (m, 2H), 7.02 (d, J = 8.3 Hz, 1H), 6.63 (s, 1H), 6.36 (dd, J = 2.4, 8.2 Hz, 1H), 5.09 (d, J = 13.5 Hz, 1H), 4.87 (d, J = 13.5 Hz, 1H), 4.36 (tt, J = 4.3, 8.3 Hz, 1H), 4.25-4.23 (m, 1H), 3.76 (s, 3H), 3.09 (d, J = 10.6 Hz, 1H), 2.97 (d, J = 10.6 Hz, 1H), 2.67 (s, 3H), 2.50–2.42 (m, 4H), 2.16 (s, 3H), 2.12–1.97 (m, 3H), 1.85–1.75 (m, 4H), 1.56 (s, 3H), 0.98 (dd, J = 6.9, 9.1 Hz, 6H).

4.1.49. 1-Methanesulfonylmethyl-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one methanesulfonate (7s). Flash chromatography on silica gel (dichloromethane/methanol; 100:0–98:2; gradient elution) gave the title compound as the free base. Dissolving the compound in dichloromethane and adding one equivalent of methanesulfonic acid prepared the methane sulfonate salt. The solution was then evaporated and acetone/diethyl ether added to form a precipitate, which was collected by filtration and dried to give the title compound as a white solid in 45% yield. ESI-MS m/z = 530.2 $(M+H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36–7.34 (m, 1H), 7.28–7.24 (m, 1H), 7.16–7.13 (m, 2H) 7.02 (d, J = 8.2 Hz, 1H), 6.63 (s, 1H), 6.39–6.36 (m, 1H), 5.06 (s, 2H), 4.36–4.31 (m, 1H), 4.29–4.23 (m, 1H), 3.76 (s, 3H), 3.10 (d, J = 11.0 Hz, 1H), 2.98 (s, 3H), 3.00–2.97 (m, 1H), 2.51–2.40 (m, 4H), 2.16 (s, 3H), 2.14–1.62 (m, 7H), 0.98 (dd, J = 7.3, 9.3 Hz, 6H).

4.1.50. 1-Ethoxymethyl-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one (7c). Trifluoroacetic acid (20 mL) was added to a magnetically stirred solution of **3c** (4.0 g, 10.7 mmol) in dichloromethane (120 mL). The reaction mixture was left to stir for 3 h at room temperature then neutralised with aqueous sodium carbonate solution and the organic layer collected. The organics were then washed with water and brine before drying over Na_2SO_4, filtering and concentrating under reduced pressure to give the intermediate amine as a colourless solid. The amine, **4c**, was then used without further purification in a similar manner to the procedure outlined for **7f** obtaining the title compound as a colourless solid in 65% yield. ESI-MS m/z = 496.5 $(M+H^+)$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36–7.31 (m, 1H), 7.22–7.17 (m, 1H), 7.13–7.07 (m, 2H) 7.02 (d, J = 8.2 Hz, 1H), 6.62 (d, J = 2.3 Hz, 1H), 6.36 (dd, J = 2.4, 8.2 Hz, 1H), 5.33 (s, 2H), 4.39 (tt, J = 4.3, 13.0 Hz, 1H), 4.25–4.21 (m, 1H), 3.76 (s, 3H), 3.58 (q, J = 7.0 Hz, 2H), 3.09 (d, J = 11.5 Hz, 1H), 2.98 (d, J = 11.5 Hz, 1H), 2.53–2.37 (m, 4H), 2.15 (s, 3H), 2.14–1.97 (m, 3H), 1.87-1.76 (m, 4H), 1.18 (t, J = 7.0 Hz, 3H), 0.98 (dd, J = 6.8, 9.7 Hz, 6H).

4.1.51. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-(2-oxo-butyl)-1,3-dihydro-benzimidazol-2-one methanesulfonate (7d). Trifluoroacetic acid (1.6 mL) was added to a magnetically stirred solution of **3d** (800 mg, 2.1 mmol) in dichloromethane (16 mL). The reaction mixture was left to stir for 3 h at room temperature. The mixture was then evaporated to a

low volume and diethyl ether added until a white solid formed, which was filtered and then dried. The trifluoroacetic acid salt of the amine, **4d** (~600 mg), was then used without further purification in a similar manner to the procedure outlined for **7f**. Flash chromatography on silica gel (dichloromethane/ethanol; 100:0–98:2; gradient elution) gave the product as a colourless solid, which was then dissolved in acetone and methanesulfonic acid (72 mg) added followed by diethyl ether to form a precipitate which was collected by filtration to give the title compound as a colourless solid in 34% yield. ESI-MS m/z = 508.3 $(M+H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 11.28 (br s, 1H, NH), 7.72 (d, J = 7.9 Hz, 1H), 7.17 (t, J = 7.4 Hz, 1H), 7.10–7.03 (m, 2H), 6.78 (d, J = 7.5 Hz, 1H), 6.42–6.39 (m, 2H), 4.70 (tt, J = 4.2, 12.6 Hz, 1H), 4.61 (s, 2H), 4.27–4.23 (m, 1H), 3.86 (d, J = 10.9 Hz, 1H), 3.78 (s, 3H), 3.78–3.73 (m, 1H), 3.27–3.05 (m, 4H), 2.88 (s, 3H), 2.85–2.77 (m, 2H), 2.51 (q, J = 7.3 Hz, 2H), 2.40-2.34 (m, 1H), 2.33–2.13 (m, 1H), 2.14 (s, 3H), 1.11 (t, J = 7.3 Hz, 3H), 1.00 (dd, J = 6.8, 17.5 Hz, 6H).

4.1.52. (3-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetic acid methyl ester hydrochloride (7h). Trifluoroacetic acid (35 mL) was added to a magnetically stirred solution of **3h** (7.0 g, 18.0 mmol) in dichloromethane (200 mL). The reaction mixture was left to stir for 3 h at room temperature then neutralised with aqueous sodium bicarbonate solution and the organic layer collected. The organics were then washed with water and brine before drying over Na_2SO_4, filtering and concentrating under reduced pressure to give the intermediate amine, **4h**, as a colourless solid. The amine was then used without further purification in a similar manner to the procedure outlined for **7f**. Flash chromatography on silica gel (dichloromethane/methanol; 100:0–98:2; gradient elution) gave the title compound as a colourless solid. The compound was dissolved in dichloromethane and 2.0 M hydrochloric acid in diethyl ether added until a precipitate formed. The solid was collected by filtration, crystallised from acetone and dried to give the hydrochloride salt as a colourless solid in 45 % yield. ESI-MS m/z = 510.4 $(M+H)^+$. ^1H NMR (400 MHz, CD_3OD): δ 7.29 (d, J = 6.6 Hz, 1H), 7.18-7.09 (m, 3H), 7.04 (d, J = 8.5 Hz, 1H), 6.52 (d, J = 2.3 Hz, 1H), 6.43 (dd, J = 2.4, 8.3 Hz, 1H), 4.72 (s, 2H), 4.58–4.51 (m, 1H), 4.37–4.33 (m, 1H), 3.76 (s, 3H), 3.75 (s, 3H), 3.78–3.69 (m, 3H), 3.28–3.12 (m, 3H), 3.85–2.74 (m, 2H), 2.16 (s, 3H), 2.17–2.03 (m, 5H), 1.03 (dd, J = 6.8, 14.7 Hz, 6H).

4.1.53. Acetic acid 2-(3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-ethyl ester hydrochloride (7i). A magnetically stirred solution of **2** (20.0 g, 63 mmol), in anhydrous *N*,*N*-dimethylformamide (60 mL) was treated with sodium hydride (60% dispersion in mineral oil, 3.0 g, 76 mmol) portion-wise under a nitrogen atmosphere. The reaction mixture was heated to 50 °C for 3 h to give a colourless solution. On cooling to room temperature 2-bromoethyl acetate (9 mL, 80 mmol) was added dropwise, with cooling to maintain ambient temperature, and the resultant mixture stirred overnight. The reaction mixture was quenched with ice, poured into water and extracted with ethyl acetate. The organic extracts were washed with water followed by brine, dried (Na_2SO_4) and concentrated under reduced pressure to a low volume. This was then cooled in ice and the resultant solid filtered, washed with ethyl acetate and dried to give the crude precursor **3i** as an orange gum. Trifluoroacetic acid (40 mL) was added to a magnetically stirred solution of crude **3i** (6.0 g, 14.8 mmol) in dichloromethane (200 mL). The reaction mixture was left to stir for 2 h at room temperature then neutralised with aqueous sodium carbonate solution and the organic layer collected. The organics were then washed with water and brine before drying over Na_2SO_4, filtering and concentrating under reduced pressure to give the intermediate amine as a yellow gum. The amine **4i** was then used without further purification in a similar manner to the procedure outlined for **7f**. Flash chromatography on silica gel (dichloromethane/methanol; 100:0–98:2; gradient elution) gave the title compound as a colourless solid. The compound was dissolved in dichloromethane and 2.0 M hydrochloric acid in diethyl ether added until a precipitate formed. The solid was collected by filtration, crystallised from acetone and dried to give the hydrochloride salt as a colourless solid in 14% yield. ESI-MS m/z = 524 $(M+H)^+$. ^1H NMR (400 MHz, CD_3OD): δ 7.34–7.32 (m, 1H), 7.23–7.21 (m, 1H), 7.17–7.13 (m, 2H), 7.03 (d, J = 8.2 Hz, 1H), 6.52 (d, J = 2.3 Hz, 1H), 6.43 (dd, J = 2.3, 8.2 Hz, 1H), 4.57 (tt, J = 4.1, 12.1 Hz, 1H), 4.37 (t, J = 5.3 Hz, 2H), 4.36–4.34 (m, 1H), 4.15 (t, J = 5.2 Hz, 2H), 3.75 (s, 3H), 3.77-3.72 (m, 2H), 3.31–3.19 (m, 4H), 2.83 (ddd, J = 3.8, 13.7, 13.8 Hz, 2H), 2.16 (s, 3H), 2.19–2.14 (m, 2H), 2.09–2.05 (m, 3H), 1.85 (s, 3H), 1.03 (dd, J = 6.8, 14.6 Hz, 6H).

4.1.54. (3-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetic acid hydrochloride (7p). A magnetically stirred solution of **7h** (3.80 g, 7.10 mmol) in ethanol (100 mL) was treated with 4 M aqueous sodium hydroxide solution (2.0 mL), and the mixture heated at 60 °C for 4 h. The solvents were removed under reduced pressure, and the residue treated with 10 M aqueous hydrochloric acid solution. The aqueous was extracted with dichloromethane, and the organic extracts combined, dried (Na_2SO_4) and filtered. The filtrate was treated with 2 M hydrochloric acid in diethyl ether solution and concentrated under reduced pressure. Precipitation with diethyl ether followed by filtration gave the title compound as a yellow solid (2.5 g, 66%). ESI-MS m/z = 496.2 $(M+H)^+$. ^1H NMR (400 MHz, CD_3OD): δ 7.33–7.32 (m, 1H), 7.16–7.08 (m, 3H), 7.04 (d, J = 8.2 Hz, 1H), 6.52–6.51 (m, 1H), 6.44–6.42 (m, 1H), 4.65 (s, 2H), 4.61–4.55 (m, 1H), 4.38–4.34 (m, 1H), 3.76 (s, 3H), 3.77–3.72 (m, 2H), 2.88–2.77 (m, 2H), 2.16 (s, 3H), 2.19–2.03 (m, 5H), 1.03 (dd, J = 6.8, 14.7 Hz, 6H).

4.1.55. 1-(2-Hydroxy-ethyl)-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one hydrochloride (7q). Prepared following a similar procedure to **7p** using **7i** in place

of **7h**. Two molar hydrochloric acid in diethyl ether solution was added to the crude material and concentrated under reduced pressure. Precipitation with diethyl ether followed by crystallisation from methanol/dichloromethane gave the title compound as a white solid in 52% yield. ESI-MS m/z = 482.5 $(M+H)^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 7.34–7.32 (m, 1H), 7.24–7.21 (m, 1H), 7.15–7.10 (m, 2H), 7.03 (d, J = 8.1 Hz, 1H), 6.52 (d, J = 2.3 Hz, 1H), 6.43 (dd, J = 2.3, 8.3 Hz, 1H), 4.58 (tt, J = 4.2, 12.2 Hz, 1H), 4.36 (dd, J = 5.6, 10.9 Hz, 1H), 3.99 (t, J = 5.6 Hz, 2H), 3.80 (t, J = 5.6 Hz, 2H), 3.75 (s, 3H), 3.77–3.71 (m, 2H), 3.32–3.19 (m, 4H), 2.84 (ddd, J = 3.9, 13.6, 13.8 Hz, 2H), 2.15 (s, 3H), 2.20–2.14 (m, 2H), 2.09–2.05 (m, 3H), 1.03 (dd, J = 6.8, 14.6 Hz, 6H).

4.1.56. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-(2-methylamino-ethyl)-1,3-dihydro-benzimidazol-2-one (7t). A solution of **7n** (1.90 g, 3.3 mmol) in AR methanol was hydrogenated over a palladium hydroxide catalyst for 10 h. The mixture was filtered and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (ethyl acetate/2 M ammonia in methanol; 9:1; as eluant) followed by hydrochloride salt formation and crystallisation from diethyl ether/ethanol to give the product as a colourless solid (0.16 g, 9%). ESI-MS m/z = 482.5 $(M+H)^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 7.46 (br s, 1H), 7.33–7.23 (m, 1H), 7.21–7.15 (m, 2H), 7.03 (d, J = 8.2 Hz, 1H), 6.53 (d, J = 2.1 Hz, 1H), 6.43 (dd, J = 2.3, 8.3 Hz, 1H), 4.64 (tt, J = 4.2, 12.3 Hz, 1H), 4.38 (dd, J = 5.5, 10.7 Hz, 1H), 4.25 (t, J = 5.7 Hz, 2H), 3.75 (s, 3H), 3.81–3.70 (m, 2H), 3.42 (t, J = 5.7 Hz, 2H), 3.37-3.18 (m, 3H), 2.96–2.82 (m, 2H), 2.76 (s, 3H), 2.23–2.02 (m, 6H), 2.16 (s, 3H) 1.03 (dd, J = 6.8, 14.8 Hz, 6H).

4.1.57. *N*-Ethyl-2-(3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]- piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetamide hydrochloride (8). To a solution of **7p** (250 mg, 0.47 mmol) in dichloromethane (5 mL) was added diisopropylethylamine (0.27 mL, 1.6 mmol) under nitrogen at room temperature. Oxalyl chloride (0.05 mL, 0.52 mmol) was then added dropwise and stirred for 1 h. Ethylamine solution (2.0 M in tetrahydrofuran, 0.3 mL, 0.6 mmol) was added and stirred for 24 h. The resultant mixture was partitioned between dichloromethane and water and the organics collected. The organic layer was then dried (Na_2SO_4), filtered and evaporated to dryness to give a yellow residue. Flash chromatography on silica gel (dichloromethane/methanol; 99:1; as eluant) gave the title compound as the free base. The compound was dissolved in dichloromethane and 2.0 M hydrochloric acid in diethyl ether added until a precipitate formed. The solid was collected by filtration, crystallised from acetone and dried to give the title compound as a colourless solid in 10% yield. ESI-MS m/z = 523.4 $(M+H)^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 7.31 (d, J = 6.7 Hz, 1H), 7.16–7.10 (m, 2H), 7.06–7.02 (m, 2H), 6.51 (d, J = 2.3 Hz, 1H), 6.43 (dd, J = 2.3, 8.3 Hz, 1H), 4.60–4.53 (m, 1H), 4.53 (s, 2H), 4.37–4.33 (m, 1H), 3.75 (s, 3H), 3.74–3.70 (m, 1H), 3.24 (q, J = 7.3 Hz, 2H), 3.19–3.17 (m, 5H), 2.86–2.75 (m, 2H), 2.16 (s, 3H), 2.18–2.05 (m, 5H), 1.13 (t, J = 7.3 Hz, 3H), 1.03 (dd, J = 6.8, 14.5 Hz, 6H).

The following compounds were prepared by the same procedure as compound **8**, substituting the relevant amine.

4.1.58. 2-(3-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4- yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-*N*-propyl-acetamide hydrochloride (9). The hydrochloride salt was collected by filtration and dried to give the title compound as a colourless solid in 11% yield. ESI-MS m/z = 537.5 $(M+H)^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 7.72–7.30 (m, 1H), 7.16–7.10 (m, 2H), 7.07–7.02 (m, 2H), 6.52 (d, J = 2.3 Hz, 1H), 6.43 (dd, J = 2.3, 8.3 Hz, 1H), 4.61–4.56 (m, 1H), 4.54 (s, 2H), 4.37–4.34 (m, 1H), 3.76 (s, 3H), 3.74–3.71 (m, 2H), 3.31–3.21 (m, 3H), 3.18 (t, J = 7.1 Hz, 2H), 2.81 (ddd, J = 3.7, 13.8, 14.0 Hz, 2H), 2.16 (s, 3H), 2.18–2.00 (m, 6H), 1.58–1.49 (m, 2H), 1.03 (dd, J = 6.8, 14.6 Hz, 6H), 0.92 (t, J = 7.4 Hz, 3H).

4.1.59. *N*-Isopropyl-2-(3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetamide hydrochloride (11). The hydrochloride salt was collected by filtration and dried to give the title compound as a colourless solid in 30% yield. ESI-MS m/z = 537.0 $(M+H)^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 7.31 (d, J = 7.6 Hz, 1H), 7.16–7.10 (m, 2H), 7.05–7.03 (m, 2H), 6.52 (d, J = 2.1 Hz, 1H), 6.43 (dd, J = 2.3, 8.2 Hz, 1H), 4.61–4.54 (m, 1H), 4.51 (s, 2H), 4.38–4.34 (m, 1H), 4.04–3.95 (m, 1H), 3.76 (s, 3H), 3.77–3.73 (m, 2H), 3.29–3.19 (m, 3H), 2.87–2.76 (m, 2H), 2.16 (s, 3H), 2.19–2.03 (m, 5H), 1.17 (d, J = 6.6 Hz, 6H), 1.03 (dd, J = 6.8, 14.8 Hz, 6H).

4.1.60. *N-tert*-Butyl-2-(3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetamide hydrochloride (12). The hydrochloride salt was collected by filtration and dried to give the title compound as a colourless solid in 35% yield. ESI-MS m/z = 550.8 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.84 (m, 1H, NH), 7.54–7.50 (m, 1H), 7.08–6.99 (m, 4H), 6.56 (d, J = 2.2 Hz, 1H), 6.42 (dd, J = 2.2, 8.2 Hz, 1H), 4.61–4.55 (m, 1H), 4.38 (s, 2H), 4.38–4.35 (m, 1H), 3.73 (s, 3H), 3.67–3.61 (m, 2H), 3.21–3.15 (m, 4H), 2.83–2.74 (m, 2H), 2.11 (s, 3H), 2.11–2.08 (m, 2H), 2.13–1.89 (m, 3H), 1.26 (s, 9H), 0.96 (dd, J = 6.8, 16.8 Hz, 6H).

4.1.61. *N*-Allyl-2-(3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetamide hydrochloride (15). The hydrochloride salt was collected by filtration and dried to give the title compound as a colourless solid in 24% yield. ESI-MS m/z = 585.3 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.31 (m, 1H, NH), 7.46–7.45 (m, 1H), 7.08–7.03 (m, 4H), 6.56 (m, 1H), 6.42 (dd, J = 2.3, 8.2 Hz, 1H), 5.84–5.73 (m, 1H), 5.16 (dd, J = 1.6, 17.2 Hz, 1H), 5.07 (dd, J = 1.5, 10.3 Hz, 1H), 4.60–4.55 (m, 1H), 4.48 (s, 2H), 4.38–4.34 (m, 1H), 3.72 (s, 3H), 3.72 (s, 2H), 3.71–3.62 (m, 2H), 3.22–3.17 (m, 4H), 2.79–2.66 (m, 2H), 2.11 (s, 3H), 2.01–1.95 (m, 5H), 0.96 (dd, J = 6.8, 16.9 Hz, 6H).

4.1.62. *N*-Cyclopropylmethyl-2-(3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetamide hydrochloride (17). The hydrochloride salt was collected by filtration, crystallised from ethyl acetate/diethyl ether and dried to give the title compound as a colourless solid in 15% yield. ESI-MS $m/z = 549.2$ $(M+H)^+$. ^1H NMR (400 MHz, CD_3OD): δ 7.32–7.31 (m, 1H), 7.16–7.10 (m, 2H), 7.07–7.02 (m, 2H), 6.52–6.51 (m, 1H), 6.43 (dd, $J = 2.4$, 8.2 Hz, 1H), 4.56 (s, 2H), 4.60–4.56 (m, 1H), 4.37–4.35 (m, 1H), 3.76 (s, 3H), 3.76–3.74 (m, 2H), 3.29–3.18 (m, 3H), 3.08 (d, $J = 6.7$ Hz, 2H), 2.86–2.76 (m, 2H), 2.16 (s, 3H), 2.19–2.03 (m, 6H), 1.03 (dd, $J = 6.8$, 14.6 Hz, 6H), 0.97–0.95 (m, 1H), 0.52–0.49 (m, 2H), 0.23–0.21 (m, 2H).

4.1.63. *N*-Cyclopropyl-2-(3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetamide hydrochloride (18). The hydrochloride salt was collected by filtration and dried to give the title compound as a colourless solid in 24% yield. ESI-MS $m/z = 535.0$ $(M+H)^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 8.26 (m, 1H, NH), 7.50–7.48 (m, 1H), 7.09–7.03 (m, 4H), 6.56 (d, $J = 2.1$ Hz, 1H), 6.42 (dd, $J = 2.3$, 8.2 Hz, 1H), 4.62–4.55 (m, 1H), 4.38 (s, 2H), 4.41–4.35 (m, 1H), 3.73 (s, 3H), 3.67–3.59 (m, 2H), 3.23–3.13 (m, 4H), 2.81–2.71 (m, 2H), 2.67–2.60 (m, 1H), 2.10 (s, 3H), 2.08–2.07 (m, 2H), 2.10–1.87 (m, 3H), 0.96 (dd, $J = 6.8$, 16.9 Hz, 6H), 0.65–0.60 (m, 2H), 0.45–0.41 (m, 2H).

4.1.64. *N*-Butyl-2-(3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]- piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetamide hydrochloride (10). A solution of **7h** (0.25 g, 0.49 mmol) and *n*-butylamine (0.05 mL, 0.52 mmol) in methanol (10 mL) was heated at 65 °C in a sealed tube for 48 h, then cooled to room temperature and concentrated under reduced pressure. The residue was partitioned between dichloromethane and aqueous sodium chloride solution. The layers were separated and the organic layer evaporated to dryness. Flash chromatography on silica gel (dichloromethane/methanol; 99:1; as eluant) gave the title compound as the free base. The compound was dissolved in dichloromethane and 2.0 M hydrochloric acid in diethyl ether added until a precipitate formed. The solid was collected by filtration and dried to give the title compound as a colourless solid in 18% yield. ESI-MS $m/z = 551.3$ $(M+H)^+$. ^1H NMR (400 MHz, CD_3OD): δ 7.32–7.30 (m, 1H), 7.16–7.10 (m, 2H), 7.06–7.02 (m, 2H), 6.52 (d, $J = 2.3$ Hz, 1H), 6.43 (dd, $J = 2.3$, 8.2 Hz, 1H), 4.54 (s, 2H), 4.60–4.51 (m, 1H), 4.37–4.34 (m, 1H), 3.76 (s, 3H), 3.72–3.69 (m, 2H), 3.22 (t, $J = 7.0$ Hz, 2H), 3.28–3.16 (m, 4H), 2.81 (ddd, $J = 3.7$, 13.7, 13.8 Hz, 2H), 2.16 (s, 3H), 2.18–2.03 (m, 5H), 1.58–1.47 (m, 2H), 1.40–1.31 (m, 2H), 1.03 (dd, $J = 6.8$, 14.5 Hz, 6H), 0.93 (t, $J = 7.3$ Hz, 3H).

The following compounds were prepared by the same procedure as compound **10**, substituting the relevant amine.

4.1.65. *N*-(2-Hydroxy-ethyl)-2-(3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetamide hydrochloride (13). The hydrochloride salt was collected by filtration and dried to give the title compound as a colourless solid in 46% yield. ESI-MS $m/z = 539.4$ $(M+H)^+$. ^1H NMR (400 MHz, CD_3OD): δ 7.32–7.31 (m, 1H), 7.16–7.06 (m, 3H), 7.03 (d, $J = 8.1$ Hz, 2H), 6.52–6.51 (m, 1H), 6.43 (dd, $J = 2.3$, 8.2 Hz, 1H), 4.57 (s, 2H), 4.60–4.53 (m, 1H), 4.38–4.34 (m, 1H), 3.76 (s, 3H), 3.76–3.73 (m, 2H), 3.61 (t, $J = 5.7$ Hz, 2H), 3.34 (t, $J = 5.7$ Hz, 2H), 3.29–3.17 (m, 4H), 2.86–2.76 (m, 2H), 2.16 (s, 3H), 2.18–2.03 (m, 5H), 1.03 (dd, $J = 6.8$, 14.8 Hz, 6H).

4.1.66. *N*-(2-Methoxy-ethyl)-2-(3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetamide hydrochloride (14). The hydrochloride salt was collected by filtration and dried to give the title compound as a colourless solid in 36 % yield. ESI-MS $m/z = 553.2$ $(M+H)^+$. ^1H NMR (400 MHz, CD_3OD): δ 7.32 (d, $J = 7.9$ Hz, 1H), 7.16–7.10 (m, 2H), 7.07–7.02 (m, 2H), 6.52 (d, $J = 2.1$ Hz, 1H), 6.43 (dd, $J = 2.3$, 8.3 Hz, 1H), 4.56 (s, 2H), 4.60–4.54 (m, 1H), 4.37–4.34 (m, 1H), 3.75 (s, 3H), 3.74–3.73 (m, 2H), 3.46 (t, $J = 4.9$ Hz, 2H), 3.39 (t, $J = 5.1$ Hz, 2H), 3.34 (s, 3H), 3.30–3.17 (m, 4H), 2.86–2.76 (m, 2H), 2.15 (s, 3H), 2.18–2.03 (m, 5H), 1.03 (dd, $J = 6.8$, 15.1 Hz, 6H).

4.1.67. *N*-Benzyl-2-(3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]- piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetamide (16). The hydrochloride salt was collected by filtration and dried to give the title compound as a colourless solid in 23% yield. ESI-MS $m/z = 585.3$ $(M+H)^+$. ^1H NMR (400 MHz, CD_3OD): δ 7.44 (m, 2H), 7.31–7.24 (m, 4H), 7.16–7.09 (m, 2H), 7.06–7.02 (m, 2H), 6.52 (m, 1H), 6.44–6.42 (m, 1H), 4.60 (s, 2H), 4.58–4.55 (m, 1H), 4.40 (s, 2H), 4.36–4.35 (m, 1H), 3.76 (s, 3H), 3.76–3.74 (m, 2H), 3.31–3.19 (m, 4H), 2.87–2.78 (m, 2H), 2.16 (s, 3H), 2.19–2.05 (m, 5H), 1.03 (dd, $J = 6.7$, 14.4 Hz, 6H).

4.1.68. *N*-Cyclobutyl-2-(3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetamide hydrochloride (19). The hydrochloride salt was collected by filtration and dried to give the title compound as a colourless solid in 26% yield ESI-MS m/z = 549.0 $(M+H)^+$. ^1H NMR (400 MHz, CD_3OD): δ 7.31 (d, $J = 8.4$ Hz, 1H), 7.16–7.09 (m, 2H), 7.07–7.02 (m, 2H), 6.52 (d, $J = 2.3$ Hz, 1H), 6.43 (dd, $J = 2.3$, 8.3 Hz, 1H), 4.60–4.49 (m, 1H), 4.51 (s, 2H), 4.37–4.26 (m, 2H), 3.76 (s, 3H), 3.76–3.71 (m, 2H), 3.26–3.17 (m, 4H), 2.81 (ddd, J = 3.7, 13.8, 13.9 Hz, 2H), 2.16 (s, 3H), 2.19–2.18 (m, 7H), 1.77–1.67 (m, 2H), 1.03 (dd, J = 6.9, 14.6 Hz, 6H).

4.1.69. 8-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one (20). A magnetically stirred suspension of **6** (3.50 g, 13.6 mmol), 1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one (1.25 g, 5.4 mmol), triethylamine (1.0 mL, 14.0 mmol) and sodium iodide (catalytic) in anhydrous *N*,*N*-dimethylformamide (25 mL) was heated at 125 °C overnight.

The reaction mixture was allowed to cool and then partitioned between ethyl acetate and water. The organic layer was collected and the aqueous layer was again extracted with ethyl acetate. The combined organic layers were washed with water, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was subjected to flash chromatography on silica (ethyl acetate as eluant) to give a dark red oil (1.37 g). The oil was dissolved in the minimum volume of diethyl ether, diluted with heptane (50 mL) and left to stand overnight. Trituration and cooling followed by filtration afforded the title compound as a colourless solid (0.69 g, 28%). ESI-MS m/z = 452.1 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.25–7.21 (m, 2H), 7.00–6.95 (m, 3H), 6.82 (t, J = 7.3 Hz, 1H), 6.53 (d, J = 2.3 Hz, 1H), 6.36 (dd, J = 2.4, 8.2 Hz, 1H), 4.81 (s, 3H), 4.67 (s, 2H), 4.26 (dt, J = 4.7, 6.8 Hz, 1H), 3.82–3.70 (m, 2H), 3.75 (s, 3H), 3.59–3.46 (m, 2H), 2.91–2.73 (m, 4H), 2.70–2.57 (m, 2H), 2.12 (s, 3H), 2.04–1.95 (m, 1H), 1.91–1.83 (m, 2H), 1.73–1.64 (m, 2H), 1.00 (dd, J = 6.8, 12.0 Hz, 6H).

4.1.70. 2-{8-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-4-oxo-1-phenyl-1,3,8-triaza-spiro[4.5]dec-3-yl}-*N*-methyl-acetamide (21). A magnetically stirred solution of **20** (250 mg, 0.55 mmol) in anhydrous *N*,*N*-dimethylformamide (3 mL) was treated with sodium hydride (60% dispersion in mineral oil, 22 mg, 0.55 mmol) portionwise under a nitrogen atmosphere. The reaction mixture was stirred for 1 h to give a pale green solution. 2-Chloro-*N*-methyl acetamide (59 mg, 0.55 mmol) in anhydrous *N*,*N*-dimethylformamide (0.5 mL) was added dropwise, and the resultant mixture stirred for 4 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic extracts were washed with water, brine, dried (Na_2SO_4), filtered, concentrated under reduced pressure, and the oily residue purified by flash chromatography on silica gel (ethyl acetate/methanol; 95:5; as eluant) to give the title compound as a colourless solid (140 mg, 49 %). ESI-MS m/z = 523.3. ^{1}H NMR (400 MHz, CD_3OD): δ 7.31 (dd, J = 7.4, 8.6 Hz, 2H), 7.05 (d, J = 8.1 Hz, 1H), 7.02 (d, J = 8.2 Hz, 2H), 6.98 (t, J = 7.3 Hz, 1H) 6.50 (d, J = 2.3 Hz, 1H), 6.42 (dd, J = 2.4, 8.2 Hz, 1H), 4.81 (s, 3H), 4.67 (s, 2H), 4.33 (dt, J = 5.3, 11.3 Hz, 1H), 4.12 (s, 2H), 3.75 (s, 3H), 3.29–3.23 (m, 2H), 2.77 (s, 3H), 2.73–2.57 (m, 2H), 2.16–2.08 (m, 4H), 2.13 (s, 3H), 2.08–2.01 (m, 1H), 1.73–1.64 (m, 2H), 1.08 (dd, J = 6.8, 15.6 Hz, 6H).

4.2. Molecular modelling

All computational studies were performed on Silicon Graphics desktop workstations using Sybyl software.[28] *N*-methylacetamide substituted benzimidazol-2-one piperidine and 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one moieties were built within Sybyl in the ionised form with the ionised amine capped with a methyl group. The molecules were converted to 3D with Concord[28] then energy-minimised using the MMFF94s forcefield and charges to a gradient less than 0.005 kcal/mol/Å. Non-bonded cutoffs at 8.0 Å and a constant dielectric of ε = 1 were applied. The energy-minimised conformations of both moieties were found to be consistent with the conformations of these fragments within structures in the Cambridge Structural Database (CSD).[23] For the benzimidazol-2-one piperidine moiety the energy-minimised conformation was consistent with the predominant conformation of the fragment observed in the CSD with the piperidine C4 proton in a *cis*-conformation relative to the carbonyl carbon of the benzimidazol-2-one. An additional conformation was also observed in the CSD differing in the orientation of the piperidine ring to the benzimidazol-2-one ring with the C4 proton in a *trans*-conformation relative to the carbonyl carbon. This alternative *trans*-conformation of the benzimidazol-2-one piperidine moiety was generated by manual rotation of the torsion between the two ring systems followed by energy minimisation as previously described. RMS superposition of the 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one moiety onto both conformations of the benzimidazol-2-one piperidine was performed with the Sybyl Fit Atoms function. Molecules were superimposed via three key pharmacophoric points the aromatic centroids, the ionisable amines and the protons attached to the ionisable amines. Only the superposition with the *cis*-conformation of the benzimidazol-2-one piperidine could suitably align the key pharmacophoric points.

4.3. Receptor binding and functional assay

CHO cells expressing the human NOP receptor and δC6 cells expressing the human DOP receptor were grown in-house and membrane preparations obtained using standard methods. MOP receptor membranes were purchased from Perkin-Elmer (RB-HOMM expressed in CHO-K1 cells). Homogenates of guinea pig brain cortical tissue were used as a source of KOP receptors. All binding assays were conducted under equilibrium conditions and optimised in 96-well formats to achieve the best signal-to-noise ratio. The test compounds were examined at six concentrations (10^{-6}–10^{-11} M) and assays terminated by vacuum filtration through glass fibre filters with adequate washing steps to minimise non-specific binding. Filterplates were air-dried before addition of scintillation fluid and counting in either a Packard TopCount or Wallac Microbeta Trilux scintillation counter. Data were analysed using curve fitting and a minimum sum of squares method to produce IC_{50} values that were converted to K_i values using the Cheng–Prusoff equation.[29]

4.3.1. [^{3}H]nociceptin competition assay. Binding to in-house human NOP receptors was conducted in 2 mL 96-well plates (Beckmann) in 50 mM Tris–HCl, pH 7.4, in a total volume of 1.5 mL. Test drugs (150 μL), assay buffer (450 μL), and a final concentration of 0.05 nM [^{3}H]nociceptin (300 μL, Amersham; 0.02 mCi/mL, specific activity 144–166 Ci/mmol) were incubated with prepared hNOP cell homogenates (600 μL; ~60 μg protein/well) for 1 h. Non-specific binding was determined in the presence of a final concentration of 100 nM Noc(1–13)NH_2. Binding was terminated using a Tomtec harvester followed by 3× 1.5 mL washes. K_i values were calculated by the equation: $K_i = IC_{50}/(1 + [L]/Kd)$.[29]

4.3.2. [^{3}H]diprenorphine competition assay. Membranes from CHO cells stably expressing human MOP receptors were purchased from Perkin-Elmer (Product code: RBHOMM). Binding to human μ-opioid receptors was conducted in 200 μL 96-well plates (Beckmann) in 50 mM Tris–HCl/5 mM $MgCl_2$, pH 7.4, in a total volume of 100 μL. Test drugs (50 μL), assay buffer (50 μL), and a final concentration of 0.5 nM [^{3}H]diprenorphine (50 μL, Perkin-Elmer; 1.0 mCi/mL, specific activity 50 Ci/mmol) were incubated with MOP receptor membranes for 150 min. Non-specific binding was determined in the presence of a final concentration of 200 μM naloxone. Binding was terminated using a Tomtec harvester followed by 3× 0.5 mL washes. K_i values were calculated by the equation: $K_i = IC_{50}/(1 + [L]/Kd)$.[29]

4.3.3. [^{3}H]naltrindole competition assay. Binding to in-house human DOP receptors was conducted in 2 mL 96-well plates (Beckmann) in 50 mM Tris–HCl/5 mM $MgCl_2$, pH 7.4, in a total volume of 1.5 mL. Test drugs (150 μL), assay buffer (450 μL), and a final concentration of 0.5 nM [^{3}H]naltrindole (50 μL, Tocris; 1 mCi/mL, specific activity 60 Ci/mmol) were incubated with DOP receptor membranes for 150 min. Non-specific binding was determined in the presence of a final concentration of 200 μM naloxone. Binding was terminated using a Tomtec harvester followed by 3× 0.5 mL washes. K_i values were calculated by the equation: $K_i = IC_{50}/(1 + [L]/Kd)$.[29]

4.3.4. [^{3}H]U69593 competition assay. Binding to native guinea-pig KOP receptors was conducted in 2 mL 96-well plates (Beckmann) in 50 mM Tris–HCl/5 mM $MgCl_2$, pH 7.4, in a total volume of 1.0 mL. Test drugs (100 μL), assay buffer (400 μL), and a final concentration of 0.5 nM [^{3}H]U69593 (200 μL, Perkin-Elmer; 1 mCi/mL, specific activity 41.4 Ci/mmol) were incubated with cerebral cortex membrane homogenates from male guinea-pigs (250–300 g; Dunkin–Hartley strain; Bantin and Kingman Ltd., UK) for 3 h. Non-specific binding was determined in the presence of a final concentration of 200 μM naloxone. Binding was terminated using a Brandell harvester followed by 3× 1.5 mL washes. K_i values were calculated by the equation: $K_i = IC_{50}/(1 + [L]/Kd)$.[29]

All functional assays were conducted using the Adenylyl Cyclase Activation Flashplate® Assay purchased from Perkin-Elmer (Cat No: SMP004A). This assay directly measures levels of [^{125}I]cAMP that competes with endogenous forskolin-induced cAMP. CHO cells expressing the human NOP receptor were grown in-house and assays conducted according to manufacturer's guidelines. The test compounds were examined at six concentrations (10^{-6}–10^{-11} M) and were added, together with forskolin, to the cells to cause stimulation. A detection mix was added (containing [^{125}I]cAMP) after 30 min. The FlashPlates were read on a Packard TopCount scintillation counter, after a 2-h incubation. The data are used to construct a standard curve from which the cAMP values produced by the stimulated cells are obtained by interpolation. Data were analysed using curve fitting and a minimum sum of squares method to produce IC_{50} values. Test compounds were screened in parallel with NC, the endogenous ligand for the NOP receptor. The efficacy of the test compounds is calculated as a % of the NC response.

4.4. Mouse vas deferens

Male ICR mice, bodyweight 25–30 g, were sacrificed by exposure to CO_2. After a midline abdominal incision, two incisions were made to the scrotum to expose the testes. The testes, epididymis, and vasa deferentia were then teased out along with the surrounding fatty tissue. The tissues were bathed with physiological buffer (120 mM NaCl, 25 mM $NaHCO_3$, 5 mM KCl, 1.2 mM KH_2PO_4, 11.5 mM D-glucose, and 1.25 mM $CaCl_2$) and warmed to 37 °C to prevent drying out. Each vas deferens was then cleared of connective tissue and blood vessels and removed to a dish containing warmed physiological buffer. A syringe fitted with a 26-G needle and filled with warmed physiological buffer was then used to flush out any residual fluid. The tissues were cut to approximately 14 mm length and each end secured with 2/0 siliconised silk braided sutures and placed in a 10-mL tissue bath with physiological buffer at 37 °C, then bubbled with a mixture of 95% oxygen and 5% CO_2. The sutures connected the vas deferens to a tissue holder and a Gould FT03 force–displacement transducer, respectively. The electrical signal from the transducer was amplified using a Gould 13-4615-50 amplifier and the developed force was registered on a Gould 8188 strip chart recorder. A small pre-load of approximately 2 mN was applied to the tissues for an equilibration period of around 30 min. The pre-load was then adjusted to approximately 6 mN for a further 30 min. The chart recorder was running at a speed of 10 mm/min. The vas deferens was stimulated under the following parameters: Constant current of 400 mA; trains of 3 pulses/train at 10 Hz; pulse duration of 2 ms; 0.1 Hz interval between trains (every 10 s). After 15 minutes of stimulation, 1 μM naloxone was added to one of the two baths. After 30 minutes, this was followed by a cumulative concentration–response curve (CCRC) with (+)**7f**. Concentration range: 10^{-10}–10^{-5} M, 0.5 log unit increments, 3 min intervals between administrations, 50 μL injection volume.

4.5. Loss of righting reflex

Experiments were performed in male ICR mice 22–35 g. In the first experiment, animals received doses of 4.0–8.0 μmol/kg of (+)-**7f** administered intravenously over 10 s (10 mL/kg; $n = 8$ animals per group). The animals were placed on their side/back in an individual Perspex compartment on top of a heated mat covered with wood shavings. If the animal remained on its side/back for at least 30 s it was deemed to have LRR. If immediate LRR did not occur, close observation of the animal continued and by repeatedly placing it on its side/back any slow onset of anaesthesia was recorded. The injection time was recorded, as well as time to LRR and time to gain of righting reflex (GRR), if appropriate. From the times recorded, the onset and sleep time for each mouse could be determined, if applicable. From the per-

centage of mice in each group showing LRR for 30 s or longer, a Probit Analysis (Minitab) was performed to yield an HD_{50} value with 95% confidence limits.

4.6. Formalin paw test

Experiments were performed in male ICR mice 21–30 g. In the first experiment, animals received increasing doses of (+)-**7f** (0.03–3.0 μmol/kg) or vehicle (saline; 10 mL/kg) administered intravenously 15 min. before injection of formalin (20 μL; 3%) into the plantar surface of the left hindpaw, ($n = 6$–8 animals per group). The time spent licking the hindpaw after injection of formalin was then measured. The total time spent licking was measured for two epochs of time 0–5 min (first phase) and 20–30 min (second phase). The means and SEM values for each treatment group were then calculated and compared between groups using the Kruskal–Wallis one-way analysis of variance, a non-parametric statistical test. If statistical significance ($p < 0.05$) was observed with this test, the vehicle group and each of the treatment groups were compared using the non-parametric Dunn's test. To calculate the dose which inhibited licking by 50% (ED_{50}), the data were normalised by expressing the time spent licking for each animal as a percentage of the mean time spent licking by the vehicle-treated mice. The percent inhibition data were calculated for both epochs of time and used to calculate ED_{50} values for both phases of licking using a non-linear regression fit, sigmoidal dose response with constants of 0 and 100 for the bottom and top, respectively (XLFit software).

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