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Structure–activity relationships and CoMFA of N-3 substituted phenoxypropyl piperidine benzimidazol-2-one analogues as NOP receptor agonists with analgesic properties

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Abstract—The N-3 position of a series of 3-phenoxypropyl piperidine benzimidazol-2-one analogues was optimised using the predictive power of a CoMFA model. The model was used to prioritise compounds for synthesis culminating in the triazole (+)-**24**. (+)-**24** was found to be a high affinity, potent NOP agonist and demonstrated both antinociceptive and antiallodynic effects when administered iv to rodents.

1. Introduction

The discovery in 1994 of the fourth member of the opioid receptor family, opioid receptor-like receptor (ORL1, NOP), has led to extensive research into its biological significance.[1] Its endogenous ligand, nociceptin (or Orphanin FQ), is a 17-amino acid neuropeptide first isolated from the brain in 1995.[2] The peptide binds the receptor with a very high affinity although activation of NOP is functionally complex.[3] Given the nociceptin receptor is widely distributed throughout the central nervous system it participates in a variety of roles. Supraspinally NOP appears to have potent anti-analgesic actions[2,4] whereas spinally analgesic properties are observed.[5] Other accounts also demonstrate the NOP system plays a significant role in pathways of anxiety, learning, memory, reward, tolerance, cardiovascular and respiratory function. Despite significant sequence homology of NOP with the classical opioid receptors, opioids such as dynorphin A do not show appreciable affinity for the NOP receptor.[2]

Opioids such as morphine are the most effective treatments for postoperative pain.[6] However, drugs that act via the mu opioid (MOP) receptor also suffer from opioid related side-effects such as constipation, nausea and vomiting, thus leading to non-compliance with patients.[7] It is believed that the development of new ligands with a high degree of selectivity and potency for NOP would provide a novel treatment for the management of pain without the unfavourable side-effects observed with MOP agonists.

Several small-molecule NOP ligands have now been reported in the literature.[8] Some of these ligands have high selectivity and potency for the NOP receptor versus the other opioid receptors.[9] Although many different classes of NOP ligands have been reported limited pharmacophore models have been defined for NOP binding and selectivity compared with opioid receptors.

In earlier work we had identified a series of 3-phenoxypropyl piperidine benzimidazol-2-ones[10] that led to the optimised compounds (+)-**1** and (+)-**2** (Fig. 1). These agonists have high affinity for NOP ($pK_i = 9.30$ and 8.70, respectively) with excellent selectivity over the other opioid receptors, in particular MOP ($pK_i = 7.27$,

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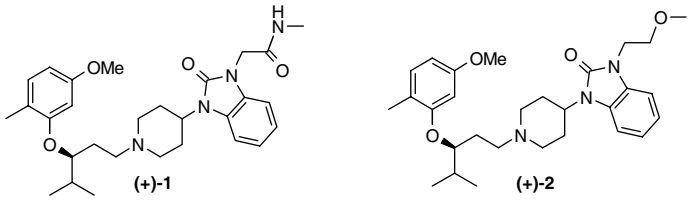


Figure 1. Structure of ORL1 agonist lead compounds.

NOP/MOP = 108 for (+)-**1**). We attributed the higher affinity and selectivity of **1** to both the H-bond donating and accepting capacity of the terminal amide appended from the N-3 position of the benzimidazol-2-one. In order to explore structure–activity relationships (SAR) and further optimise this series we focussed on replacing the amide with suitable heterocyclic (bio)isosteres.

Given the diverse nature of heterocycles we were able to employ a CoMFA model based on the analysis of the SAR observed in our own series of NOP ligands. This technique allowed chemists to ultimately design and predict activities of molecules to help prioritise the chemistry efforts. Herein we report the culmination of this series, with the synthesis and antinociceptive effects in vivo.

2. Results and discussion

2.1. CoMFA modelling

To aid the prioritisation of synthesis a CoMFA 3D QSAR model was developed. The full model encompassed a dataset of 113 3-phenoxypropyl piperidine benzimdazol-2-one compounds all with NOP affinity data (see Supplementary data). The majority of these compounds have been disclosed in previous publications around this series.[10] The 113 compounds were superimposed onto an X-ray structure of (+)-**2**[11] by electrostatic and steric similarity. Initial efforts using a smaller dataset indicated that a predictive model could be obtained. The full model was generated using default CoMFA steric and electrostatic fields and default CoMFA parameters except for 1 Å grid spacing. Models were generated using partial least squares with default CoMFA scaling and a 2 kcal minimum σ cut-off. The statistics of the best model were as follows: Q^2(10 groups) = 0.75, cross validated s.e. = 0.48, R^2 = 0.95, s.e. = 0.22, $F_{6,106}$ = 305.15 ($p \ll 0.05$), n = 113, 6 components, steric contribution = 0.55, electrostatic contribution = 0.45.

The CoMFA steric field contour plot (Fig. 2) indicates that 2,5-disubstitution of the phenoxy moiety is most favourable (green) but substitution is not tolerated at the 5-position of the benzimidazol-2-one ring system (yellow). The region in the vicinity of the benzimidazol-2-one N-3 position is of great interest. The steric contours suggest that some steric bulk in this region is favourable however the nearby yellow contours indicate that larger groups in this region are likely to have reduced affinity. The CoMFA electrostatic field

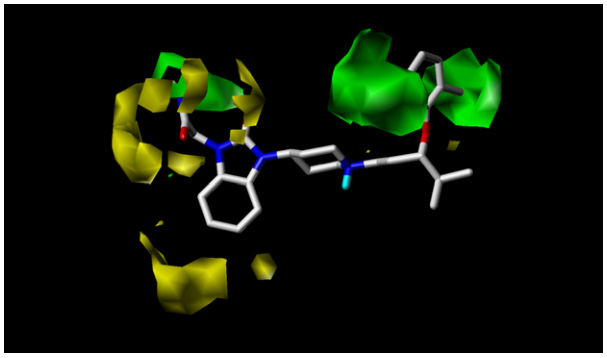


Figure 2. CoMFA steric field standard deviation × coefficient contour map surrounding (+)-**1**. Green contours (80% contribution) indicate regions where steric bulk is favourable. Yellow contours (20%) indicate regions where steric bulk is not favourable.

contour plot (Fig. 3) is more difficult to interpret. The red contours favouring negative charge at the 5-position of the phenoxy moiety are consistent with the preference for a methoxy substituent. The region surrounding the benzimidazol-2-one N-3 substituent shows favourable regions for both positive and negative charge whereas favourable positive charge at the benzimidazol-2-one 5-position is likely to reflect the preference for the unsubstituted analogue.

The CoMFA model was used to provide predictions prior to synthesis at the idea generation stage. Compounds maintaining the 3-phenoxypropyl piperidine benzimdazol-2-one scaffold but with variations of the phenoxy substituents or benzimidazol-2-one N-3 substituent were predicted using the model. Subsequent prior-

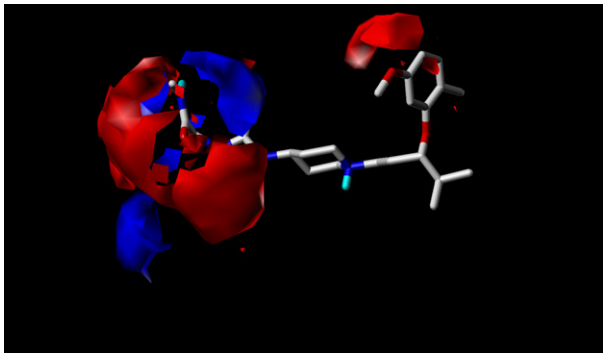


Figure 3. CoMFA electrostatic field standard deviation × coefficient contour map surrounding (+)-**1**. Blue contours (80% contribution) indicate regions where positive charge is favourable. Red contours (20%) indicate regions where negative charge is favourable.

itisation, synthesis and NOP affinity evaluation yielded a dataset of more than 30 compounds ranging in NOP pK_i from 6.74 to 8.92 and a standard error of prediction of 0.82 log units highlighting the predictive tendency of the model. Among the compounds predicted and prioritised for synthesis were a number of heterocycle replacements for the *N*-methyl acetamide of **1**, only the predictions and experimental data for these compounds are shown in Table 1 (all other prediction data are not shown). The model was only used in a truly predictive manner to aid the prioritisation process prior to synthesis. Some compounds in Table 1 were not predicted prior to synthesis and therefore have no predicted pK_i data.

2.2. Chemistry

Compounds were initially prioritised for synthesis on the basis of the CoMFA model and further compounds synthesised to explore SAR within the series. The synthesis of benzimidazol-2-ones substituted at N-3 with heterocycles are shown in Schemes 1–4. These Schemes can be divided into two categories. The first includes displacement of an activated heterocycle by benzimidazol-2-one **3**. The second involves manipulation of functional groups already present on the benzimidazol-2-one to derive the heterocycle.

In Scheme 1 N-Boc benzimidazol-2-one **3**[10] is deprotonated with sodium hydride and treated with a series of heteroaromatic halomethyls. Many of the halomethyls were commercially available, however those that could not be purchased were prepared according to literature protocols.[12–17] In some instances the more stable mesylate was prepared. The Boc group of **4** was then removed with acid and the free amine **5** coupled to intermediate phenoxypropyl chloride **6**[10] affording the final compounds **7a**–**f**. In some instances the activated aromatic was attached directly to the coupled benzimidazolone **8**[10] (Scheme 2) to afford the target compounds **7l**–**o**.

N-linked heterocycles were introduced from the methylene mesylate intermediate (Scheme 3). Following the methods of Davoll et al.,[18] treatment of the benzimidazolone **3** with formalin under reflux yielded the methylene alcohol **9** in moderate yield. This could be converted to the mesylate followed by treatment with the nucleophilic heterocycle to give imidazoles **4p** and **4r**, pyrazole **4q** and triazole **4s**. In a similar manner to that outlined in Scheme 1, these intermediates were deprotected and coupled to the phenoxypropyl chloride **6** to give final compounds **7p**–**s**.

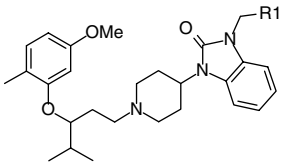
Modification of the ester **11** derived from alkylation of **3** with chloro-ethyl acetate allowed access to both 1,2,4-triazole[19] **14** and 1,2,4-oxadiazole[20] **16** (Scheme 4). Treatment of the ester **11** with hydrazine affords the hydrazide **12**, which was condensed with acetamidine[19] using sodium hydride to give the cyclised 1,2,4-triazole **13**. Treating the ester **11** with *N*-hydroxyacetamidine[21] under similar conditions yielded the 1,2,4-oxadiazole **15**. Both intermediates were then coupled to **6** as outlined in Scheme 1 to give **14** and **16**, respectively. Alkylation of the benzimidazol-2-one **3** with chloroacetonitrile gives the nitrile intermediate **17**. Treatment with hydroxylamine under reflux conditions gave the amidoxime **18** which when reacted with sodium hydride and ethyl acetate cyclises to the 1,2,4-oxadiazole **19**.[22] Again this is readily converted to the desired final compound **20** via de-protection with acid and coupling to the phenoxypropyl chloride intermediate **6** in a similar manner to that outlined in Scheme 1. The unsubstituted triazole **24** was prepared following the methods of Ladduwahetty and co-workers.[23] The benzimidazol-2-one **3** was alkylated with the appropriate (chloromethyl)amidrazone and subsequently heated to 130 °C to effect cyclisation to the desired compound **21**. Treatment of the triazole **21** with methyl iodide under basic conditions affords both the 1-methyl-1,2,4-trazole[24] **22** and 2-methyl-1,2,4-trazole[24] **23** following chromatographic separation of the two regioisomers. These intermediates can then be coupled to phenoxypropyl chloride **6** in the usual manner to give triazoles **24**–**26**.

Both tetrazole[25] **28** and oxadiazole[26] **29** can be accessed from the readily available intermediate **27**. Treatment of **27** with tributyltinazide (Scheme 5) converts the nitrile to the tetrazole **28**. Refluxing **27** in hydroxylamine results in the amidoxime **30** which readily cyclises to the unsubstituted oxadiazole **29** on heating with triethylorthoformate in the presence of a Lewis acid. Alkylation of **8** with propargyl bromide gives the terminal alkyne **32** which upon treatment with tributyltinazide following the methods of Dillard et al.[27] resulted in the 1,2,3-triazole **31**. Triazolone **33** is accessed from the alkylation of **8** with *N*-carbomethoxy-2-chloroactamidrazone[23] derived from chloroacetonitrile and methyl hydrazinocarboxylate to give the intermediate amidrazone which without isolation was heated neat to effect cyclisation to the final compound triazolone **33**. The thiadiazole **34** was accessed from the chloro precursor **35** using hydrogenation conditions to extrude the halogen. All compounds were prepared as either the hydrochloride or methanesulfonate salt to increase solubility in water.

2.3. Affinity and functional data

Table 1 shows the receptor binding affinities and NOP agonist potency data resulting from the replacement of the *N*-methylacetamide of **1**[10] with a variety of heterocycles. The oxazol-2-yl analogue **7h** shows both a reduction in NOP affinity and an increase in MOP affinity in comparison to **1**, resulting in almost an 8-fold decrease in selectivity. A decrease in NOP affinity and selectivity is also observed for the oxazol-4-yl derivative **7g**. Replacement of the oxygen of **7g** to give thiazole **7a** yielded an approximate 2-fold increase in affinity for both NOP and MOP over the oxazole. 2-Methyl substitution of the thiazol-4-yl (**7d**) however resulted in a 14-fold decrease in NOP affinity and unchanged MOP affinity in comparison to the parent heterocycle suggesting steric intolerance in this region. Thiadiazole **34** shows similar NOP affinity to the thiazole **7k** but a slight decrease in MOP affinity leads to a 58-fold selectivity window between NOP and MOP. Interestingly oxadiazole **29** shows similar NOP affinity and no decrease in MOP affinity relative to the oxazole **7g**. Methyl

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



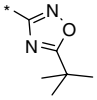
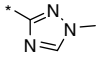
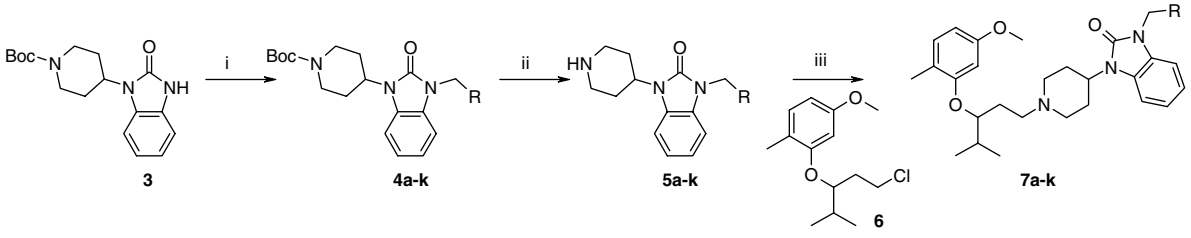
Compound	R1	ORL1 pK_i	CoMFA pK_i	MOP pK_i	MOP/ORL1	cAMP IC_{50} (nM) (% NC resp.)
1[*]	CONHMe	9.00	ND	6.78	164	10 (113)
2[*]	CH_2OMe	8.39	ND	6.73	46	60 (109)
7a		8.92	8.69	7.37	35.5	31.1 (105)
7b		8.82	ND	7.41	25.7	192.8(103)
7c		8.31	ND	7.28	10.7	95.4 (105)
7d		7.76	8.30	7.41	2.2	ND
7e		7.54	ND	7.37	1.5	815.5(98)
7f		5.93	ND	6.28	0.4	ND
7g		8.60	8.62	6.98	41.7	22.3 (116)
7h		8.41	8.87	7.08	21.4	29.1 (114)
7i		7.66	ND	6.84	6.6	1301.67(90)
7j		8.45	ND	7.25	15.8	186.69(102)
7k		8.85	ND	7.35	32.6	150.3 (96)
7l		8.02	ND	7.32	5.0	329.6(102.7)
7m		8.23	ND	7.12	12.9	467.6(106)
7o		8.43	8.72	6.67	57.5	133.73 (105)
7p		8.38	8.85	6.92	28.8	296.6 (110)
7q		8.48	8.79	6.65	67.6	102.7 (106)

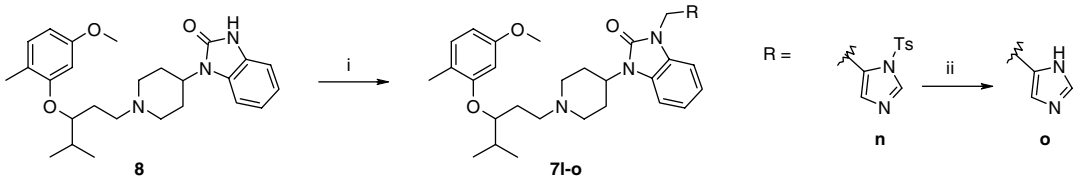
Table 1 (*continued*)

Compound	R1	ORL1 pK_i	CoMFA pK_i	MOP pK_i	MOP/ORL1	cAMP IC_{50} (nM) (% NC resp.)
7r		8.31	ND	6.91	25.1	361.3(105)
7s		8.73	ND	7.41	20.9	39.5(108)
14		7.38	ND	6.92	2.9	506.4 (97)
16		7.53	8.17	7.02	3.2	394(91)
20		7.43	8.68	6.69	5.8	ND
24		8.76	9.22	6.86	79.4	10.8 (109)
(+)-**24**		9.05	ND	7.44	40.7	11.5 (109)
25		7.93	ND	7.32	4.1	77.9 (108)
26		7.02	ND	7.00	1.0	ND
28		6.04	ND	5.99	1.1	ND
29		8.62	8.93	7.11	32.4	32.8 (107)
31		8.10	8.92	6.74	22.9	253.7 (100)
33		8.23	ND	7.60	4.3	60.4(106)
34		8.77	ND	7.00	58.9	ND

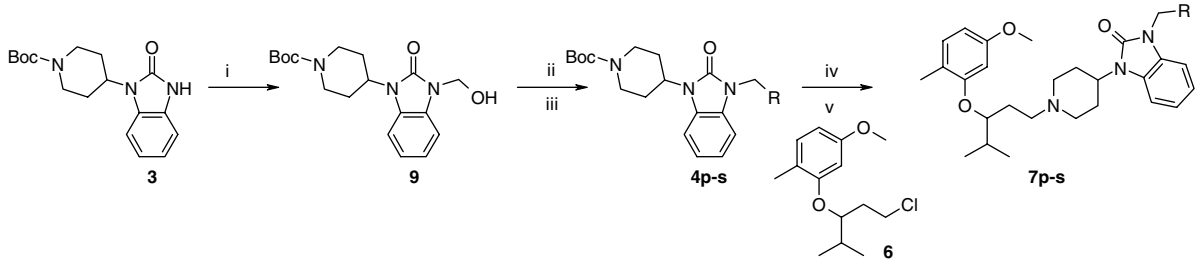
ND, not determined.
* Data for racemate.



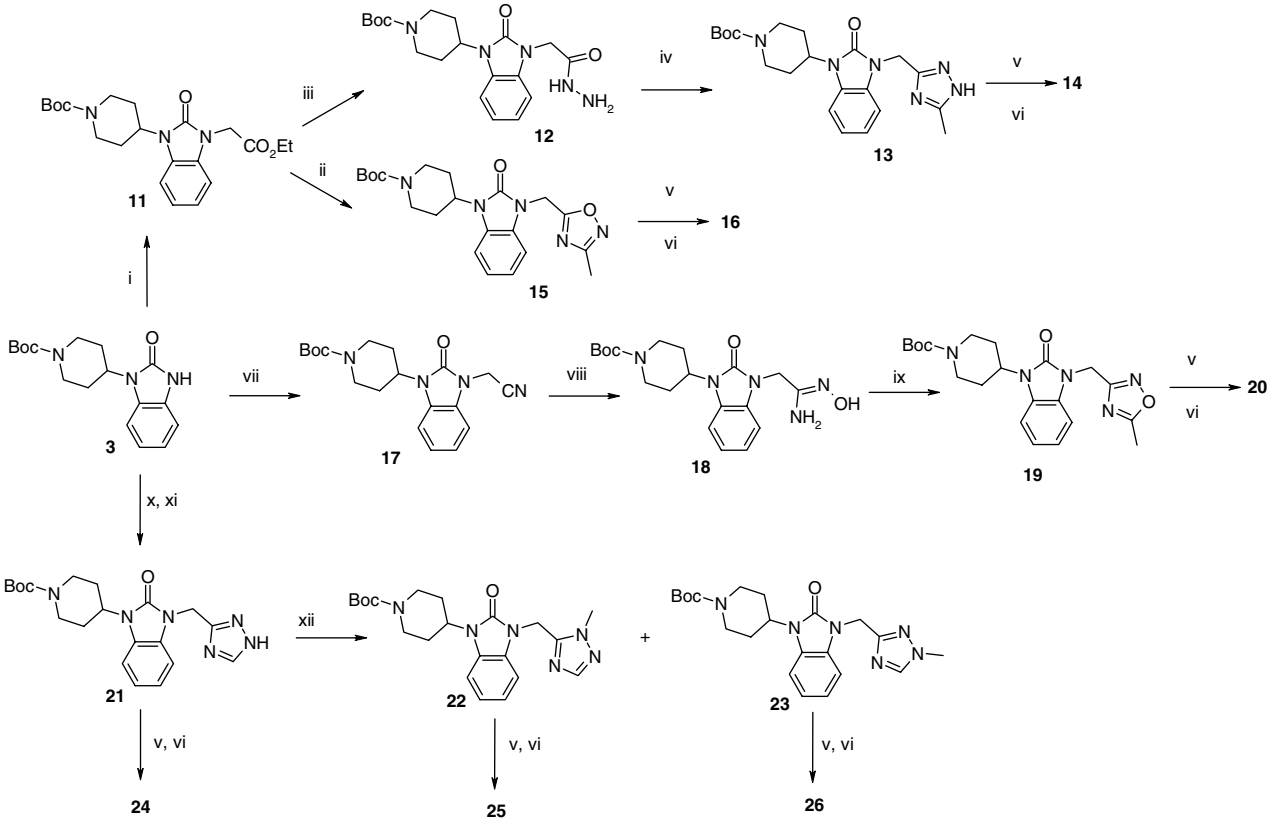
Scheme 1. Reagents and conditions: (i) 60% NaH, DMF, 1 h, then RCH_2Cl or RCH_2OMs, rt, 16 h; (ii) TFA, DCM, 16 h, rt; (iii) **6**, DiPEA, NaI, CH_3CN, 80 °C, 20 h.



Scheme 2. Reagents and conditions: (i) 60% NaH, DMF, 1 h, then RCH_2Cl or RCH_2OMs, rt, 16 h; (ii) HCl, DCM, rt, 1 h.

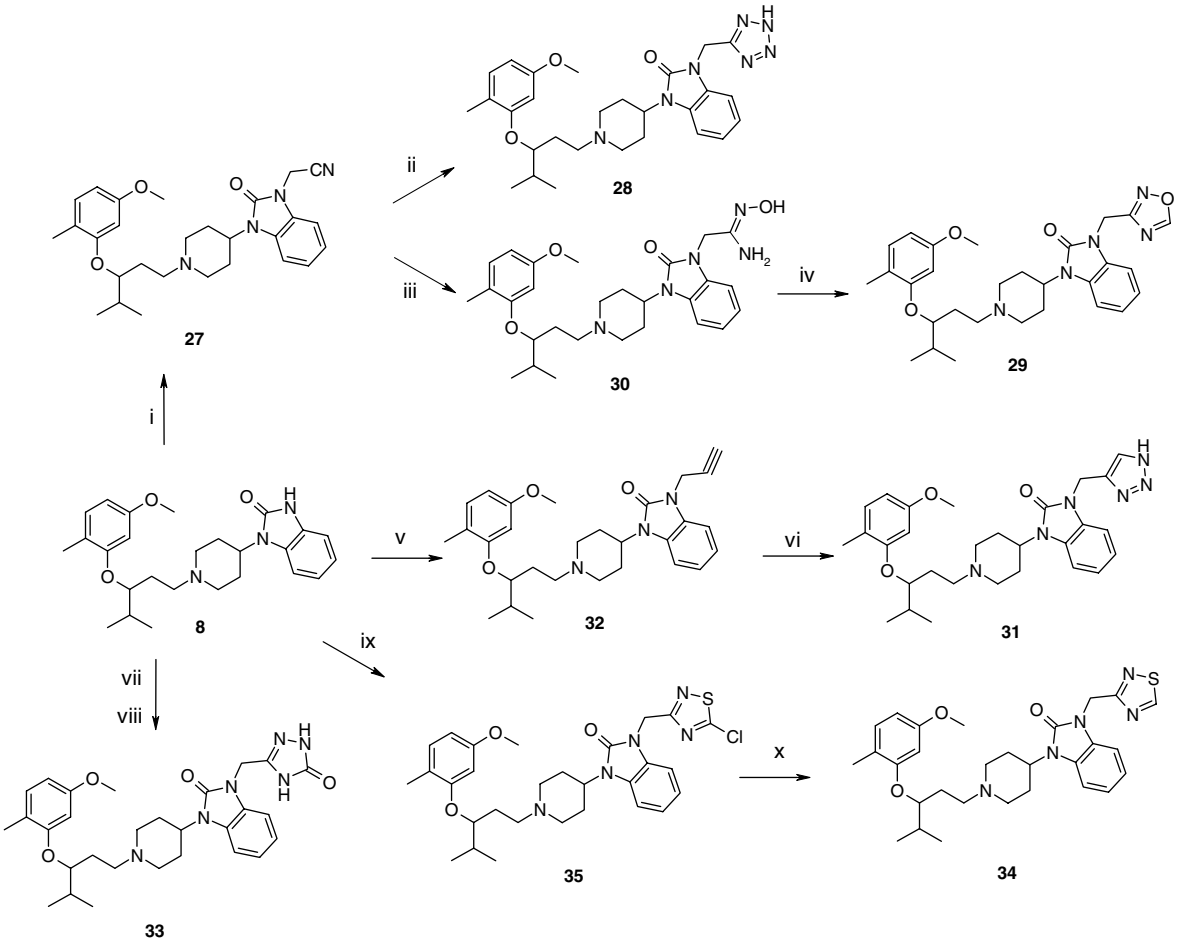


Scheme 3. Reagents and conditions: (i) 35% formalin, 1,4-dioxane, reflux, 20 h; (ii) MsCl, DCM, 1.5 h, 0 °C; (iii) HNHet, DCM, rt, 3 days; (iv) TFA, DCM, 16 h, rt; (v) **6**, DiPEA, NaI, CH_3CN, 80 °C, 20 h.



Scheme 4. Reagents and conditions: (i) 60% NaH, DMF, 15 min, then $ClCH_2CO_2Et$, rt, 16 h; (ii) 4 Å MS, *N*-hydroxyacetamidine, NaH, THF, reflux, 2 h; (iii) $NH_2NH_2{\cdot}H_2O$, EtOH; (iv) acetamidine·HCl, NaOMe, then $MeOCH_2CH_2OH$, heat; (v) TFA, DCM, 16 h rt; (vi) **6**, DiPEA, NaI, CH_3CN, 80 °C, 20 h; (vii) 60% NaH, DMF, 15 min, then $CNCH_2Cl$, rt, 16 h; (viii) $NH_2OH{\cdot}HCl$, *i*-Pr_2EtN, EtOH, reflux, 16 h; (ix) 4 Å MS, 60% NaH, THF, 50 °C, 2 h then EtOAc, reflux, 2 h; (x) 60% NaH, DMF, 15 min, then *N*-formyl-2-chloroacetamidrazone, rt, 16 h; (xi) 130 °C neat; (xii) MeI, K_2CO_3, acetone, reflux, 15 min.

substituted oxadiazoles **16** and **20** both show reduced NOP affinities which is consistent with the thiazole SAR. Further increasing the bulk in this region exemplified by the *tert*-butyl analogue **7f** gives a dramatic 490-fold decrease in NOP affinity and a modest 6.7-fold decrease in MOP affinity relative to the unsubstituted oxadiazole **29**. This clearly highlights that further steric bulk in this region is highly unfavourable for NOP receptor binding, although the MOP receptor appears to be somewhat more tolerant. This seems to be consistent



Scheme 5. Reagents and conditions: (i) 60% NaH, DMF, 15 min, then $CNCH_2Cl$, rt, 16 h; (ii) Bu_3SnN_3, xylene, 140 °C, 24 h; (iii) $NH_2OH{\cdot}HCl$, DiPEA, EtOH, reflux, 16 h; (iv) BF_3OEt, triethylorthoformate, 115 °C, 30 min; (v) 60% NaH, DMF, 50 °C, 1 h, then propargyl bromide, rt, 16 h; (vi) Bu_3SnN_3, xylene, 150 °C, 16 h; (vii) 60% NaH, DMF, 15 min, then *N*-carboxymethyl-2-chloroacetamidrazone; (viii) 130 °C neat; (ix) 60% NaH, DMF, 50 °C, 15 min, then $HetCH_2Cl$, rt, 18 h; (x) 10% Pd-C, AcOH, H_2, 28 h.

with the steric contours of the CoMFA model which indicate that some steric bulk is favourable but extension beyond this favourable region is likely to be detrimental to NOP affinity. The 5-methyl isoxazol-3-yl compound **7i** unsurprisingly exhibits a similar affinity profile to the oxadiazole analogue **20**. However, methyl substitution at the positions adjacent to the ring attachment point as in compound **7j** shows good NOP affinity but a modest 16-fold selectivity.

Removal of the hydrogen bond capability of the heterocycle to afford the thiophenes **7k** and **7b** resulted in high NOP affinity similar to the *N*-methylacetamide **1** but reduced selectivity over MOP and reduced NOP functional potency in a cAMP assay. Pyrazole **7q** and imidazole **7o** show similar profiles with high NOP receptor affinity and selectivity over MOP, however functional potency decreases by an order of magnitude relative to **1**. N-linked imidazole **7p** shows similar NOP affinity to the C-linked imidazole **7o** but an increase in MOP affinity leads to a decreased selectivity of 29-fold, functional potency is also further decreased. 2-Methyl substituted imidazole **7r** fails to show any improvement in selectivity or potency. Insertion of a further nitrogen into the ring system to afford the 1,2,4-triazol-1-yl analogue **7s** resulted in a 2.2-fold increase in NOP affinity over the imidazole **7p** and a restoration of functional potency but selectivity over MOP dropped to 21-fold. 1,2,3-Triazol-4-yl **31** showed decreased NOP affinity, however the 1,2,4-triazol-3-yl **24** yielded high NOP affinity, good selectivity over MOP and a functional potency equivalent to the *N*-methylacetamide **1**. It is noted that the 1,2,4-triazol-3-yl heterocycle was predicted prior to synthesis to exhibit the highest NOP affinity of all the analogues that were predicted by the CoMFA model therefore highlighting this particular heterocycle as a priority compound for synthesis.

Methyl substituted triazole analogues **14**, **25** and **26** all show decreased NOP affinities compared to **24** with the most pronounced decreases for **14** and **26** exhibiting 24- and 55-fold reduced NOP affinities, respectively. The decreased NOP affinities coupled with similar or increased MOP affinities relative to **24** effectively abolish selectivity for all 3 methyl triazoles. Insertion of a carbonyl into the triazole ring system of **24** to give **33** resulted in a small decrease in NOP affinity but a 5.5-fold increase in MOP affinity leading to a significantly reduced 4-fold selectivity ratio. Tetrazole **28** unsurprisingly showed a loss of NOP and MOP binding affinities

to give a similar profile to the carboxylic acid analogue described previously.[10] A limited number of 6 membered heterocyclic systems were explored without further improvement of the in vitro profile. The 2-pyridyl compound **7c** exhibited good NOP affinity, a modest 10-fold selectivity over MOP and a functional potency of 95.4 nM in the cAMP assay. However the 3-pyridyl, 4-pyridyl and pyrazine analogues (**7l**, **7e** and **7m**) all showed similar or reduced NOP affinity relative to **7c** but significant decreases in functional potency. Comparing the CoMFA predicted affinities with the experimental affinities it can be seen that generally the model showed good predictivity. Of the 12 heterocycles shown in Table 1 that were predicted in the CoMFA model prior to synthesis 10 were found to be well predicted with an absolute error less than 0.7 log units, only oxadiazole **20** and triazole **31** showed poorer predictions.

Results, taken together, suggest that hydrogen bond acceptor and donor capabilities adjacent to the heterocyclic ring attachment point appear to yield an optimal combination of NOP affinity, selectivity over MOP and NOP agonist potency. This is consistent with previous SAR findings indicating that the donor and acceptor functionality of the *N*-methylacetamide was required to yield a similar optimal combination of affinity, selectivity and potency.[10] In general the heterocycles improved solubility over the parent *N*-methylacetamide (e.g., **7o** had solubility of 200 mg/L). Given the activity of **24**, this was separated into its component enantiomers using chiral HPLC. It was shown that the (+) enantiomer [(+)-**24**] was the eutomer as previously observed with NOP ligands within this series.[10] However, triazole (+)-**24** exhibited a solubility of 4 mg/L as the hydrochloride salt.

2.4. Behavioural effects of compound (+)-24

The antinociceptive properties of compound (+)-**24** were evaluated in the mouse formalin paw test[28] (FPT), a model that assesses behavioural responses to continuous, noxious stimulation generated by injection of a dilute solution of formalin into one hindpaw, and in a model of post-surgical pain, the Brennan model. In the FPT test, (+)-**24** administered iv (0.03–3.0 μmol/kg) 5 min prior to injection of formalin 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 phases 1 and 2, respectively). The calculated ED_{50} value for the inhibition of licking during the first phase it was 2.62 μmol/kg (95% CI 0.63–4.40 μmol/kg) and the second phase was 0.65 μmol/kg (95% CI 0.31–1.61 μmol/kg). Previously, we have demonstrated within this series that this effect is mediated by NOP by blocking the inhibition of licking by pre-treatment with a NOP antagonist.[10]

In the Brennan model, mechanical allodynia was induced by making a 1.5 cm incision, under isoflurane anaesthesia, through the skin and fascia of the plantar surface of a rat's hindpaw, elevating the underlying muscle and using 2 sutures to close the wound. Withdrawal thresholds using von Frey filaments were measured prior to the surgery, 2 h after surgery at which time the compound was administered and at regular intervals after compound administration. Compound (+)-**24** reversed the mechanical allodynia that develops in this model (Fig. 5). After intravenous administration the calculated ED_{50} value was 12.1 μmol/kg. In this assay it should be noted that although it appeared that 100% reversal of mechanical allodynia was observed after administration of the top dose 30 μmol/kg this was due to the fact that the animals lost their righting reflex at this dose and could not respond to the stimulus. However, after administration of the 10 μmol/kg dose a significant reversal of allodynia (Dunn's vehicle versus 10 μmol/kg, $p < 0.05$) was observed without any loss of righting reflex at the time points assessed.

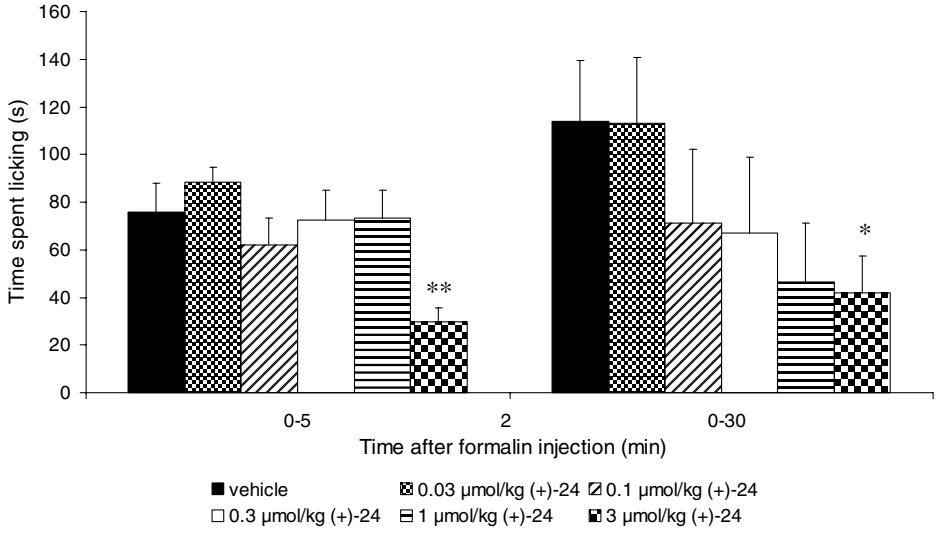


Figure 4. Effect of vehicle or increasing doses of (+)-24 administered intravenously, 5 min prior to the injection of formalin, on the time spent licking (s). *,** $p < 0.05$ and 0.01 when compound (+)-24 treated mice were compared to vehicle treated mice (Kruskal–Wallis test followed by Dunn's post hoc test).

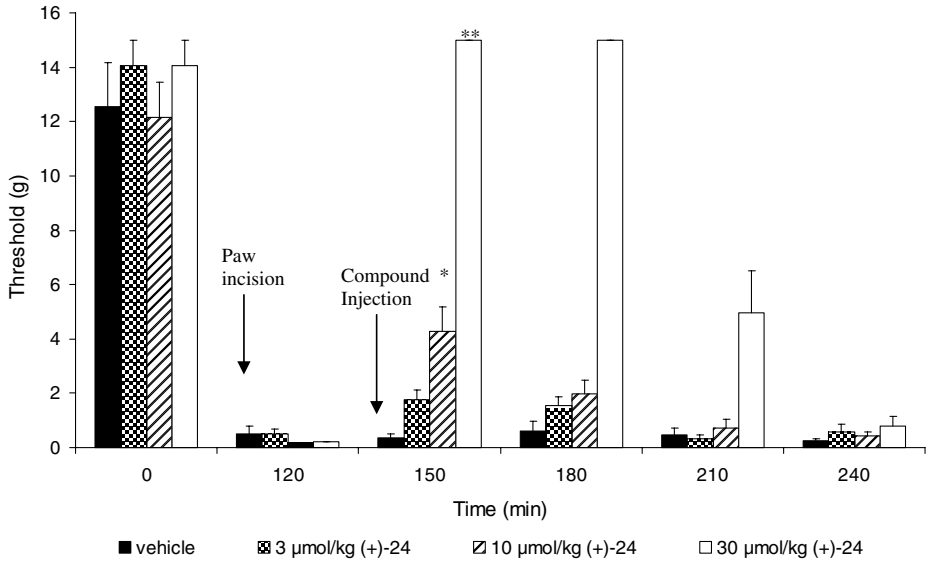


Figure 5. Effect of vehicle or increasing doses of (+)-24, administered intravenously 2 h after paw incision, on mechanical allodynia. $^{*,**}p < 0.05$ and 0.01, respectively (Kruskal–Wallis test followed by Dunn's post hoc test). N.B., statistical comparisons were only carried out at the time of maximum effect (150 min).

3. Conclusion

The main drive within this investigation was to find a follow-up compound to the *N*-methyl acetamide **1**. A CoMFA model was developed focussing on the N-3 substitution of 3-phenoxypropyl piperidine benzimidazol-2-one analogues. The model allowed the chemist to predict activities of the heterocyclic replacements prior to synthesis, thus allowing the prioritisation of key compounds and ultimately accessing triazole derivative (+)-**24**. (+)-**24** was shown to be a potent agonist at NOP and selective over the other classical opioid receptors (NOP $pK_i = 9.05$, MOP $pK_i = 7.44$, DOP $pK_i = 5.89$, KOP $pK_i = 6.06$).

The SAR indicates that the presence of both hydrogen bond acceptor and donor functions adjacent to the heterocyclic ring attachment point provides optimal affinity and selectivity for NOP. Furthermore, when (+)-**24** is given iv the compound produces antinociceptive effects comparable to morphine (Fig. 6) in the FPT and in the Brennan model was shown to reverse mechanical allodynia indicating that this compound could have a potential utility in treating postoperative pain.

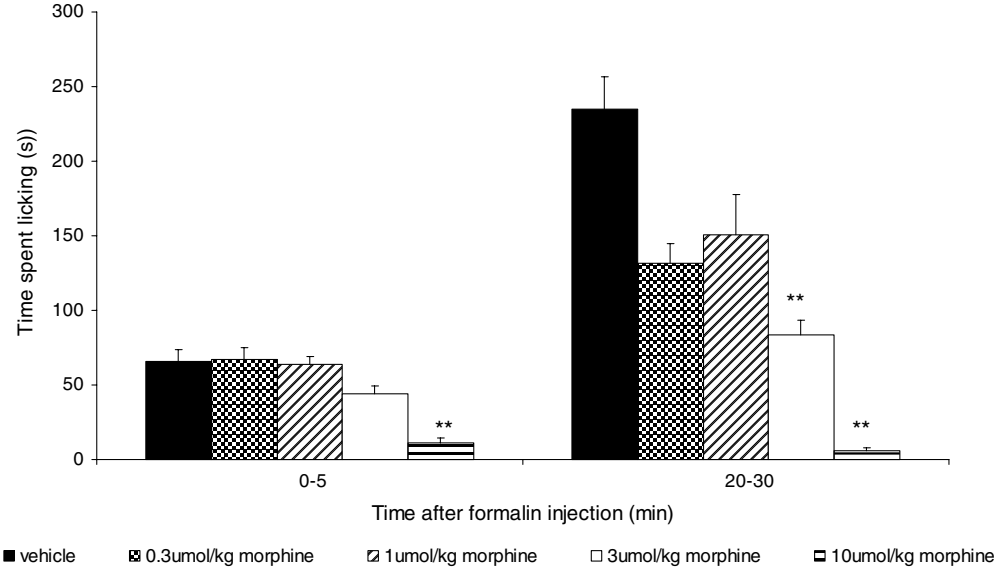


Figure 6. Effect of vehicle or increasing doses of morphine administered intravenously, 5 min prior to the injection of formalin, on the time spent licking (s). $^{**}p < 0.01$ when morphine treated mice were compared to vehicle treated mice (Kruskal–Wallis test followed by Dunn's post hoc test).

4. Experimental

4.1. Chemistry

4.1.1. General information. NMR spectra were recorded using a Bruker DPX 400 or DRX 400 instrument with tetramethylsilane used as internal standard. The chemical shifts are reported in δ values (ppm). The following notations are used for ^{1}H NMR signal patterns: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Optical rotations were recorded on an Optical Activity AA-1000 polarimeter. Mass spectrometry was carried out on either a PE SCIEX API 150EX or ESCi micromassZQ machine. High resolution mass spectra (HRMS) were recorded on an Applied Biosystems Inc Mariner (TOF) instrument, error limit <5 ppm. HPLC analyses were recorded on a Perkin-Elmer Integral 4000 Quaternary HPLC system with UV detection or a Perkin-Elmer Series 200 HPLC system with 200 Diode Array detectors. Max RP C12 (Phenomenex) columns (150 × 4.6 mm, 4 μm) or Xterra RP C18 (Waters) columns (100 × 4.6 mm, 5 μm) for purity determinations. Thin layer chromatography (TLC) was carried out using Kieselgel 60 F_{254} aluminium backed plates (Merck). Matrix silica gel, particle size 0.040–0.063 mm, was used for column chromatography. Solvents used were commercial grades from either Aldrich or BDH and used without further purification. All chemicals and reagents were obtained from commercial suppliers and used without further purification. All compounds passed in-house purity analysis of >90% as determined by NMR and LC.

4.1.2. 4-(2-Oxo-3-thiazol-4-ylmethyl-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (4a). Sodium hydride (60% suspension in mineral oil, 180 mg, 4.50 mmol) was added in portions to a solution of 4-(2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester **3**[10] (1.30 g, 4.10 mmol) in *N*,*N*-dimethylformamide (13 mL) under ice-water bath cooling and stirred at 50 °C for 15 min. The reaction mixture was then cooled to 5 °C, and a solution of 4-chloromethylthiazole (657 mg, 4.92 mmol) in tetrahydrofuran (2 mL) added dropwise. The resulting mixture was stirred at room temperature for 16 h before diluting with brine (300 mL) and extracted with ethyl acetate (4× 100 mL). The organic layers were combined, washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (dichloromethane/methanol; 99.4:0.6; as eluant) to give the title compound as a colourless solid (1.30 g, 76%). ESI-MS m/z = 415.0 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.78–1.90 (m, 2H), 2.24–2.42 (m, 2H), 2.76–2.98 (m, 2H), 4.20–4.42 (br s, 2H), 4.45–4.57 (m, 1H), 5.25 (s, 2H), 7.03–7.09 (m, 2H), 7.10–7.17 (m, 2H), 7.19–7.22 (m, 1H), 7.25–7.28 (m, 1H), 8.76–8.80 (m, 1H).

The following compounds were prepared in the same manner as **4a**, using the appropriate alkylating reagent.

4.1.3. 4-(2-Oxo-3-thiophen-3-ylmethyl-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (4b). Using 3-bromomethylthiophene[13] as the alkylating reagent the residue was purified by flash column chromatography on silica gel (heptane/ethyl acetate; 50:50; as eluant) to give the title compound as a pale yellow solid in 36% yield. ESI-MS m/z = 414.3 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.78–1.88 (m, 2H), 2.26–2.41 (m, 2H), 2.78–2.97 (m, 2H), 4.20–4.44 (br s, 2H), 4.45–4.58 (m, 1H), 5.06 (s, 2H), 6.94–6.98 (m, 1H), 7.01–7.08 (m, 3H), 7.10–7.15 (m, 1H), 7.17–7.21 (m, 1H), 7.25–7.29 (m, 1H).

4.1.4. 4-(2-Oxo-3-pyridin-2-ylmethyl-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (4c). Using 2-chloromethylpyridine as the alkylating reagent the residue was purified by precipitating from diethyl ether to give the title compound as a white solid in 66% yield. ESI-MS m/z = 409 $[M+H]^+$, 432 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.84–1.87 (m, 2H), 2.34 (dd, J = 12.8 and 4.4 Hz, 2H), 2.85–2.91 (m, 2H), 4.33 (br s, 2H), 4.53 (tt, J = 12.4 and 4.4 Hz, 1H), 5.19 (s, 2H), 6.99–7.07 (m, 3H), 7.11–7.20 (m, 3H), 7.61 (dt, J = 7.6 and 1.6 Hz, 1H), 8.56–8.58 (m, 1H).

4.1.5. 4-[3-(2-Methylthiazol-4-ylmethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (4d). Using 4-chloromethyl-2-methylthiazole hydrochloride as alkylating agent, the crude product was purified by chromatography on silica gel (dichloromethane/ethanol; 98:2; as eluant) to give the title compound as a yellow oil in quantitative yield. ESI m/z = 429.4 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.84 (br d, J = 12.8 Hz, 2H), 2.34 (dq, J = 12.5 and 4.5 Hz, 2H), 2.68 (s, 3H), 2.86 (br t, J = 12.8 Hz, 2H), 4.26–4.37 (m, 2H), 4.51 (tt, J = 12.5 and 4.2 Hz, 1H), 5.15 (s, 2H), 6.87 (s, 1H), 7.02–7.07 (m 2H), 7.08–7.15 (m, 2H).

4.1.6. 4-(2-Oxo-3-pyridin-4-ylmethyl-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (4e). Using 4-chloromethylpyridine as the alkylating reagent the residue was purified by precipitating from diethyl ether to give the title compound as a yellow solid in 89% yield. ESI-MS m/z = 409 $[M+H]^+$, 432 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.84–1.87 (m, 2H), 2.35 (dd, J = 12.4 and 4.4 Hz, 2H), 2.84–2.90 (m, 2H), 4.33 (br s, 2H), 4.51 (tt, J = 12.5 and 4.4 Hz, 1H), 5.06 (s, 2H), 6.81 (d, J = 8.0 Hz, 1H), 7.00–7.09 (m, 2H), 7.16–7.18 (m, 3H), 8.55 (dd, J = 4.4 and 1.6 Hz, 2H).

4.1.7. 4-[3-(5-*tert*-Butyl-1,2,4-oxadiazol-3-ylmethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (4f). Using 5-*tert*-butyl-3-chloromethyl-1,2,4-oxadiazole as alkylating agent, the crude product was purified by chromatography on silica gel (heptane/ethyl acetate; 1:1; as eluant) to give the title compound as a clear oil in quantitative yield. ESI m/z = 456.4 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.34 (s, 9H), 1.50 (s, 9H), 1.85 (br d, J = 12.6 Hz, 2H), 2.34 (dq, J = 12.6 and 4.5 Hz, 2H), 2.87 (br t, J = 12.5 Hz, 2H), 4.23–4.39 (m, 2H), 4.51 (tt, J = 12.8 and 4.0 Hz, 1H), 5.16 (s, 2H), 7.04–7.12 (m, 3H), 7.12–7.18 (m, 1H).

4.1.8. 4-(3-Oxazol-4-ylmethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (4g). Using methanesulfonic acid oxazol-4-ylmethyl ester[14] as alkylating agent, the crude product was chromatographed on silica gel (dichloromethane/ethanol; 99:1 then 98:2; gradient as eluant) to give the title compound as a pale gum in 44% yield. ESI m/z = 399.3 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.80 (d, J = 12.2 Hz, 2H), 2.24–2.39 (m, 2H), 2.83–2.93 (m, 2H), 4.30–4.39 (m, 2H), 4.41–4.51 (m, 1H), 5.01 (s, 2H), 7.07–7.15 (m, 3H), 7.18–7.26 (m, 1H), 7.62 (s, 1H), 7.82 (s, 1H).

4.1.9. 4-(3-Oxazol-2-ylmethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (4h). Using methanesulfonic acid oxazol-2-ylmethyl ester[15] as the alkylating reagent, the residue was purified by flash column chromatography on silica gel (dichloromethane/methanol; 98:2; as eluant) to give the title compound as a yellow solid in 16% yield. ESI-MS m/z = 399.5 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.82–1.85 (m, 2H), 2.28–2.37 (m, 2H), 2.83–2.88 (m, 2H), 4.31 (br s, 1H), 4.47–4.53 (m, 1H), 5.19 (s, 2H), 5.29 (s, 1H), 7.01–7.09 (m, 4H), 7.12–7.18 (m, 1H), 7.59–7.62 (m, 1H).

4.1.10. 4-[3-(5-Methyl-isoxazol-3-ylmethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (4i). Using 3-chloromethyl-5-methyl-isoxazole as alkylating agent, the crude compound was obtained in quantitative yield and used without further purification. ESI m/z = 413.1 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.85 (d, J = 12.3 Hz, 2H), 2.32 (dq, J = 12.3 and 4.3 Hz, 2H), 2.35 (s, 3H), 2.87 (br t,J = 12.3 Hz, 2H), 4.31 (br s, 2H), 4.47–4.53 (m, 1H), 5.07 (s, 2H), 5.96 (s, 1H), 7.06–7.18 (m, 4H).

4.1.11. 4-[3-(3,5-Dimethyl-isoxazol-4-ylmethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (4j). Using 4-(chloromethyl)-3,5-dimethyl-isoxazole as the alkylating reagent, the residue was purified by flash column chromatography on silica gel (ethyl acetate; as eluant) to give the title compound as a yellow solid in 45% yield. ESI-MS m/z = 427 $[M+H]^+$, 327 $[M\text{-Boc}]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.78–1.81 (m, 2H), 2.19 (s, 3H), 2.27–2.41 (m, 2H), 2.44 (s, 3H), 2.82–2.88 (m, 2H), 4.32 (br s, 2H), 4.37–4.50 (m, 1H), 4.80 (s, 2H), 6.81–6.84 (m, 1H), 7.00–7.08 (m, 2H), 7.12–7.15 (m, 1H).

4.1.12. 4-(2-Oxo-3-thiophen-2-ylmethyl-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (4k). Using 2-chloromethylthiophene[12] as the alkylating reagent the residue was purified by flash column chromatography on silica gel (dichloromethane/methanol; 99.7:0.3; as eluant) to give the title compound as a pale yellow solid in 69% yield. ESI-MS m/z = 414.1 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.76–1.88 (m, 2H), 2.24–2.40 (m, 2H), 2.76–2.96 (m, 2H), 4.12–4.44 (br s, 2H), 4.44–4.56 (m, 1H), 5.21 (s, 2H), 6.91–6.96 (m, 1H), 7.02–7.15 (m, 5H), 7.18–7.22 (m, 1H).

4.1.13. 4-(3-Hydroxymethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (9). A mixture of **3** (10.0 g, 31.5 mmol), 35% formalin (30 mL), and 1,4-dioxane (100 mL) was stirred at reflux temperature for 20 h. The reaction mixture was concentrated in vacuo, and the resultant residue was dissolved in water (200 mL) and extracted with dichloromethane (4× 250 mL). The organic layers were combined, washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (*n*-heptane/ethyl acetate; 75:25; as eluant) to give a colourless solid. The solid was then washed with a mixture of *n*-heptane/ethyl acetate 1:1 to give the title compound as a colourless solid (6.39 g, 59%). ESI-MS m/z = 348.3 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.72–1.82 (m, 2H), 2.22–2.38 (m, 2H), 2.74–2.94 (m, 2H), 3.17 (t, J = 7.7 Hz, 1H), 4.20–4.38 (br s, 2H), 4.38–4.49 (m, 1H), 5.43 (d, J = 7.7 Hz, 2H), 7.06–7.16 (m, 3H), 7.17–7.22 (m, 1H).

4.1.14. 4-(3-Imidazol-1-ylmethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (4p). Methanesulfonyl chloride (317 mg, 2.76 mmol) was added dropwise to a solution of **9** (800 mg, 2.30 mmol) in dichloromethane (8 mL) with ice-water bath cooling and stirred for 1.5 h. The reaction mixture was then diluted with dichloromethane (40 mL) and washed with cold water. The combined organics were dried ($MgSO_4$), filtered and concentrated in vacuo to give the intermediate mesylate as an oil which was used without further purification. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.75–1.90 (m, 2H), 2.20–2.40 (m, 2H), 2.75–2.95 (m, 2H), 4.20–4.50 (m, 3H), 5.76 (s, 2H), 7.05–7.25 (m, 4H).

The mesylate from the previous step was dissolved in dichloromethane (8 mL), and imidazole (783 mg, 11.5 mmol) added to the solution under ice-water bath cooling. The resulting mixture was stirred at room temperature for 3 days. The reaction mixture was diluted with dichloromethane (40 mL), and the resulting mixture was washed with 5% aqueous solution of sodium carbonate. The organic layer was separated and extracted further with dichloromethane (4× 100 mL). The organic layers were combined, washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was then purified by flash column chromatography on silica gel (dichloromethane/methanol; 96:4; as eluant) to give the title compound as a yellow solid (740 mg, 81%). ESI-MS m/z = 398.1 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.77–1.88 (m, 2H), 2.24–2.38 (m, 2H), 2.76–2.94 (m, 2H), 4.20–4.39 (br s, 2H), 4.39–4.50 (m, 1H), 5.95 (s, 2H), 7.02–7.22 (m, 6H), 7.78 (s, 1H).

The following compounds were prepared in the same manner as **4p**, using the appropriate *N*-heterocycle.

4.1.15. 4-(2-Oxo-3-pyrazol-1-ylmethyl-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (4q). Using pyrazole as the amine heterocycle the resultant residue was purified by flash column chroma-

tography on silica gel (dichloromethane/methanol; 98:2; as eluant) to give the title compound as a colourless solid in 74% yield. ESI-MS m/z = 398.1 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.74–1.86 (m, 2H), 2.23–2.38 (m, 2H), 2.76–2.93 (m, 2H), 4.22–4.39 (br s, 2H), 4.39–4.50 (m, 1H), 6.13 (s, 2H), 6.24–5.29 (m, 1H), 7.06–7.14 (m, 3H), 7.44–7.49 (m, 1H), 7.49–7.53 (m, 1H), 7.69–7.82 (m, 1H).

4.1.16. 4-[3-(2-Methyl-imidazol-1-ylmethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (4r). Using 2-methyl-imidazole as the amine heterocycle the resultant residue was purified by flash column chromatography on silica gel (dichloromethane/methanol; 97:3; as eluant) to give the title compound as a white solid in 79% yield. ESI-MS m/z = 412.3 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.78–1.88 (m, 2H), 2.26–2.44 (m, 2H), 2.59 (s, 3H), 2.78–2.96 (m, 2H), 4.20–4.38 (br s, 2H), 4.38–4.50 (m, 1H), 5.88 (s, 2H), 6.89–6.93 (m, 1H), 7.05–7.19 (m, 5H).

4.1.17. 4-(2-Oxo-3-1,2,4-triazol-1-ylmethyl-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (4s). Using 1,2,4-triazole as the amine heterocycle the resultant residue was purified by flash column chromatography on silica gel (dichloromethane/methanol; 98:2; as eluant) to give the title compound as colourless oil in 26% yield. ESI-MS m/z = 399.3 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.76–1.88 (m, 2H), 2.24–2.40 (m, 2H), 2.76–2.94 (m, 2H), 4.24–4.38 (br s, 2H), 4.38–4.50 (m, 1H), 6.17 (s, 2H), 7.10–7.19 (m, 3H), 7.41–7.47 (m, 1H), 7.93 (s, 1H), 8.40 (s, 1H).

4.1.18. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-thiazol-4-ylmethyl-1,3-dihydro-benzimidazol-2-one hydrochloride (7a). Trifluoroacetic acid (3.0 mL) was added dropwise to a solution of **4a** (600 mg, 1.45 mmol) in dichloromethane (30 mL) under ice-water cooling and stirred at room temperature for 16 h. Aqueous solution (1 M) of sodium hydroxide (30 mL) was then added carefully to the reaction mixture, and the resulting organic layer was separated. The aqueous layer was extracted with dichloromethane (4× 100 mL). The combined organics were washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo to give the free amine **5a** as a colourless solid (420 mg, 92%). ESI-MS m/z = 315.1 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.62 (br s, 1H), 1.78–1.92 (m, 2H), 2.28–2.44 (m, 2H), 2.74–2.88 (m, 2H), 3.20–3.34 (m, 2H), 4.42–4.56 (m, 1H), 5.25 (s, 2H), 7.03–7.14 (m, 3H), 7.17–7.20 (m, 1H), 7.26–7.31 (m, 1H), 8.77 (s, 1H).

A mixture of 2-[1-(2-chloro-ethyl)-2-methyl-propoxy]-4-methoxy-1-methyl-benzene **6**[10] (624 mg, 2.00 mmol), **5a** (420 mg, 1.34 mmol), diisopropylethylamine (259 mg, 2.00 mmol), sodium iodide (30 mg) and acetonitrile (15 mL) was stirred at gentle reflux temperature for 20 h. The reaction mixture was diluted with 1 M aqueous solution of sodium hydroxide (20 mL), then the resulting mixture was extracted with dichloromethane (4× 100 mL). The organic layers were combined, washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The resultant residue was purified by flash column chromatography on silica gel (dichloromethane/methanol; 98:2; as eluant). The obtained free base of the title compound was dissolved in diethyl ether, and 1 M solution of hydrochloric acid in diethyl ether added dropwise. The resulting solution was concentrated in vacuo to give the title compound as a colourless solid (320 mg, 39%). ESI-MS m/z = 535.3 $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 6.8 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H), 1.98–2.24 (m, 8H), 2.80–2.96 (m, 2H), 3.18–3.38 (m, 4H), 3.70–3.83 (m, 5H), 4.32–4.40 (m, 1H), 4.56–4.70 (m, 1H), 5.26 (s, 2H), 6.43 (dd, J = 2.5, 8.3 Hz, 1H), 6.52 (d, J = 2.5 Hz, 1H), 7.03 (d, J = 8.3 Hz, 1H), 7.04–7.17 (m, 3H), 7.36–7.43 (m, 1H), 7.53 (d, J = 2.0 Hz, 1H), 9.07 (d, J = 2.0 Hz, 1H).

The following compounds were prepared in the same manner as **7a**.

4.1.19. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-thiophen-3-ylmethyl-1,3-dihydro-benzimidazol-2-one hydrochloride (7b). Starting with **4b** the title compound was purified by flash column chromatography on silica gel (dichloromethane/methanol; 99.4:0.6; as eluant). The obtained free base was dissolved in diethyl ether, and 1 M solution of hydrochloric acid in diethyl ether added dropwise. The resulting solution was concentrated in vacuo to give the title compound as a pale yellow solid in 34% yield. ESI-MS m/z = 534.3 $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 7.2 Hz, 3H), 1.05 (d, J = 7.2 Hz, 3H), 2.00–2.24 (m, 8H), 2.78–2.94 (m, 2H), 3.14–3.44 (m, 4H), 3.68–3.84 (m, 5H), 4.32–4.41 (m, 1H), 4.54–4.66 (m, 1H), 5.08 (s, 2H), 6.43 (dd, J = 8.2 and 2.3 Hz, 1H), 6.52 (d, J = 2.3 Hz, 1H), 7.00–7.18 (m, 5H), 7.26–7.40 (m, 3H).

4.1.20. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-pyridin-2-ylmethyl-1,3-dihydro-benzimidazol-2-one hydrochloride (7c). Starting with **4c** the title compound was purified by flash column chromatography on silica gel (dichloromethane/ethanol; 98:2; as eluant). The obtained free base was dissolved in diethyl ether, and 1 M solution of hydrochloric acid in diethyl ether added dropwise. The resulting solution was concentrated in vacuo to give the title compound as a white solid in 41% yield. ESI-MS m/z = 409.6 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 0.97 (d, J = 6.8 Hz, 3H), 1.01 (d, J = 6.8 Hz, 3H), 1.98–2.16 (m, 6H), 2.25–2.44 (m, 2H), 2.85–2.95 (m, 2H), 3.11–3.16 (m, 2H), 3.31–3.48 (m, 2H), 3.64–3.66 (m, 1H), 3.78 (s, 3H), 3.77–3.80 (m, 1H), 4.24–4.28 (m, 1H), 4.75–4.81 (m, 1H), 5.58 (s, 2H), 6.39–6.44 (m, 2H), 7.01–7.11 (m, 2H), 7.18–7.24 (m, 2H), 7.60–7.65 (m, 2H), 7.93 (d, J = 8.0 Hz, 1H), 8.08 (t, J = 7.6 Hz, 1H), 8.69 (d, J = 4.8 Hz, 1H).

4.1.21. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-(2-methylthiazol-4-ylmethyl)-1,3-dihydro-benzimidazol-2-one hydrochloride (7d). Starting with **4d** the title compound was purified by flash column chromatography on silica gel (dichloromethane/ethanol; 98:2 then 96:4 gradient; as eluant). The free base of the title compound (64% yield) was dissolved

in dichloromethane, and 1 M solution of hydrochloric acid in diethyl ether added. The hydrochloride salt was then precipitated twice from acetone/ether to give an off-white solid in 73% yield from free base. ESI-MS m/z = 549.3 $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.02 (d, J = 7.0 Hz, 3H), 1.04 (d, J = 7.0 Hz, 3H), 2.03–2.23 (m, 5H), 2.16 (s, 3H), 2.65 (s, 3H), 2.81–2.94 (m, 2H), 3.20–3.29 (m, 2H), 3.76 (s, 3H), 4.36 (q, J = 5.5 Hz, 1H), 4.63 (tt, J = 12.2 and 3.9 Hz, 1H), 5.13 (s, 2H), 6.42 (dd, J = 12.2 and 3.9 Hz, 1H), 6.52 (br s, 1H), 7.02 (d, J = 8.3 Hz, 1H), 7.06–7.15 (m, 3H), 7.21 (s, 1H), 7.39 (d, J = 7.3 Hz, 1H).

4.1.22. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-pyridin-4-ylmethyl-1,3-dihydro-benzimidazol-2-one hydrochloride (7e). Starting with **4e** the title compound was purified by flash column chromatography on silica gel (dichloromethane/methanol; 98:2; as eluant). The obtained free base was dissolved in diethyl ether, and 1 M solution of hydrochloric acid in diethyl ether added dropwise. The resulting solution was concentrated in vacuo to give the title compound as a white solid in 12% yield. ESI-MS m/z = 409.5 [M+H]. ^1H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 6.8 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H), 2.03–2.23 (m, 8H), 2.87–2.97 (m, 2H), 3.25–3.34 (m, 4H), 3.75–3.80 (m, 5H), 4.36–4.39 (m, 1H), 4.67–4.73 (m, 1H), 4.77–4.81 (m, 2H), 5.43 (s, 2H), 6.42 (dd, J = 8.0 and 2.0 Hz, 1H), 6.53 (s, 1H), 7.02 (d, J = 8.0 Hz, 1H), 7.07–7.14 (m, 2H), 7.19 (t, J = 7.6 Hz, 1H), 7.53 (d, J = 8.0 Hz, 1H), 7.92 (d, J = 5.6 Hz, 2H), 8.79 (s, 2H).

4.1.23. 1-(5-*tert*-Butyl-1,2,4-oxadiazol-3-ylmethyl)-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one methansulfonate (7f). Starting with **4f** the title compound was purified by flash column chromatography on silica gel (dichloromethane/ethanol; 98:2 then 96:4 gradient; as eluant). The obtained free base of the title compound (18% yield) was dissolved in dichloromethane, and 1 equivalent of methanesulfonic acid was added. The methanesulfonate salt was crystallised from acetone to give a white solid in 74% yield from free base. ESI-MS m/z = 576.3 $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.02 (d, J = 7.0 Hz, 3H), 1.04 (d, J = 7.0 Hz, 3H), 1.39 (s, 3H), 2.05–2.20 (m, 5H), 2.16 (s, 3H), 2.70 (s, 3H), 2.76–2.88 (m, 2H), 3.16–3.32 (m, 4H), 3.75 (s, 3H), 4.35 (q, J = 5.5 Hz, 1H), 4.58 (tt, J = 12.2 and 4.0 Hz, 1H), 5.20 (s, 2H), 6.44 (dd, J = 8.2 and 2.2 Hz, 1H), 6.52 (br s, 1H), 7.02 (d, J = 8.8 Hz, 1H), 7.07–7.19 (m, 3H), 7.33 (d, J = 7.8 Hz, 1H).

4.1.24. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-oxazol-4-ylmethyl-1,3-dihydro-benzimidazol-2-one methanesulfonate (7g). Starting with **4g** the title compound was purified by flash column chromatography on silica gel (dichloromethane/ethanol; 98:2 then 96:4 gradient; as eluant). The obtained free base (25% yield) was dissolved in dichloromethane, and converted to the methanesulfonate salt by addition of one equivalent of methanesulfonic acid. The salt was crystallised twice from acetone/diethyl ether to give a white solid (68% yield from free base). ESI-MS m/z = 519.0 $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 6.8 Hz, 3H), 1.06 (d, J = 6.8 Hz, 3H), 2.03–2.19 (m, 5H), 2.16 (s, 3H), 2.71 (s, 3H), 2.84 (dq, J = 12.8 and 2.5 Hz, 2H), 3.24 (br t, J = 12.6 Hz, 2H), 3.73–3.76 (m, 2H), 3.76 (s, 3H), 4.37 (q, J = 5.0 Hz, 1H), 4.58 (tt, J = 12.2 and 4.0 Hz, 1H), 5.03 (s, 2H), 6.44 (dd, J = 2.3 and 8.3 Hz, 1H), 6.52 (d, J = 2.3 Hz, 1H), 7.04 (d, J = 8.3 Hz, 1H), 7.08–7.15 (m, 2H), 7.21 (d, J = 7.5 Hz, 1H), 7.32 (d, J = 7.5 Hz, 1H), 7.93 (s, 1H), 8.11 (s, 1H).

4.1.25. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-oxazol-2-ylmethyl-1,3-dihydro-benzimidazol-2-one methanesulfonate (7h). Starting with **4h** the title compound was purified by flash column chromatography on silica gel (dichloromethane/ethanol; 97:3; as eluant). The obtained free base was converted to the methanesulfonate salt by addition of one equivalent of methanesulfonic acid. The salt was crystallised twice from acetone/diethyl ether to give a white solid in 47% yield. ESI-MS m/z = 519.6 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 0.96–0.99 (m, 6H), 1.70–1.83 (m, 4H), 1.99–2.15 (m, 6H), 2.38–2.51 (m, 4H), 2.96–2.98 (m, 1H), 3.07–3.10 (m, 1H), 3.75 (s, 3H), 4.23–4.24 (m, 1H), 4.38–4.44 (m, 1H), 5.19 (s, 2H), 6.36 (d, J = 8.0 Hz, 1H), 6.62 (s, 1H), 7.01 (d, J = 8.4 Hz, 1H), 7.05–7.07 (m, 4H), 7.32 (d, J = 6.8 Hz, 1H), 7.58 (s, 1H).

4.1.26. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-(5-methyl-isoxazol-3-ylmethyl)-1,3-dihydro-benzimidazol-2-one methanesulfonate (7i). Starting with **4i** the title compound was purified by flash column chromatography on silica gel (dichloromethane/ethanol; 98.5:1.5; as eluant). The obtained free base of (46% yield) was dissolved in dichloromethane and 1 equivalent of methanesulfonic acid was added. The methanesulfonate salt was precipitated three times from acetone by adding copious amounts of diethyl ether to give a white solid in 64% yield from free base. ESI-MS m/z = 533.3 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 0.98 (d, J = 7.0 Hz, 3H), 1.03 (d, J = 7.0 Hz, 3H), 1.96–2.18 (m, 4H), 2.15 (s, 3H), 2.19–2.40 (m, 4H), 2.35 (s, 3H), 2.80–2.95 (m, 2H), 3.03–3.28 (m, 4H), 3.74 (d, J = 10.0 Hz, 1H), 3.77 (s, 3H), 3.87 (d, J = 10.0 Hz, 1H), 4.26 (m, 1H), 4.73 (m, 1H), 5.06 (s, 2H), 5.92 (s, 1H), 6.41 (br s, 2H), 7.04 (d, J = 8.2 Hz, 1H), 7.06–7.11 (m, 2H), 7.13–7.18 (m, 1H), 7.68 (d, J = 8.0 Hz, 1H).

4.1.27. 1-(3,5-Dimethyl-isoxazol-4-ylmethyl)-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one hydrochloride (7j). Starting with **4j** the title compound was purified by flash column chromatography on silica gel (dichloromethane/ethanol; 99:1; as eluant). The obtained free base was dissolved in diethyl ether, and 1 M solution of hydrochloric acid in diethyl ether added dropwise. The resulting solution was concentrated in vacuo to give the title compound as a pale yellow solid in 5% yield. ESI-MS m/z = 547.6 [M+H]. ^1H NMR (400 MHz, CD_3OD): δ 0.96 (d, J = 6.8 Hz, 3H), 0.99 (d, J = 6.8 Hz, 3H), 1.72–1.85 (m, 4H), 1.97–2.14 (m, 3H), 3.15 (s, 3H), 2.19 (s, 3H), 2.38–2.49 (m, 7H), 2.96–2.98 (m, 1H),

3.07–3.10 (m, 1H), 3.75 (s, 3H), 4.22–4.26 (m, 1H), 4.34–4.40 (m, 1H), 4.79 (s, 2H), 6.36 (dd, J = 8.0 and 2.4 Hz, 1H), 6.61 (d, J = 2.4 Hz, 1H), 6.81 (d, J = 7.2 Hz, 1H), 7.00–7.08 (m, 3H), 7.31 (d, J = 7.6 Hz, 1H).

4.1.28. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-thiophen-2-ylmethyl-1,3-dihydro-benzimidazol-2-one hydrochloride (7k). Starting with **4k** the title compound was purified by flash column chromatography on silica gel (dichloromethane/methanol; 99.2:0.8; as eluant). The obtained free base was dissolved in diethyl ether, and 1 M solution of hydrochloric acid in diethyl ether added dropwise. The resulting solution was concentrated in vacuo to give the title compound as a pale yellow solid in 45% yield. ESI-MS m/z = 534.3 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 7.0 Hz, 3H), 1.05 (d, J = 7.0 Hz, 3H), 2.00–2.23 (m, 8H), 2.77–2.94 (m, 2H), 3.16–3.40 (m, 4H), 3.66–3.82 (m, 5H), 4.32–4.41 (m, 1H), 4.54–4.65 (m, 1H), 5.25 (s, 2H), 6.43 (dd, J = 2.2 and 8.2 Hz, 1H), 6.52 (d, J = 2.2 Hz, 1H), 6.94 (dd, J = 5.2 and 3.2 Hz, 1H), 7.03 (d, J = 8.2 Hz, 1H), 7.06–7.23 (m, 4H), 7.26–7.39 (m, 2H).

4.1.29. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-pyridin-3-ylmethyl-1,3-dihydro-benzimidazol-2-one hydrochloride (7l). Using 3-chloromethylpyridine as the alkylating reagent **4l** was prepared in a similar manner to **4a**. The residue was purified by precipitating the solid from diethyl ether to give the intermediate compound as a yellow solid in 77% yield. ESI-MS m/z = 409.5 $[M+H]^+$. The material was used without further purification. Starting with **4l** the title compound was prepared in a similar manner to **7a**. The residue was purified by flash column chromatography on silica gel (dichloromethane/ethanol; 97:3; as eluant). The obtained free base of the title compound was dissolved in diethyl ether, and 1 M solution of hydrochloric acid in diethyl ether added dropwise. The resulting solution was concentrated in vacuo to give the title compound as a white solid in 19% yield. ESI-MS m/z = 409.4 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 6.8 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H), 2.03–2.12 (m, 8H), 2.85–2.96 (m, 2H), 3.23–3.33 (m, 2H), 3.74–3.80 (m, 5H), 4.35–4.39 (m, 1H), 4.62–4.70 (m, 1H), 4.77–4.81 (m, 2H), 5.35 (s, 2H), 6.42 (dd, J = 8.0 and 2.4 Hz, 2H), 6.53 (d, J = 2.4 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 7.08–7.21 (m, 3H), 7.49 (d, J = 8.0 Hz, 1H), 8.06 (dd, J = 8.0 and 5.6 Hz, 1H), 8.55 (d, J = 8.4 Hz, 1H), 8.80 (d, J = 5.6 Hz, 1H), 8.90 (s, 1H).

4.1.30. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-pyrazin-2-ylmethyl-1,3-dihydro-benzimidazol-2-one hydrochloride (7m). Using 2-chloromethylpyrazine[16] was used as the alkylating agent for substrate **8**[10] and following the methods for **4a**. The final compound was purified by column chromatography on silica gel (dichloromethane/methanol; 98.5:1.5; as eluant) to give the free base. This was then dissolved in diethyl ether, and mixed with 1 M solution of hydrochloric acid in diethyl ether. The resulting mixture was concentrated in vacuo to give the title compound as a brown solid in a yield of 36%. ESI-MS m/z = 530.3 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 6.6 Hz, 3H), 1.05 (d, J = 6.6 Hz, 3H), 1.98–2.24 (m, 8H), 2.78–2.94 (m, 2H), 3.18–3.38 (m, 4H), 3.70–3.83 (m, 5H), 4.32–4.41 (m, 1H), 4.57–4.68 (m, 1H), 5.27 (s, 2H), 6.43 (dd, J = 8.2 and 2.0 Hz, 1H), 6.52 (d, J = 2.0 Hz, 1H), 7.03 (d, J = 8.2 Hz, 1H), 7.04–7.19 (m, 3H), 7.39 (d, J = 7.2 Hz, 1H), 8.47–8.55 (m, 2H), 8.61 (s, 1H).

4.1.31. 1-(1*H*-Imidazol-4-ylmethyl)-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one hydrochloride (7o). Using 1-(4-methylphenylsulfonyl)-4-methanesulfonyloxymethyl imidazole[17] as the alkylating agent for substrate **8**,[10] and following the methods outlined for **4a**, the intermediate **7n** was purified by column chromatography on silica gel (dichloromethane/ethanol; 98.5:1.5; as eluant) to give the free base in 23% yield. ESI-MS m/z = 672.5 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (d, J = 7.2 Hz, 3H), 0.98 (d, J = 7.2 Hz, 3H), 1.75–1.90 (m, 4H), 1.95–2.12 (m, 2H), 2.15 (s, 3H), 2.35–2.50 (m, 5H), 2.92–2.98 (m, 1H), 3.05–3.10 (m, 1H), 3.76 (s, 3H), 4.20–4.23 (m, 1H), 4.33–4.40 (m, 1H), 4.95 (s, 2H), 6.36 (d, J = 8.2 Hz, 1H), 6.62 (s, 1H), 7.00–7.13 (m, 3H), 7.17 (s, 1H), 7.32–7.34 (m, 3H), 7.77 (d, J = 8.0 Hz, 2H), 7.92 (s, 1H). This material was dissolved in dichloromethane and treated with excess 1 M solution of hydrochloric acid in diethyl ether and stirred for 1 h. The solution was evaporated to dryness and dissolved in dichloromethane, diethyl ether was added slowly to effect precipitation and the solid collected by filtration. The white solid was crystallised from dichloromethane and acetone to give the detosylated compound **7o** (100% yield). ESI-MS m/z = 518.3 $[M+H]^+$. ^{1}H NMR (400 MHz, D_2O): δ 0.90 (d, J = 8.0 Hz, 3H), 0.93 (d, J = 8.0 Hz, 3H), 1.96–2.01 (m, 1H), 2.11 (s, 3H), 2.13–2.11 (m, 4H), 2.60–2.71 (m, 2H), 3.12–3.20 (m, 2H), 3.23–3.29 (m, 2H), 3.68–3.70 (m, 2H), 3.75 (s, 3H), 4.30–4.33 (m, 1H), 4.55–4.61 (m, 1H), 5.20 (s, 2H), 6.50 (d, J = 8.0 Hz, 1H), 6.58 (s, 1H), 7.10 (d, J = 8.3 Hz, 1H), 7.12–7.23 (m, 3H), 7.33 (d, J = 8.3Hz, 1H), 7.40 (s, 1H), 8.6 (s, 1H).

4.1.32. 1-Imidazol-1-ylmethyl-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one hydrochloride (7p). Starting with **4p** the title compound was purified by flash column chromatography on silica gel (dichloromethane/methanol; 98:2; as eluant). The obtained free base was dissolved in diethyl ether, and 1 M solution of hydrochloric acid in diethyl ether added dropwise. The resulting solution was concentrated in vacuo to give the title compound as a colourless solid in 15% yield. ESI-MS m/z = 518.3 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 6.6 Hz, 3H), 1.05 (d, J = 6.6 Hz, 3H), 1.98–2.28 (m, 8H), 2.78–2.98 (m, 2H), 3.16–3.40 (m, 4H), 3.68–3.86 (m, 5H), 4.32–4.44 (m, 1H), 4.56–4.70 (m, 1H), 6.37 (s, 2H), 6.43 (dd, J = 8.3 and 2.4 Hz, 1H), 6.50–6.56 (m, 1H), 7.03 (d, J = 8.3 Hz, 1H), 7.16–7.28 (m, 2H), 7.42–7.54 (m, 2H), 7.58 (s, 1H), 7.76 (s, 1H), 9.22 (s, 1H).

4.1.33. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-pyrazol-1-ylmethyl-1,3-dihydro-benzimidazol-2-one hydrochloride (7q). Starting with **4q** the title compound was purified by flash column chromatography on silica gel (dichloromethane/methanol; 98:2; as eluant). The obtained free base was dissolved in diethyl ether, and 1 M solution of hydrochloric acid in diethyl ether added dropwise. The resulting solution was concentrated in vacuo to give the title compound as a yellow solid in 23% yield. ESI-MS $m/z = 518.3$ $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.01 (d, $J = 6.6$ Hz, 3H), 1.05 (d, $J = 6.6$ Hz, 3H), 1.96–2.23 (m, 8H), 2.75–2.91 (m, 2H), 3.15–3.38 (m, 4H), 3.70–3.82 (m, 5H), 4.30–4.40 (m, 1H), 4.51–4.63 (m, 1H), 6.18 (s, 2H), 6.32 (s, 1H), 6.43 (dd, $J = 8.6$ and 2.2 Hz, 1H), 6.52 (d, $J = 2.2$ Hz, 1H), 7.03 (d, $J = 8.6$ Hz, 1H), 7.09–7.20 (m, 2H), 7.27–7.34 (m, 1H), 7.38–7.46 (m, 1H), 7.46–7.53 (m, 1H), 7.85 (s, 1H).

4.1.34. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-(2-methyl-imidazol-1-ylmethyl)-1,3-dihydro-benzimidazol-2-one hydrochloride (7r). Starting with **4r** the title compound was purified by flash column chromatography on silica gel (dichloromethane/methanol; 98:2; as eluant). The obtained free base was dissolved in diethyl ether, and 1 M solution of hydrochloric acid in diethyl ether added dropwise. The resulting solution was concentrated in vacuo to give the title compound as a pale brown solid in 11% yield. ESI-MS $m/z = 532.3$ $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.01 (d, $J = 7.0$ Hz, 3H), 1.05 (d, $J = 7.0$ Hz, 3H), 1.98-2.26 (m, 8H), 2.76–2.98 (m, 5H), 2.87 (s, 3H), 3.15–3.40 (m, 4H), 3.66–3.85 (m, 5H), 4.30–4.42 (m, 1H), 4.54–4.71 (m, 1H), 6.28 (s, 2H), 6.42 (dd, $J = 8.4$ and 2.8 Hz, 1H), 6.47–6.58 (m, 1H), 7.03 (d, $J = 8.4$ Hz, 1H), 7.16–7.28 (m, 2H), 7.39–7.54 (m, 3H), 7.62–7.70 (m, 1H).

4.1.35. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-(1,2,4-triazol-1-ylmethyl)-1,3-dihydro-benzimidazol-2-one hydrochloride (7s). Starting with **4s** the title compound was purified by flash column chromatography on silica gel (dichloromethane/methanol; 99:1; as eluant). The obtained free base was dissolved in diethyl ether, and 1 M solution of hydrochloric acid in diethyl ether added dropwise. The resulting solution was concentrated in vacuo to give the title compound as a pale yellow solid in 19% yield. ESI-MS $m/z = 519.3$ $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.01 (d, $J = 6.6$ Hz, 3H), 1.05 (d, $J = 6.6$ Hz, 3H), 1.96–2.30 (m, 8H), 2.72–2.96 (m, 2H), 3.10–3.43 (m, 4H), 3.66–3.86 (m, 5H), 4.28–4.44 (m, 1H), 4.50–4.67 (m, 1H), 6.35 (s, 2H), 6.39–6.60 (m, 2H), 7.03 (d, $J = 8.0$ Hz, 1H), 7.11–7.28 (m, 2H), 7.31–7.55 (m, 2H), 8.13 (br s, 1H), 8.98 (br s, 1H).

4.1.36. 4-(3-Ethoxycarbonylmethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (11). Prepared according to the method outlined for **4a** using ethyl chloroacetate as the alkylating reagent. The residue was purified by flash column chromatography on silica gel (dichloromethane/methanol; 98:2; as eluant) to give the title compound as a colourless solid in 84% yield. ESI-MS $m/z = 404.3$ $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.27 (t, $J = 7.0$ Hz, 3H), 1.50 (s, 9H), 1.78–1.89 (m, 2H), 2.24–2.39 (m, 2H), 2.77–2.94 (m, 2H), 4.22–4.38 (m, 3H), 4.41–4.53 (m, 1H), 4.61 (s, 2H), 6.85–6.92 (m, 1H), 7.02–7.16 (m, 3H).

4.1.37. 4-(3-Hydrazinocarbonylmethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (12). Compound **11** (2.4 g, 5.96 mmol) was treated with a large excess of hydrazine hydrate (55%, 4.0 mL) in ethanol (25 mL) for 45 min at reflux. The reaction mixture was cooled and poured into water and the resulting solid filtered off and washed well with water. The solid was dissolved in dichloromethane, washed with water then brine and the organics dried (Na_2SO_4). The dichloromethane was removed under vacuum and replaced with diethyl ether. The resultant solid was filtered off and dried to give the title compound as a dense white solid (2.0 g, 86% yield). ESI $m/z = 390.1$ $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.82 (br d, $J = 12.6$ Hz, 2H), 2.30 (dq, $J = 12.3$ and 4.5 Hz, 2H), 2.87 (br t, $J = 12.5$ Hz, 2H), 3.72–3.97 (br m, 2H), 4.24–4.37 (m, 2H), 4.43 (tt, $J = 12.5$ and 4.2 Hz, 1H), 4.52 (s, 2H), 7.04–7.18 (m, 4H), 7.46 (br s, 1H).

4.1.38. 4-[3-(5-Methyl-2*H*-1,2,4-triazol-3-ylmethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (13). Acetamidine hydrochloride (486 mg, 5.14 mmol) was dissolved in ethanol (6.0 mL) and treated with sodium methoxide (280 mg, 5.18 mmol) in ethanol (3.0 mL) and stirred for 20 min. The precipitated white solid was filtered off and **12** (1.0 g, 2.57 mmol) was added to the filtrate and the mixture stirred for 2 h. The intermediate acyl amidrazone was then filtered off and heated in methoxyethanol at 140 °C for 1 h in the presence of 4 Å molecular sieves. Removal of solvent under high vacuum gave the title compound as a gum (620 mg, 58% yield). ESI $m/z = 413.0$ $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.81 (br d, $J = 12.5$ Hz, 2H), 2.29 (dq, $J = 12.5$ and 4.5 Hz, 2H), 2.39 (s, 3H), 2.83 (br t, $J = 12.5$ Hz, 2H), 4.23–4.37 (br m, 2H), 4.48 (tt, $J = 12.5$ and 4.0 Hz, 1H), 5.13 (s, 2H), 7.02–7.08 (m, 2H), 7.08–7.13 (m, 1H), 7.13–7.18 (m, 1H).

4.1.39. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-(5-methyl-1*H*-1,2,4-triazol-3-ylmethyl)-1,3-dihydro-benzimidazol-2-one hydrochloride (14). Prepared according to the method outlined for **7k** using **13**, the title compound was purified by flash column chromatography on silica gel (dichloromethane/ethanol; 98:2 then 92:8 gradient; as eluant). The obtained free base of the title compound (58% yield) was dissolved in dichloromethane plus a few drops of ethanol and 1 M solution of hydrochloric acid in diethyl ether added. The hydrochloride salt was crystallised twice from acetone to give a creamy white solid in quantitative yield from the free amine. ESI-MS $m/z = 533.3$ $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.02 (d, $J = 7.0$ Hz, 3H), 1.04 (d, $J = 7.0$ Hz, 3H), 2.03–2.21 (m, 5H), 2.16 (s, 3H), 2.62 (s, 3H), 2.85–2.97 (m, 2H), 3.21–3.34 (m, 4H), 3.73–3.81 (m, 2H), 3.76 (s, 3H),

4.36 (q, J = 5.5 Hz, 1H), 4.66 (tt, J = 12.5 and 4.2 Hz, 1H), 5.33 (s, 2H), 6.42 (dd, J = 8.3 and 2.2 Hz, 1H), 6.52 (d, J = 2.2 Hz, 1H), 7.04 (d, J = 8.3 Hz, 1H), 7.10–7.21 (m, 3H), 7.47 (d, J = 7.5 Hz, 1H).

4.1.40. 4-[3-(3-Methyl-1,2,4-oxadiazol-5-ylmethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (15). Molecular sieves (4 Å powdered, 200 mg) were added to a suspension of *N*-hydroxyacetamidine[21] (222 mg, 3.00 mmol) in tetrahydrofuran (10 mL), and the resulting mixture stirred at room temperature for 30 min. Sodium hydride (60% suspension in mineral oil, 120 mg, 3.00 mmol) was added to the mixture and stirred at 50 °C for 20 min. After the reaction mixture had been cooled to room temperature, **11** (403 mg, 1.00 mmol) was added to the reaction mixture and stirred at reflux temperature for 2 h. The reaction mixture was concentrated in vacuo, and the resultant residue poured into water (200 mL) and extracted with dichloromethane (4× 100 mL). The organic layers were combined, washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (dichloromethane/methanol; 99:1; as eluant) to give the title compound as a colourless solid (110 mg, 27%). ESI-MS m/z = 414.1 $[M+H]^+$, 437.1 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.78–1.90 (m, 2H), 2.24–2.46 (m, 5H), 2.72–2.98 (m, 2H), 4.16–4.42 (br s, 2H), 4.42–4.56 (m, 1H), 5.27 (s, 2H), 6.94–7.20 (m, 4H).

4.1.41. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]piperidin-4yl}-3-[(3-methyl[1,2,4]oxadiazol-5-yl)-methyl]-1,3-dihydro-benzimidazol-2-one hydrochloride (16). Prepared according to the method outlined for **7a** using **15**, the title compound was purified by flash column chromatography on silica gel (dichloromethane/methanol; 99:1; as eluant). The obtained free base was dissolved in isopropanol and diethyl ether, and 1 M solution of hydrochloric acid in diethyl ether added dropwise. The resulting solution was concentrated in vacuo to give the title compound as a pale yellow solid in 38% yield. ESI-MS m/z = 534.2 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 6.4 Hz, 3H), 1.05 (d, J = 6.4 Hz, 3H), 2.00–2.23 (m, 8H), 2.31 (s, 3H), 2.78–2.94 (m, 2H), 3.16–3.38 (m, 4H), 3.69–3.83 (m, 5H), 4.33–4.41 (m, 1H), 4.56–4.67 (m, 1H), 5.38 (s, 2H), 6.43 (dd, J = 8.4 and 2.0 Hz, 1H), 6.52 (d, J = 2.0 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 7.10–7.22 (m, 3H), 7.36–7.45 (m, 1H).

4.1.42. 4-(3-Cyanomethyl-2-oxo-2,3-dihydro-benzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (17). Prepared according to the method outlined for **4a** using chloroacetonitrile as the alkylating reagent. The residue was purified by flash column chromatography on silica gel (*n*-heptane/ethyl acetate; 60:40; as eluant) to give the title compound as a yellow oil in 83% yield. ESI-MS m/z = 357.1 $[M+H]^+$, 380.1 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.78–1.88 (m, 2H), 2.25–2.40 (m, 2H), 2.78–2.94 (m, 2H), 4.24–4.38 (br s, 2H), 4.38–4.50 (m, 1H), 4.81 (s, 2H), 7.11–7.22 (m, 4H).

4.1.43. 4-[3-(*N*-Hydroxycarbamimidoylmethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (18). Hydroxylamine hydrochloride (351 mg, 5.05 mmol) was added to a solution of **17** (900 mg, 2.53 mmol), diisopropylethylamine (653 mg, 5.05 mmol), and ethanol (23 mL), and the resulting mixture stirred at reflux for 16 h. The reaction mixture was concentrated in vacuo, and the resultant residue dissolved in dichloromethane. The resulting mixture was washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was then purified by flash column chromatography on silica gel (dichloromethane/methanol; 97:3; as eluant) to give the title compound as a pale yellow solid (715 mg, 73%). ESI-MS m/z = 390.3 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.44 (s, 9H), 1.66–1.76 (m, 2H), 2.12–2.28 (m, 2H), 2.72–3.04 (br s, 2H), 4.00–4.20 (m, 2H), 4.32–4.46 (m, 3H), 5.43 (s, 2H), 6.98–7.14 (m, 3H), 7.20–7.29 (m, 1H), 9.20 (s, 1H).

4.1.44. 4-[3-(5-Methyl-1,2,4-oxadiazol-3-ylmethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (19). Molecular sieves (4 Å powdered, 200 mg) were added to a suspension of **18** (500 mg, 1.28 mmol) in tetrahydrofuran (10 mL), and the resulting mixture stirred at room temperature for 30 min. Sodium hydride (60% suspension in mineral oil, 77 mg, 1.93 mmol) was added to the mixture and stirred at 50 °C for 20 min. After the reaction mixture had been cooled to room temperature, ethyl acetate (452 mg, 5.13 mmol) was added to the mixture and stirred at reflux for 2 h. The reaction mixture was poured into water (200 mL) and extracted with dichloromethane (4× 100 mL). The organic layers were combined, washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The resultant oil was purified by flash column chromatography on silica gel (dichloromethane/methanol; 99:1; as eluant) to give the title compound as a colourless oil (432 mg, 81%). ESI-MS m/z = 414.2 $[M+H]^+$, 437.2 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.79–1.90 (m, 2H), 2.25–2.39 (m, 2H), 2.55 (s, 3H), 2.76–2.96 (m, 2H), 4.16–4.44 (br s, 2H), 4.46–4.57 (m, 1H), 5.16 (s, 2H), 7.02–7.18 (m, 4H).

4.1.45. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]piperidin-4yl}-3-[(5-methyl[1,2,4]oxadiazol-3-yl)-methyl]-1,3-dihydro-benzimidazol-2-one hydrochloride (20). Prepared according to the method outlined for **7a** using **19**, the title compound was purified by flash column chromatography on silica gel (dichloromethane/methanol; 99:1; as eluant). The obtained free base was dissolved in isopropanol and diethyl ether, and 1 M solution of hydrochloric acid in diethyl ether added dropwise. The resulting solution was concentrated in vacuo to give the title compound as a pale yellow solid in 46% yield. ESI-MS m/z = 534.2 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 6.8 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H), 1.98–2.28 (m, 8H), 2.53 (s, 3H), 2.77–2.95 (m, 2H), 3.15–3.39 (m, 4H), 3.70–3.84 (m, 5H), 4.32–4.42 (m, 1H), 4.54–4.68 (m, 1H), 5.19 (s, 2H), 6.38–6.46 (m, 1H), 6.47–6.56 (m, 1H), 7.03 (d, J = 8.4 Hz, 1H), 7.06–7.20 (m, 3H), 7.32–7.44 (m, 1H).

4.1.46. 4-[2-Oxo-3-(2H-1,2,4-triazol-3-ylmethyl)-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (21). Prepared according to the method outlined for **4a** using *N*-formyl-2-chloroacetamidrazone[23] as alkylating agent, the intermediate product was not isolated, but ring closure was effected by heating the reaction mixture in *N*,*N*-dimethylformamide at 140 °C for 1 h. The resulting mixture was chromatographed on silica gel (dichloromethane/ethanol; 98:2 then 94:6; gradient as eluant) to give the title compound as a yellow gum in 27% yield. ESI *m*/*z* = 399.3 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.78 (d, J = 12.5 Hz, 2H), 2.31 (dq, J = 12.5 and 4.2 Hz, 2H), 2.84 (t, J = 12.2 Hz, 2H), 4.29 (m, 2H), 4.47 (tt, J = 12.3 and 4.1 Hz, 1H), 5.21 (s, 2H), 7.05–7.14 (m, 3H), 7.19–7.24 (m, 1H), 8.06 (br s, 1H).

4.1.47. 4-[3-(2-Methyl-2*H*-1,2,4-triazol-3-ylmethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (22) and 4-[3-(1-Methyl-1*H*-1,2,4-triazol-3-ylmethyl)-2-oxo-2,3-dihydro-benzimidazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (23). Compound **21** (500 mg, 1.25 mmol) was refluxed in acetone (20 mL) in the presence of potassium carbonate (207 mg, 1.5 mmol) and methyliodide (0.177 mL, 2.8 mmol). After 15 min, the reaction mixture was cooled and the inorganic material filtered off. Acetone was evaporated from the filtrate and the component of the residue soluble in dichloromethane was chromatographed on silica gel (dichloromethane/ethanol; 98:2 then 96:4 gradient; as eluant). Pure samples of **22** (150 mg, 29%) and **23** (200 mg, 39%) were isolated. Isomer assignment was based on HMBC NMR studies. **22**: ESI *m*/*z* = 413.0 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.82 (br d, J = 12.5 Hz, 2H), 2.32 (dq, J = 12.5 and 4.5 Hz, 2H), 2.86 (br t, J = 12.5 Hz, 2H), 3.97 (s, 3H), 4.25–4.38 (br m, 2H), 4.45 (tt, J = 12.5 and 4.0 Hz, 1H), 5.22 (s, 2H), 7.06–7.13 (m, 3H), 7.32–7.35 (m, 1H), 7.83 (s, 1H). **23**: ESI *m*/*z* = 413.1 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H), 1.83 (br d, J = 12.5 Hz, 2H), 2.33 (dq, J = 12.5 and 4.6 Hz, 2H), 2.88 (br t, J = 12.5 Hz, 2H), 3.86 (s, 3H), 4.23–4.38 (br m, 2H), 4.52 (tt, J = 12.5 and 4.0 Hz, 1H), 5.14 (s, 2H), 7.01–7.06 (m, 2H), 7.07–7.14 (m, 2H), 7.93 (s, 1H).

4.1.48. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-(1*H*-1,2,4-triazol-3-ylmethyl)-1,3-dihydro-benzimidazol-2-one hydrochloride (24). Starting with **21** the title compound was purified by flash column chromatography on silica gel (dichloromethane/ethanol; 96:4 then 92:8; gradient, as eluant). The obtained free base of the title compound (40% yield) was dissolved in dichloromethane plus a few drops of ethanol and 1 M solution of hydrochloric acid in diethyl ether added. The hydrochloride salt was precipitated twice from ethanol plus acetone by adding copious amounts of diethyl ether to give a yellow solid in 95% yield. ESI-MS *m*/*z* = 519.3 $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.02 (d, J = 7.0 Hz, 3H), 1.06 (d, J = 7.0 Hz, 3H), 2.03–2.23 (m, 5H), 2.16 (s, 3H), 2.84–2.96 (m, 2H), 3.20–3.34 (m, 4H), 3.72–3.82 (m, 2H), 3.76 (s, 3H), 4.33–4.39 (m, 1H), 4.59–4.69 (m, 1H), 5.34 (s, 2H), 6.42 (dd, J = 8.5 and 2.5 Hz, 1H), 6.53 (br s, 1H), 7.02 (d, J = 8.0 Hz, 1H), 7.07–7.18 (m, 3H), 7.44 (d, J = 7.6 Hz, 1H), 9.02 (s, 1H).

Racemic **24** was subjected to chiral chromatography on a Chiralpak® AD column (2 cm × 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 equivalent of methanesulfonic acid in dichloromethane and then precipitated twice from a concentrated acetone solution by flooding with diethyl ether to give (+)-**24**. Purity by HPLC 98.68%, enantiomeric ratio 100:0. $[\alpha]_D$ +26.7° in methanol. ^1H NMR (CD_3OD): δ 8.92 (1H, s), 7.44 (1H, d, J = 7.5 Hz), 7.13–7.18 (1H, m), 7.08–7.13 (2H, m), 7.02 (1H, d, J = 9.0 Hz), 6.53 (1H, d, J = 2.0 Hz), 6.42 (1H, dd, J = 2.5 and 8.0 Hz), 5.32 (2H, s), 4.64 (1H, tt, J = 4.0 and 12.5 Hz), 4.37 (1H, q, J = 5.5 Hz), 3.76 (3H, s), 3.72–3.81 (2H, m), 3.26–3.34 (2H, m), 3.25 (2H, t, br, J = 13.0 Hz), 2.89 (2H, dq, J = 3.5 and 13.0 Hz), 2.16 (3H, s), 2.02–2.24 (5H, m), 1.05 (3H, d, J = 6.5 Hz), 1.02 (3H, d, J = 6.5 Hz). HRMS (TOF) $(M+H)^+$ calculated for $C_{29}H_{39}N_6O_3$ 519.3078, found 519.3061.

4.1.49. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-(2-methyl-2*H*-1,2,4-triazol-3-ylmethyl)-1,3-dihydro-benzimidazol-2-one hydrochloride (25). Starting with **22** the title compound was purified by flash column chromatography on silica gel (dichloromethane/ethanol; 98:2 then 95:5 gradient; as eluant). The obtained free base of the title compound (46% yield) was dissolved in dichloromethane plus a few drops of ethanol and 1 M solution of hydrochloric acid in diethyl ether added. The hydrochloride salt was precipitated twice from acetone by adding copious amounts of diethyl ether to give a filterable solid in 58% yield. ESI-MS *m*/*z* = 533.3 $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.02 (d, J = 7.0 Hz, 3H), 1.06 (d, J = 7.0 Hz, 3H), 2.03–2.22 (m, 5H), 2.16 (s, 3H), 2.79–2.92 (m, 2H), 3.18–3.31 (m, 4H), 3.71–3.81 (m, 2H), 3.76 (s, 3H), 3.98 (s, 3H), 4.36 (q, J = 5.5 Hz, 1H), 4.60 (tt, J = 12.5 and 4.0 Hz, 1H), 5.29 (s, 2H), 6.44 (dd, J = 8.2 and 2.3 Hz, 1H), 6.52 (br s, 1H), 7.02 (d, J = 8.0 Hz, 1H), 7.08–7.21 (m, 3H), 7.37 (d, J = 7.5 Hz, 1H), 7.81 (s, 1H).

4.1.50. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-(1-methyl-1*H*-1,2,4-triazol-3-ylmethyl)-1,3-dihydro-benzimidazol-2-one hydrochloride (26). Starting with **23** the title compound was purified by flash column chromatography on silica gel (dichloromethane/ethanol; 98:2 then 95:5 gradient; as eluant). The obtained free base of the title compound (31% yield) was dissolved in dichloromethane plus a few drops of ethanol and 1 M solution of hydrochloric acid in diethyl ether added. The hydrochloride salt was precipitated twice from acetone by adding copious amounts of diethyl ether to give a filterable solid in 35% yield. ESI-MS *m*/*z* = 533.3 $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 7.0 Hz, 3H), 1.06 (d, J = 7.0 Hz, 3H), 2.04–2.22 (m, 5H), 2.16 (s, 3H), 2.79–2.93 (m, 2H), 3.18–3.35 (m, 4H), 3.71–3.81 (m, 2H),

3.76 (s, 3H), 3.87 (s, 3H), 4.37 (q, J = 5.5 Hz, 1H), 4.61 (tt, J = 12.3 and 3.7 Hz, 1H), 5.14 (s, 2H), 6.44 (dd, J = 8.3 and 2.2 Hz, 1H), 6.52 (d, J = 1.8 Hz, 1H), 7.01–7.14 (m, 4H), 7.35 (d, J = 7.5 Hz, 1H), 8.31 (s, 1H).

4.1.51. (3-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetonitrile hydrochloride (27). Starting with **8**[10] and following the methods outlined for **17**, the title compound was purified by column chromatography on silica gel (dichloromethane/methanol; 99.4:0.6; as eluant) to give the free base of the title compound as an oil (5.60 g, 53%). ESI-MS m/z = 477.3 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.00 (d, J = 6.8 Hz, 3H), 1.03 (d, J = 6.8 Hz, 3H), 1.70–2.08 (m, 5H), 2.11–2.23 (m, 5H), 2.44–2.60 (m, 4H), 3.00–3.08 (m, 1H), 3.09–3.18 (m, 1H), 3.74 (s, 3H), 4.26–4.39 (m, 2H), 4.99 (s, 2H), 6.38 (dd, J = 8.2 and 2.4 Hz, 1H), 6.59 (d, J = 2.4 Hz, 1H), 6.99 (d, J = 8.2 Hz, 1H), 7.11–7.28 (m, 3H), 7.38–7.50 (m, 1H). A portion of this oil (255 mg) was dissolved in diethyl ether, then the resulting solution was mixed with 1 M solution of hydrochloric acid in diethyl ether. The resulting mixture was concentrated in vacuo to give the title compound (195 mg). ESI-MS m/z = 477.3 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 6.8 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H), 2.02–2.22 (m, 8H), 2.75–2.91 (m, 2H), 3.14–3.38 (m, 4H), 3.68–3.82 (m, 5H), 4.31–4.40 (m, 1H), 4.52–4.64 (m, 1H), 5.00 (s, 2H), 6.43 (dd, J = 8.2 and 2.4 Hz, 1H), 6.52 (d, J = 2.4 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 7.18–7.33 (m, 3H), 7.34–7.41 (m, 1H).

4.1.52. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-(1*H*-tetrazol-5-ylmethyl)-1,3-dihydro-benzimidazol-2-one hydrochloride (28). Tributylstannylazide (700 mg, 2.11 mmol) was added to a solution of **27** (200 mg, 0.420 mmol) in xylene (10 mL), and the resulting mixture was stirred at 140 °C for 24 h. The reaction mixture was then concentrated in vacuo, and the residue mixed with 2 M aqueous hydrochloric acid (50 mL) and methanol (50 mL). The mixture was stirred at room temperature for 30 min then concentrated in vacuo. The residue was mixed with 5% aqueous solution of sodium carbonate (50 mL) and the pH adjusted to 5 by addition of 0.5 M hydrochloric acid. The resulting mixture was extracted with dichloromethane (4× 100 mL) and the combined organics washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (dichloromethane/methanol; 90:10; as eluant) to give a pale brown oil. This oil was dissolved in methanol and mixed with 1M solution of hydrochloric acid in diethyl ether. The resulting mixture was concentrated in vacuo to give the title compound as a brown oil (142 mg, 61%). ESI-MS m/z = 520.2 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 7.0 Hz, 3H), 1.05 (d, J = 7.0 Hz, 3H), 2.01–2.25 (m, 8H), 2.80–2.95 (m, 2H), 3.17–3.39 (m, 4H), 3.69–3.82 (m, 5H), 4.33–4.41 (m, 1H), 4.56–4.69 (m, 1H), 5.43 (s, 2H), 6.42 (dd, J = 8.1 and 2.7 Hz, 1H), 6.52 (d, J = 2.7 Hz, 1H), 7.03 (d, J = 8.1 Hz, 1H), 7.06–7.19 (m, 3H), 7.35–7.45 (m, 1H).

4.1.53. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-1,2,4-oxadiazol-3-ylmethyl-1,3-dihydro-benzimidazol-2-one hydrochloride (29). Boron trifluoride etherate (1 drop) was added to a suspension of **30** (60 mg, 0.118 mmol) in triethyl orthoformate (500 mg, 3.37 mmol), then the resulting mixture was stirred at 105–115 °C for 30 min. The reaction mixture was diluted with 5% aqueous solution of sodium carbonate and resulting mixture extracted with dichloromethane (4× 100 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (dichloromethane/methanol; 98:2; as eluant) to give an oil. This oil was dissolved in methanol and mixed with 1 M solution of hydrochloric acid in diethyl ether. The resulting mixture was concentrated in vacuo to give the title compound as a colourless solid (32 mg, 49%). ESI-MS m/z = 520.2 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 7.0 Hz, 3H), 1.05 (d, J = 7.0 Hz, 3H), 2.02–2.24 (m, 8H), 2.78–2.94 (m, 2H), 3.15–3.40 (m, 4H), 3.68–3.82 (m, 5H), 4.32–4.41 (m, 1H), 4.55–4.66 (m, 1H), 5.30 (s, 2H), 6.40–6.46 (m, 1H), 6.50–6.55 (m, 1H), 7.03 (d, J = 8.0 Hz, 1H), 7.08–7.20 (m, 3H), 7.33–7.42 (m, 1H), 9.19 (s, 1H).

4.1.54. *N*-Hydroxy-2-(3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-2-oxo-2,3-dihydro-benzimidazol-1-yl)-acetamidine hydrochloride (30). A mixture of **27** (170 mg, 0.357 mmol), hydroxylamine hydrochloride (50 mg, 0.720 mmol) and diisopropylethylamine (93 mg, 0.720 mmol) in ethanol (4 mL) was stirred at reflux temperature for 16 h. The reaction mixture was concentrated in vacuo, and the residue was mixed with 5% aqueous solution of sodium carbonate and extracted with dichloromethane (4× 100 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (dichloromethane/methanol; 97:3; as eluant) to give the free base of the title compound as a colourless solid (135 mg, 74%). ESI-MS m/z = 510.5 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.00 (d, J = 6.4 Hz, 3H), 1.03 (d, J = 6.4 Hz, 3H), 1.73–2.06 (m, 5H), 2.11–2.24 (m, 5H), 2.45–2.60 (m, 4H), 3.01–3.09 (m, 1H), 3.10–3.19 (m, 1H), 3.74 (s, 3H), 4.26–4.41 (m, 2H), 4.49 (s, 2H), 6.38 (dd, J = 8.2 and 2.7 Hz, 1H), 6.58 (d, J = 2.7 Hz, 1H), 6.99 (d, J = 8.2 Hz, 1H), 7.06–7.14 (m, 2H), 7.19–7.24 (m, 1H), 7.38–7.42 (m, 1H).

The free base was dissolved in methanol and the resulting solution was mixed with 1 M solution of hydrochloric acid in diethyl ether. The resulting mixture was concentrated in vacuo to give the hydrochloric acid salt as a colourless solid. ESI-MS m/z = 510.5 $[M+H]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 6.8 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H), 1.98–2.23 (m, 8H), 2.78–2.94 (m, 2H), 3.18–3.38 (m, 4H), 3.71–3.83 (m, 5H), 4.31–4.39 (m, 1H), 4.55–4.67 (m, 1H), 4.89 (s, 2H), 6.43 (dd, J = 8.3 and 2.2 Hz, 1H), 6.49–6.54 (m, 1H), 7.03 (d, J = 8.3 Hz, 1H), 7.13–7.25 (m, 3H), 7.38–7.46 (m, 1H).

4.1.55. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-(3*H*-1,2,3-triazol-4-ylmethyl)-1,3-dihydro-benzimidazol-2-one hydrochloride (31). A mixture of **32** (260 mg, 0.547 mmol), tributylstannylazide (908 mg, 2.73 mmol) in xylene (5 mL) was stirred at 150 °C for 16 h. The reaction mixture was evaporated to dryness and subjected to flash column chromatography on silica gel (dichloromethane/methanol; 90:10; as eluant). The oil obtained was dissolved in methanol (10 mL), and then poured onto a 2 M solution of hydrochloric acid in water (10 mL). This was then stirred at room temperature for 1 h. The mixture was concentrated in vacuo, and the oily residue taken up in 5% aqueous solution of sodium carbonate (100 mL) and extracted with dichloromethane (4× 100 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The oil obtained was dissolved in methanol and 1 M solution of hydrochloric acid in diethyl ether added and concentrated in vacuo to give the title compound as a brown solid (72 mg, 22%). ESI-MS m/z = 519.3 $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 7.0 Hz, 3H), 1.05 (d, J = 7.0 Hz, 3H), 2.02–2.22 (m, 8H), 2.78–2.92 (m, 2H), 3.22–3.38 (m, 4H), 3.69–3.83 (m, 5H), 4.32–4.41 (m, 1H), 4.54–4.65 (m, 1H), 5.20 (s, 2H), 6.43 (dd, J = 8.6 and 2.2 Hz, 1H), 6.52 (d, J = 2.2 Hz, 1H), 7.03 (d, J = 8.6 Hz, 1H), 7.07–7.22 (m, 3H), 7.27–7.37 (m, 1H), 7.76 (br s, 1H).

4.1.56. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-prop-2-ynyl-1,3-dihydro-benzimidazol-2-one hydrochloride (32). Sodium hydride (60% suspension in mineral oil, 44 mg, 1.10 mmol) was added in portions to a solution of **8** (400 mg, 0.914 mmol) in *N*,*N*-dimethylformamide (3 mL) at 0 °C. The resulting solution was then heated to 50–60 °C for 1 h. After the reaction mixture had been cooled to 5 °C, propargyl bromide (141 mg, 1.19 mmol) was added dropwise to the mixture and then allowed to warm to room temperature for 16 h. The reaction mixture was concentrated in vacuo, and the resultant residue combined with water (150 mL) and extracted with ethyl acetate (4× 100 mL). The organic layers were combined, washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The oil obtained was purified by flash column chromatography on silica gel (dichloromethane/methanol; 99.3:0.7; as eluant) to give the free base of the title compound as a brown oil (325 mg, 75%). ESI-MS m/z = 476.3 $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.00 (d, J = 7.2 Hz, 3H), 1.03 (d, J = 7.2 Hz, 3H), 1.70–2.08 (m, 5H), 2.11–2.23 (m, 5H), 2.44–2.60 (m, 4H), 2.69–2.73 (m, 1H), 3.00–3.08 (m, 1H), 3.09–3.18 (m, 1H), 3.74 (s, 3H), 4.26–4.39 (m, 2H), 4.70 (d, J = 2.4 Hz, 2H), 6.38 (dd, J = 2.4 and 8.2 Hz, 1H), 6.59 (d, J = 2.4 Hz, 1H), 6.99 (d, J = 8.2 Hz, 1H), 7.11–7.17 (m, 2H), 7.22–7.28 (m, 1H), 7.38–7.44 (m, 1H).

The free base (65 mg) was dissolved in diethyl ether (10 mL), and the resulting solution poured onto 0.1 M solution of hydrochloric acid in diethyl ether (2.5 mL). The resulting mixture was concentrated in vacuo to give the title compound as a yellow solid. ESI-MS m/z = 476.3 $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 6.6 Hz, 3H), 1.05 (d, J = 6.6 Hz, 3H), 2.02–2.22 (m, 8H), 2.73 (t, J = 2.4 Hz, 1H), 2.75–2.91 (m, 2H), 3.15–3.38 (m, 4H), 3.68–3.81 (m, 5H), 4.32–4.40 (m, 1H), 4.51–4.63 (m, 1H), 4.70 (d, J = 2.4 Hz, 2H), 6.43 (dd, J = 8.4 and 2.2 Hz, 1H), 6.52 (d, J = 2.2 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 7.13–7.21 (m, 2H), 7.25–7.37 (m, 2H).

4.1.57. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-(5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-3-ylmethyl)-1,3-dihydro-benzimidazol-2-one hydrochloride (33). Compound **8** (3.31 g, 7.56 mmol) was dissolved in *N*,*N*-dimethylformamide (40 mL) and treated with 60% dispersion of sodium hydride (333 mg, 8.34 mmol), stirring for 15 min at room temperature. *N*-Carboxymethyl-2-chloroacetamidrazone[23] was then added and the mixture stirred overnight at room temperature followed by 2 h at 50 °C. The volume of *N*,*N*-dimethylformamide was reduced under vacuum and the residue then heated at 150–160 °C for 1 h to effect ring closure. After partitioning between ethyl acetate and water, the ethyl acetate was washed with water, washed with brine and then dried (Na_2SO_4). The resulting complex mixture was chromatographed on silica (dichloromethane/ethanol; 98:2 then 92:8 gradient; as eluant). Approximately 100 mg of freebase was obtained and converted to the hydrochloride salt with 1 M hydrochloric acid in diethyl ether and precipitated twice from ethanol by adding copious amounts of diethyl ether to give a brown solid (80 mg, 1.8%). ESI-MS m/z = 535.3 $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.02 (d, J = 6.8 Hz, 3H), 1.04 (d, J = 6.8 Hz, 3H), 2.04–2.20 (m, 5H), 2.16 (s, 3H), 2.86 (dq, J = 12.8 and 3.0 Hz, 2H), 3.24 (br t, J = 12.5 Hz, 2H), 3.71–3.80 (m, 2H), 3.76 (s, 3H), 4.36 (q, J = 5.5 Hz, 1H), 4.60 (tt, J = 12.2 and 4.1 Hz, 1H), 4.97 (s, 2H), 6.44 (dd, J = 8.3 and 2.3 Hz, 1H), 6.52 (d, J = 2.3 Hz, 1H), 7.04 (d, J = 8.3 Hz, 1H), 7.10–7.18 (m, 3H), 7.36 (br d, J = 7.3 Hz, 1H).

4.1.58. 1-{1-[3-(5-Methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-3-1,2,4-thiadiazol-3-ylmethyl-1,3-dihydro-benzimidazol-2-one hydrochloride (34). A mixture of **35** (1.20 g, 2.10 mmol), 10% Pd-C (1.50 g), and acetic acid (30 mL) was stirred under a hydrogen atmosphere at room temperature for 28 h. The resulting mixture was diluted with methanol (200 mL), and Pd-C was removed carefully by filtration. The filtrate was concentrated in vacuo and the residue purified by flash column chromatography on silica gel (dichloromethane/methanol; 98.5:1.5; as eluant) to give an oil. This oil was dissolved in diethyl ether and treated with 1 M solution of hydrochloric acid in diethyl ether. The resulting mixture was concentrated in vacuo to give the title compound as a colourless solid (69 mg, 5.8%). ESI-MS m/z = 536.3 $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.01 (d, J = 6.8 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H), 2.02–2.24 (m, 8H), 2.76–2.92 (m, 2H), 3.14–3.38 (m, 4H), 3.64–3.80 (m, 5H), 4.30–4.40 (m, 1H), 4.52–4.66 (m, 1H), 5.42 (s, 2H), 6.39–6.46 (m, 1H), 6.52 (d, J = 2.4 Hz, 1H), 6.98–7.17 (m, 4H), 7.27–7.34 (m, 1H), 10.08 (s, 1H).

4.1.59. 1-(5-Chloro-1,2,4-thiadiazol-3-ylmethyl)-3-{1-[3-(5-methoxy-2-methyl-phenoxy)-4-methyl-pentyl]-piperidin-4-yl}-1,3-dihydro-benzimidazol-2-one hydrochloride (35). Prepared according to the method outlined for **4k** using 5-chloro-3-chloromethyl[1,2,4] thiadiazole as the alkylating agent, the obtained residue was purified by flash column chromatography on silica gel (dichloromethane/methanol; 99.3:0.7; as eluant) to give the free base of the title compound as a pale yellow oil in a yield of 61%. ESI-MS $m/z = 570.3$ $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 0.97 (d, $J = 6.6$ Hz, 3H), 0.99 (d, $J = 6.6$ Hz, 3H), 1.74–1.90 (m, 4H), 1.94–2.20 (m, 6H), 2.34–2.56 (m, 4H), 2.91–3.14 (m, 2H), 3.76 (s, 3H), 4.18–4.28 (m, 1H), 4.34–4.50 (m, 1H), 5.29 (s, 2H), 6.36 (dd, $J = 8.2$ and 2.3 Hz, 1H), 6.62 (d, $J = 2.3$ Hz, 1H), 6.92–7.12 (m, 4H), 7.28–7.38 (m, 1H).

The free base was dissolved in diethyl ether and the resulting solution treated with 1 M solution of hydrochloric acid in diethyl ether. The resulting mixture was concentrated in vacuo to give the title compound as a yellow solid. ESI-MS $m/z = 570.3$ $[M+H]^+$. ^1H NMR (400 MHz, CD_3OD): δ 1.01 (d, $J = 6.6$ Hz, 3H), 1.05 (d, $J = 6.6$ Hz, 3H), 2.02–2.23 (m, 8H), 2.78–2.93 (m, 2H), 3.16–3.40 (m, 4H), 3.69–3.82 (m, 5H), 4.31–4.40 (m, 1H), 4.54–4.66 (m, 1H), 5.32 (s, 2H), 6.43 (dd, $J = 8.2$ and 2.2 Hz, 1H), 6.52 (d, $J = 2.2$ Hz, 1H), 7.03 (d, $J = 8.2$ Hz, 1H), 7.06–7.19 (m, 3H), 7.30–7.40 (m, 1H).

4.2. Molecular modelling

All computational studies were performed on Silicon Graphics desktop workstations using Sybyl[29] software unless otherwise stated. Structures and data of all compounds used in the QSAR modelling are given in the supplementary data. Seventy-nine compounds all containing the 3-phenoxypropyl piperidine benzimdazol-2-one scaffold with NOP pK_i data measured in the described NOP binding assay formed the initial training and test sets. All data correspond to racemic mixtures of the compounds with a pK_i range from 5.10 to 8.84. All compounds were modelled within Sybyl as the ionised form of the higher affinity S enantiomer, converted to 3D with Concord then energy minimised with the Tripos forcefield and Gasteiger-Hückel charges to a gradient less than 0.005 kcal/mol/Å.[29] Compounds were subsequently superimposed onto an X-ray structure of compound (+)-**2** using a steric and electrostatic alignment algorithm similar to the published MIMIC.[30] CoMFA steric and electrostatic fields were calculated using default box dimensions and parameters except a 1 Å grid spacing was applied. The dataset (see supplementary data) was separated into a training set ($n = 61$) and a test set ($n = 18$) by hierarchical clustering using the CoMFA fields and the complete linkage algorithm as implemented in Sybyl. Cross validated partial least squares was performed with 10 groups, CoMFA standard scaling and a minimum σ cut-off of 2 kcal. The most appropriate model was selected on the basis of minimum cross validated standard error, maximum Q^2 and minimum number of components. The best cross validated model was identified as follows: $Q^2 = 0.62$, cross validated s.e. = 0.62, $n = 61$, components = 7. The full partial least squares model was generated without cross validation to yield: $R^2 = 0.97$, s.e. = 0.16, $n = 61$, $F_{7,53} = 288.3$ ($p \ll 0.05$), components = 7. The 18 compound test set was then predicted by the model showing a predictive $R^2 = 0.84$ and standard error of prediction = 0.42. A further 34 compounds (see supplementary data) with NOP pK_i data from the series were added to the dataset. These 34 compounds were modelled and superimposed identically to those described previously to give a complete dataset of 113 compounds with a pK_i range from 5.10 to 9.01. A final CoMFA model was generated for the 113 compounds using identical parameters to that of the initial model. Cross validated partial least squares was performed with 10 groups to determine the optimal number of components. Partial least squares without cross validation was used to generate the full CoMFA model. In both cases CoMFA standard scaling and a minimum σ cut-off of 2 kcal was applied. Potential new compounds were predicted prior to synthesis using the full model by subjecting each compound to an identical modelling and superposition procedure to that described in the generation of the model.

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 6 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.[31]

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; $K_i = IC_{50}/(1 + [L]/Kd)$.[31]

4.3.2. [^{3}H]Diprenorphine competition assay. Membranes from CHO cells stably expressing human MOP recep-

tors were purchased from Perkin-Elmer (Product Code: RBHOMM). Binding to human MOP 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; $K_i = IC_{50}/(1 + [L]/Kd)$.[31]

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; $K_i = IC_{50}/(1 + [L]/Kd)$.[31]

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; $K_i = IC_{50}/(1 + [L]/Kd)$.[31]

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 guidelines. The test compounds were examined at 6 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. Formalin paw test

Experiments were performed in male ICR mice 21–29 g. In the first experiment, animals received increasing doses of (+)-**24** (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, ($n = 8$ 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 mean and s.e.m. 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).

4.5. Brennan test

Experiments were performed in male Wistar rats weighing between 170 and 200 g; $n = 6$ animals per group. In brief, the rats' withdrawal threshold to a series of filaments was measured (baseline reading). Filaments of increasing force (2.6–167.0 mN) were applied to the plantar surface of the paw using an up and down method. The paw was touched with 1 of a series of 8 von Frey hairs with logarithmically incremental stiffness. The von Frey hair was presented perpendicular to the plantar surface with sufficient force to cause buckling against the paw and held for approximately 1–3 s. A positive response was noted if the paw was sharply withdrawn. A cut-off of 15 g was selected as the upper limit for testing, since stiffer hairs tend to raise the entire limb rather than buckling, substantially changing the nature of the stimulus.

The animals were then anaesthetised with isoflurane and an incision made in the plantar surface of the left hindpaw (0.5 cm from the heel). The plantaris muscle was then elevated using curved forceps before the wound was sutured with 4–0 nylon and the animals allowed to recover. Two hours after making the incision, paw withdrawal thresholds were re-measured. Immediately after this reading, the rats were injected intravenously with vehicle (saline) or compound (+)-**24** (3, 10 or 30 μmol/kg). Readings were then made at 30, 60, 90 and 120 min post-compound injection.

Data were expressed as means ± s.e.m. and compared between groups using the Kruskal–Wallis one-way

analysis of variance, a non-parametric statistical test. Each of the treatment groups was then compared against the vehicle group, using the non-parametric Dunn's test. The time of maximum effect (T_{max}) was calculated. The ED_{50} (dose at which allodynia is reversed by approximately 50%) values were also calculated at T_{max} using non-linear regression (sigmoidal dose–response; variable slope) with constants of 0 and 15 g (cut-off) for the bottom and top, respectively (XLFit).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.01.005.

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