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Structure-Activity Relationship Exploration of Kv1.3 Blockers Based on Diphenoxylate

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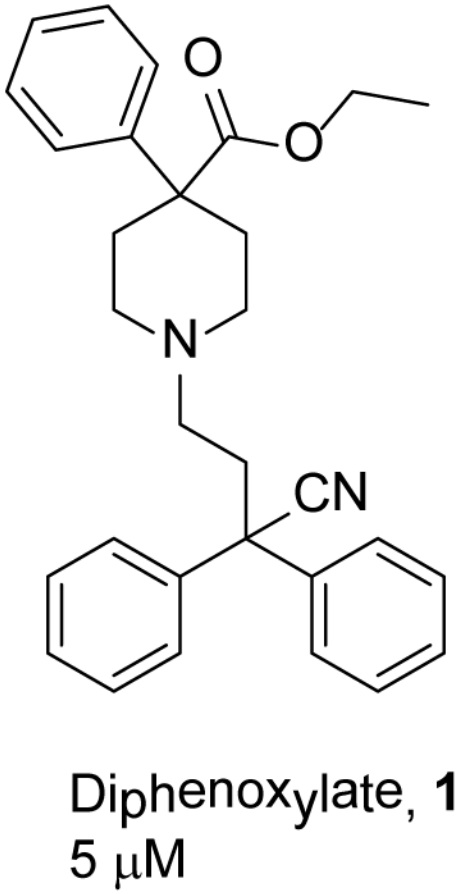
Abstract



Diphenoxylate, **1**
5 μM

Diphenoxylate, a well-known opioid agonist and anti-diarrhoeal agent, was recently found to block Kv1.3 potassium channels, which have been proposed as potential therapeutic targets for a range of autoimmune diseases. The molecular basis for this Kv1.3 blockade was assessed by the

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selective removal of functional groups from the structure of diphenoxylate as well as a number of other structural variations. Removal of the nitrile functional group and replacement of the C-4 piperidinyl substituents resulted in several compounds with submicromolar IC_{50} values.

Autoimmune disorders are characterized by an organism mistakenly mounting an immune response against itself. Examples of these disorders include multiple sclerosis (MS) psoriasis, type-1 diabetes, Crohn's Disease, and rheumatoid arthritis[1]. The aberrant immune response is mediated by autoantibodies and self-reactive lymphocytes. The lymphocytes involved are T cells and once activated they proliferate and cause tissue damage. One particular type of T cells known as effector memory (T_{EM}) cells have been linked to autoimmune disorders[2–4].

Human T cells express two types of K+ channels, the voltage-gated Kv1.3 and the calcium-activated KCa3.1 channel[5, 6]. Both Kv1.3 and KCa3.1 play a role in regulating membrane potential and calcium signalling during the activation of T cells[5]. Calcium influx, which is crucial to the process, is only possible if the T cells are able to maintain a negative membrane potential through a counterbalancing potassium efflux via Kv1.3 and/or KCa3.1 channels[5, 7, 8].

Blockade of Kv1.3 or KCa3.1 channels is a possible method for treating autoimmune and inflammatory diseases by suppressing T-cell proliferation and modulating their activities[9]. Importantly, naïve and central memory T cells (T_{CM}) upregulate KCa3.1 channels upon activation leaving the number of Kv1.3 channels largely unchanged. The opposite occurs in T_{EM} cells which upregulate Kv1.3 channels when activated[5]. Blockade of the Kv1.3 channel therefore provides an opportunity for intervention by therapeutic agents, leaving naïve and T_{CM} cells free to address other immunogenic threats (e.g. infections).

A number of studies have shown that blockade of Kv1.3 potassium channels results in functional inhibition of T cell activation/proliferation and cytokine secretion[5, 8, 10]. In one study, the potent Kv1.3 blocking peptide ShK and a series of related analogues were able to treat both adoptive transfer and chronic relapsing experimental autoimmune encephalomyelitis (EAE) in rats[11]. Small molecule blockers of Kv1.3 channels have also been investigated[12]. The most potent of these compounds is PAP-1 (IC_{50} 2 nM) which has been shown to suppress delayed type hypersensitivity (DTH) and allergic contact dermatitis (ACD) in Lewis rats when dosed orally, by i.p. injection or topically[13, 14].

In searching for new compounds that might have clinical potential as Kv1.3 channel blockers we noticed that diphenoxylate (**1**) had been shown, in a small clinical trial, to successfully treat psoriasis and other inflammatory skin conditions[15, 16]. Diphenoxylate also shows structural similarity to a number of Kv1.3 blockers (Figure 1) and when assessed it was found to block Kv1.3 channels with an IC_{50} of 5 μM[12]. With a view to optimising the Kv1.3 blockade shown by diphenoxylate, we examined which elements of the chemical structure are required for biological activity.

As our principle objective was to delineate the pharmacophoric elements of diphenoxylate with respect to Kv1.3 blockade, analogues were prepared where one or more of the functional elements of **1** were removed or altered. The first reports of diphenoxylate synthesis date back to Janssen *et al.* in 1959[17] and that synthesis provided the basis for the work described here. As exemplified in the synthesis of the methyl ester **2,** it was found that the alkylation of the key piperidine precursor (**3**) with diphenylbromopropionitrile (**4**) could be accelerated and the yield improved by microwave heating in acetonitrile (ACN) in the presence of *N,N*-diisopropylethylamine (DIPEA). This synthetic step (Scheme 1) formed the

basis for all analogues described here, with variations derived from commercial or synthesized building blocks. Full synthesis details are provided as supplementary material.

Other compounds prepared in this way included **5**, **6** and **7** in which the ester, piperidine 4-phenyl substituent and the nitrile were replaced by a hydrogen atom, respectively or **8** where both piperidine 4-substituents were removed. In compound **9** the 2-biphenylbutyronitrile portion is replaced by a simple phenylpropionyl group. Other analogues that were prepared included replacements of the piperidine ring such as piperazine (**10, 11**) and tetrahydropyridine (**12**). Replacement of the ester with a hydroxyl group (**13**) was undertaken as the 4-phenylpiperidin-4-ol group is a well-known fragment in established drugs. The activity of **13** discussed later, prompted the removal of the 4-phenyl ring to produce **14** and the related piperidine-3-ol analogue (**15**). To complement our SAR exploration, the truncated analogues **16** and **17** were also produced. A further series of analogues based on compound **14** were synthesized to produce **18**, **19**, **20** and **21**.

Compounds were assessed for their ability to block Kv1.3 channels using manual whole-cell patch-clamp as previously described[14, 18]. Briefly, L929 cells stably expressing Kv1.3 channels were subjected to depolarizing step pulses from −80 mV to +40 mV to elicit Kv1.3 currents. Compounds were manually perfused and in most cases 3–5 different concentrations were tested at least 2–3 times. All compounds were washed out again to differentiate true pharmacological effects from unspecific current “run-down”. IC_{50} values were determined by fitting the reduction of area under the current curve after reaching equilibrium block to the Hill equation. Full details are provided as supplementary material.

The compounds tested demonstrated a range of potencies, clearly showing the specific contributions of the diphenoxylate substructures. In the simplest analogue, replacement of the ethyl ester with a methyl ester (**2**) resulted in a marginal improvement in activity (Table 1). Removing the ethyl ester from diphenoxylate altogether in **5**, also slightly improved blocking potency. In contrast, removal of the phenyl ring from the piperidine (**6**) resulted in a substantial decrease in activity (~50 fold). Compound **7** which lacked the cyano group was 6-fold more active than diphenoxylate itself.

Of the more extensively pruned compounds, compound **8** was 4-fold less potent than diphenoxylate demonstrating that while the ester is not needed, further removal of the phenyl ring was not well tolerated suggesting that this aromatic group is required for activity (Table 1). Pruning of the diphenylmethane/cyano moiety of diphenoxylate had a significant effect on activity. Removal of both the cyano group and one phenyl ring from this group (**9**) resulted in a similar improvement in potency relative to compound **7**. Compound **9** has been flagged as an interesting substance for future optimization.

Having systematically removed groups from the structure of diphenoxylate, we investigated

Diphenoxylate has already been shown to be able to treat inflammatory skin conditions including the autoimmune disorder psoriasis[15, 16] and this clinical observation, though unproven in a large clinical trial, is consistent with modest Kv1.3 channel blockade[12]. Efforts to produce a suitable topical formulation of diphenoxylate would seem unlikely due to the side-effect potential of this narcotic agent.

Exploitation of diphenoxylate as a lead compound for developing Kv1.3 channel blockers presents a number of challenges[12]. Diphenoxylate has moderate potency and a relatively high molecular weight and lipophilicity. Compounds with high logP values are often found to be able to block potassium channels and the need to demonstrate a clear SAR for diphenoxylate was imperative. From a structural perspective we saw partial similarities to

the known Kv1.3 blockers UK 78,282, verapamil and PAP-1 (Figure 1). These included the basic aliphatic nitrogen, diphenylmethyl moiety, cyano group and the location of aromatic rings at both ends of the molecules. We began by pruning these functional groups on diphenoxylate to explore their effect on Kv1.3 blockade. In some cases these groups were replaced by other functional groups of varying sizes and properties.

The principle outcomes were the demonstration that Kv1.3 blockade could be maintained and even enhanced by the removal of one or more of diphenoxylate's key functional groups. For example, removal of the R^4-phenyl ring and the nitrile was tolerated such that compound **9** (IC_{50} 0.8 μM) had improved potency. The replacement of the phenyl and carboxylic ester substituents by a single hydroxyl group in compound **14** also showed improved activity (IC_{50} 0.75 μM). The combination of these modifications however, yielded a poorly active compound **17** (IC_{50} > 100 μM). This contradicted the overall pharmacophore that we had tentatively proposed[12] for Kv1.3 inhibitors. The inner cavity, which constitutes the binding site for most small molecule Kv1.3 blockers is somewhat large[19] and it seems probable that the compounds bind in various ways such that a single pharmacophore will not describe the SAR.

From a drug discovery perspective, the retention of blockade with both reduced molecular size and lipophilicity is encouraging. While PAP-1 shows good potency it has no ionizable functional groups and a logP value of 4.03[14] resulting in a relatively low oral availability of only 25% and solubility issues for formulation[20]. Both compounds **14** and **9** have reduced $ClogD_{7.4}$ values relative to diphenoxylate as well as reduced molecular weights (320.4 and 351.5, respectively). Diphenoxylate is a poorly soluble substance due to its relatively high lipophilicity ($ClogP_{7.4}$ 4.27). The lipophilicity of compound **9** was reduced ($ClogD_{7.4}$ 3.47) however, the removal of groups to generate compound **14** resulted in a substantially lower $ClogD_{7.4}$ value of 1.46.

The concept of lipophilic ligand efficiency (LipE) has recently emerged as an influential descriptor to assist lead optimization[21] and is calculated by subtracting the pIC in its IC_{50}. Also in contrast to **9**, the removal of a cyano group plus a phenyl ring (**17**) also resulted in no activity. The difference in SAR between diphenoxylate and **14** suggests that these two compounds are likely to have two distinct binding modes and/or locations within the Kv1.3 channel. It is possible that these compounds may also be binding to a different state of the channel (i.e. open or closed) which may account for their dissimilar SAR.

Another important aspect of investigating the SAR of diphenoxylate is to place focus on the ester group which is linked to its activity at opioid receptors. The ester on diphenoxylate is metabolized *in vivo* to the carboxylic acid (diphenoxin) which is the active opioid agent[22]. Any future work would need to monitor mu opioid activity and avoiding an ester would need to be considered. Compound **14** circumvents this problem, however any optimization of **9** would need to bear this in mind.

This study has identified two new series of Kv1.3 blockers derived from the anti-diarrhoeal compound diphenoxylate. Successive deletion of functional groups was able to improve activity although the SAR was not consistent between the compound classes. Removal of the ester, cyano and an aromatic ring were tolerated and in many cases improved activity. These deletions also reduced both MW and lipophilicity presenting compounds worthy of further investigation. There is a need for Kv1.3 blockers with improved selectivity and biopharmaceutical properties, and this study provides a starting point for further investigations.

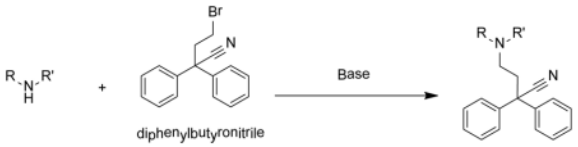
Experimental

Electrophysiology

L929 cells stably expressing Kv1.3 channels were used for all electrophysiology experiments. All experiments were conducted in the whole-cell configuration of the patch-clamp technique with a holding potential of −80 mV unless otherwise stated. Pipette resistances averaged 2.0 MΩ, and series resistance compensation of 80% was employed when currents exceeded 2 nA. Kv1.3 currents were elicited by repeated 200-ms or 500-ms pulses from −80 mV to 40 mV, applied at intervals of 30 or 60 s. Kv1.3 currents were recorded in normal Ringer solution with a Ca^{2+}-free pipette solution containing (in mM): 145 KF, 10 HEPES, 10 EGTA, 2 $MgCl_2$, pH 7.2, 300 mOsm. IC_{50}-values and Hill coefficient were determined by fitting the Hill equation to the reduction of area under the current curve. All compounds tested were >95% purity as determined by RP-HPLC.

Chemistry

Alkylation Reactions—



General method for the preparation of diphenoxylate analogues

Method A—To a solution of alkyl halide (1–2 eq) in ACN (or DMF) was added amine (1–2 eq) with diisopropylamine (2–3 eq) and refluxed overnight under N_2. Solvent was concentrated *in vacuo* yielding a crude residue, which was dissolved in EtOAc and washed with saturated $NaHCO_3$, H_2O, saturated NaCl, dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was either reacted directly or purified.

Method B—To a solution of alkyl halide (1–2 eq) in ACN (or DMF) was added amine (1–2 eq) with diisopropylamine (2–3 eq). The resulting mixture was placed in the microwave reactor (90W) and reaction commenced for 80 mins at 140°C. Solvent was concentrated *in vacuo* yielding a crude residue, which was dissolved in EtOAc and washed with saturated $NaHCO_3$, H_2O, saturated NaCl, dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was either reacted directly or purified.

Methyl 1-(3-cyano-3,3-diphenylpropyl)-4-phenylpiperidine-4-carboxylate (2)[23]

According to method A, 4-bromo-2,2-diphenylbutanenitrile (270 mg, 0.90 mmol) was treated with methyl 4-phenylpiperidine-4-carboxylate (120 mg, 0.55 mmol). The yellow oil was purified *via* RP-HPLC to afford a cream solid (149 mg, 62 %). **Mp:** 184–186 °C (lit. 180–181 °C). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.50 – 7.31 (m, 15H, Ar H), 3.75 (s, 3H, $COOCH_3$), 2.72-2.62 (m, 4H, 4 × CH_{pip}), 2.48 (t, J = 14.2 Hz, 2H, NCH_2CH_2C), 2.22 (t, J = 12.8 Hz, 2H, NCH_2CH_2C), 1.98 – 1.86 (m, 4H, 4 × CH_{pip}). **^{13}C NMR** (600 MHz, $CDCl_3$) δ 175.2, 140.9, 139.8, 138.6, 128.1, 127.1, 126.8, 126.2, 125.0, 120.9, 54.7, 51.4, 50.2, 48.7, 47.8, 36.3, 32.4. **Mass Spectrum** (ESI): *m/z* 439.5 $[M+H]^+$.

2,2-diphenyl-4-(4-phenylpiperidin-1-yl)butanenitrile (5)[24]

According to method B, 4-bromo-2,2-diphenylbutanenitrile (120 mg, 400 μmol) was treated with 4-phenylpiperidine (90 mg, 560 μmol). The crude product was purified by column chromatography using a gradient solvent system (100% hexane – 33% EtOAc in hexane) to afford a yellow oil (82 mg, 54%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.46 – 7.30 (m, 12H, Ar

H), 7.26 – 7.19 (m, 3H, Ar H), 3.06 (ad, J = 10.8 Hz, 2H, 2 × CH_{pip}), 2.76-2.65 (m, 2H, N$CH_2$$CH_2$C), 2.58 – 2.45 (m, 3H, NCH_2CH_2C, CH), 2.19 – 2.06 (m, 2H, 2 × CH_{pip}), 1.89 – 1.77 (m, 4H, 4 × CH_{pip}). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 146.07, 140.01, 128.96, 128.44, 127.96, 126.84, 126.80, 126.21, 122.08, 55.11, 54.61, 50.10, 42.52, 36.68, 33.27. **Mass Spectrum** (ESI): *m/z* 381.2 $[M+H]^+$. (HR-ESI) Found: $[M+H]^+$, 381.2311. $C_{27}H_{28}N_2$ requires $[M+H]^+$, 381.2325.

Ethyl piperidine-4-carboxylate (22)[25]

Isonipecotic acid (1.29g, 10.0 mmol) was dissolved in absolute ethanol (50 ml). The solution was cooled to 0°C and thionyl chloride (2.91 ml, 40.0 mmol) added dropwise. The solution was then stirred and refluxed for 48 h. The solvent was removed *in vacuo* yielding yellow oil, which was dissolved in EtOAc and washed with 10% NaOH. The organic layer was dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to afford a clear oil (1.48 g, 94%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.12 (q, J = 7.1 Hz, 2H, O$CH_2$$CH_3$), 3.07 (dt, J = 12.6, 3.6 Hz, 2H, 2 × CH_{pip}), 2.61 (td, J = 12.3, 2.7 Hz, 2H, 2 × CH_{pip}), 2.38 (tt, J = 11.3, 3.9 Hz, 1H, CH), 1.94 – 1.78 (m, 2H, 2 × CH_{pip}), 1.59 (adtd, J = 13.4, 11.4, 4.0 Hz, 2H, 2 × CH_{pip}), 1.24 (t, J = 7.1 Hz, 3H, OCH_2CH_3). **Mass Spectrum** (ESI): *m/z* 158.2 $[M+H]^+$.

Ethyl 1-(3-cyano-3,3-diphenylpropyl)piperidine-4-carboxylate (6)

According to method B, 4-bromo-2,2-diphenylbutanenitrile (50 mg, 167 μmol) was treated with ethyl piperidine-4-carboxylate **(22)** (31 mg, 200 μmol). The crude product was purified by column chromatography using a gradient solvent system (100% hexane – 33% EtOAc in hexane) to afford orange oil (38 mg, 61%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.48 – 7.23 (m, 10H, Ar H), 4.14 (q, J = 7.1 Hz, 2H, O$CH_2$$CH_3$), 2.86 (ad, J = 11.5 Hz, 2H, 2 × CH_{pip}), 2.68 – 2.57 (m, 2H, N$CH_2$$CH_2$C), 2.50 – 2.40 (m, 2H, NCH_2CH_2C), 2.35 – 2.22 (m, 1H, CH), 2.09 – 1.97 (m, 2H, 2 × CH_{pip}), 1.94 – 1.84 (m, 2H, 2 × CH_{pip}), 1.82 – 1.68 (m, 2H, 2 × CH_{pip}), 1.26 (t, J = 7.1 Hz, 3H, OCH_2CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.94, 140.01, 128.92, 127.93, 126.78, 122.07, 60.31, 54.96, 53.22, 50.03, 41.01, 36.72, 28.17, 14.20. **Mass Spectrum** (ESI): *m/z* 377.5 $[M+H]^+$. (HR-ESI) Found: $[M+H]^+$, 377.2232. $C_{24}H_{28}N_2O_2$ requires $[M+H]^+$, 377.2224.

Ethyl 4-phenylpiperidine-4-carboxylate (23)[26]

4-phenylpiperidine-4-carboxylic acid (1.00 g, 3.27 mmol) was added to a stirred solution of ACN (20ml) with ethyl iodide (523 μl, 6.55 mmol) and DIPEA (1.71 ml, 9.82 mmol) and stirred at room temperature overnight. ACN was then removed *in vacuo*, added to a 1:1 mixture of TFA/DCM (20 ml) and stirred for 30 mins. TFA/DCM was then removed *in vacuo*. The resulting residue was then dissolved in DCM and washed with 2M NaOH, saturated NaCl, H_2O, dried with anhydrous Na_2SO_4 and filtered. The solvent was then concentrated *in vacuo* to afford a creamy white solid (653 mg, 86%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.40 – 7.24 (m, Ar H), 4.21 (q, 2H, J = 7.0 Hz, O$CH_2$$CH_3$), 3.13 (dt, 2H, J = 3.5 Hz, 2 × CH_{pip}), 2.82 (td, J = 2.0 Hz, 2 × CH_{pip}), 2.57 (ad, J = 14 Hz, 2H, 2 × CH_{pip}), 1.87 (td, 2H, J = 4.0 Hz, 2 × CH_{pip}), 1.20 (t, 3H, J = 4 Hz, OCH_2CH_3). **Mass Spectrum** (ESI): *m/z* 234.3 $[M+H]^+$.

Ethyl 1-(3,3-diphenylpropyl)-4-phenylpiperidine-4-carboxylate (7)[27]

According to method B, (3-bromopropane-1,1-diyl)dibenzene (127 mg, 463 μmol) was treated with ethyl 4-phenylpiperidine-4-carboxylate **(23)** (120 mg, 514 μmol). The crude product was purified by column chromatography using a gradient solvent system (100% hexane – 66% EtOAc in hexane) to afford a crude oil (174 mg. 79%). 29 mg of this crude sample was then further purified using preparative TLC using a solvent system of 5% MeOH in $CHCl_3$ to afford clear gel (24 mg, 65%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.32 –

7.16 (m, 15H, Ar H), 4.05 (q, 2H, J = 7.0 Hz, OCH_2CH$_3$), 3.95 – 3.85 (m, 1H, CH), 2.76 (ad, J = 11.2 Hz, 2H, 2 × CH$_{pip}$), 2.48 (ad, J = 13.1 Hz, 2H, NCH_2CH$_2$CH), 2.23 – 2.16 (m, 4H, NCH$_2$$CH_2$CH, CH, 2 × CH$_{pip}$), 2.10 – 1.99 (m, 2H, 2 × CH$_{pip}$), 1.96 – 1.83 (m, 2H, 2 × CH$_{pip}$), 1.07 (t, J = 7.1 Hz, 3H. OCH$_2$$CH_3$). **^{13}C NMR** (101 MHz, CDCl$_3$) δ 174.43, 144.82, 128.54, 128.51, 128.46, 127.88, 127.86, 126.17, 125.82, 61.02, 60.81, 57.06, 51.56, 49.27, 49.24, 47.37, 38.27, 33.85, 32.83, 14.07. **Mass Spectrum** (ESI): *m/z* 428.5 [M+H]$^+$. (HR-ESI) Found: [M+H]$^+$, 428.2563. $C_{29}H_{33}NO_2$ requires [M+H]$^+$, 428.2584.

2,2-diphenyl-4-(piperidin-1-yl)butanenitrile (8)[23]

A mixture of diphenylacetonitrile (2.00 g, 10.35 mmol) and anhydrous sodium hydride (1.52 g, 50.67 mmol) in anhydrous DMF (30 ml) was stirred at room temperature for 30 min. 1-(2-chloroethyl)piperidine (2.85 g, 19.43 mmol) in anhydrous DMF (10 ml) was added and solution refluxed at 90 °C for 24 h. The solution was concentrated *in vacuo*, taken up in water, made basic with potassium carbonate, then extracted into ethyl acetate and concentrated *in vacuo*. 100 mg of the crude product was purified by RP-HPLC to afford 72 mg of a white solid. **Mp**: 72 – 74 °C. **^{1}H NMR** (300 MHz, CDCl$_3$) δ 7.47 – 7.35 (m, 10H, Ar H), 3.62 (t, J = 12.2 Hz, 2H, NCH_2CH$_2$C), 3.22-3.14 (m, 4H, 4 × CH$_{pip}$), 2.66 (t, J = 11.2 Hz, 2H, NCH$_2$$CH_2$C), 2.28 – 2.01 (m, 4H, 4 × CH$_{pip}$), 1.46 (m, 2H, NCH$_2$CH$_2$$CH_2$). **^{13}C NMR** (600 MHz, CDCl$_3$) δ 138.0, 129.4, 128.6, 126.4, 121.2, 53.8, 49.9, 33.0, 30.9, 22.7, 21.9. **Mass Spectrum** (ESI): *m/z* 305.4 [M+H]$^+$.

Ethyl 4-phenyl-1-(3-phenylpropyl)piperidine-4-carboxylate (9)[28]

According to method B, 4-bromo-2,2-diphenylbutanenitrile (89 μl, 585 μmol) was treated with ethyl 4-phenylpiperidine-4-carboxylate **(23)** (150 mg, 643 μmol). The crude product was purified by column chromatography using a gradient solvent system (100% DCM – 5% MeOH in DCM) to afford a yellow oil (163 mg, 80%). **^{1}H NMR** (400 MHz, CDCl$_3$) δ 7.44 – 7.17 (m, 10H, Ar H), 4.15 (q, 2H, J = 7.0 Hz, OCH_2CH$_3$), 2.78 (ad, J = 11.5 Hz, 2H, 2 × CH$_{pip}$), 2.61 – 2.55 (m, 4H, NCH$_2$CH$_2$$CH_2$, 2 × CH$_{pip}$), 2.39 – 2.29 (m, 2H, N$CH_2CH_2CH_2$), 2.21 – 2.08 (m, 2H, 2 × CH$_{pip}$), 2.05 – 1.92 (m, 2H, 2 × CH$_{pip}$), 1.86 – 1.75 (m, 2H, NCH$_2$$CH_2CH_2$), 1.20 (t, J = 7.1 Hz, 3H. OCH$_2$$CH_3$). **^{13}C NMR** (101 MHz, CDCl$_3$) δ 174.46, 143.02, 142.17, 128.49, 128.40, 128.31, 126.93, 125.82, 125.76, 60.79, 58.19, 51.52, 49.33, 33.91, 33.80, 28.73, 14.08. **Mass Spectrum** (ESI): *m/z* 352.3 [M+H]$^+$. (HR-ESI) Found: [M+H]$^+$, 352.2254. $C_{23}H_{29}NO_2$ requires [M+H]$^+$, 352.2271.

2,2-diphenyl-4-(4-phenylpiperazin-1-yl)butanenitrile (10)[29]

According to method B, 4-bromo-2,2-diphenylbutanenitrile (100 mg, 333 μmol) was treated with 1-phenylpiperazine (36 μl, 233 μmol). The crude product was purified by column chromatography using a gradient solvent system (100% hexane – 33% EtOAc in hexane) to afford a yellow oil (62 mg, 49%). **^{1}H NMR** (400 MHz, CDCl$_3$) δ 7.41 – 7.16 (m, 12H, Ar H), 6.82 (add, J = 8.8, 0.9 Hz, 2H, Ar H), 6.79 – 6.73 (m, 1H, Ar H), 3.12 – 3.06 (m, 4H, 2 × Piperazine CH$_2$), 2.61 – 2.54 (m, 2H, NCH_2CH$_2$C), 2.54 – 2.48 (m, 4H, 2 × Piperazine CH$_2$), 2.47 – 2.40 (m, 2H, NCH$_2$$CH_2$C). **^{13}C NMR** (101 MHz, CDCl$_3$) δ 151.25, 140.02, 129.11, 128.97, 128.00, 126.82, 122.10, 119.73, 116.06, 54.74, 53.38, 50.04, 49.06, 36.64. **Mass Spectrum** (ESI): *m/z* 382.5 [M+H]$^+$. (HR-ESI) Found: [M+H]$^+$, 382.2284. $C_{26}H_{27}N_3$ requires [M+H]$^+$, 382.2278.

4-(4-benzylpiperazin-1-yl)-2,2 diphenylbutanenitrile (11)[30]

According to method B, 4-bromo-2,2-diphenylbutanenitrile (100 mg, 333 μmol) was treated with 1-benzylpiperazine (81 μl, 470 μmol). The crude product was purified by column chromatography using a gradient solvent system (100% hexane – 33% EtOAc in hexane) to afford a pale yellow oil (82 mg, 62%). **^{1}H NMR** (400 MHz, CDCl$_3$) δ 7.39 – 7.09 (m, 15H,

Ar H), 3.47 (s, 2H, Benzyl CH_2), 2.62 – 2.31 (m, 12H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 139.87, 129.32, 128.96, 128.32, 127.99, 127.31, 126.78, 122.03, 62.74, 54.54, 52.99, 52.57, 49.98, 36.26. **Mass Spectrum** (ESI): *m/z* 396.6 $[M+H]^+$. (HR-ESI) Found: $[M+H]^+$, 396.2441. $C_{27}H_{29}N_3$ requires $[M+H]^+$, 396.2434.

4-phenyl-1,2,3,6-tetrahydropyridine (24)[31]

4-phenylpiperidin-4-ol (100 mg, 564 μmol) was dissolved in 9M HCl and stirred at 80°C for 24 hours. The solution was then cooled to 0°C and NaOH added and stirred till the solution was basic (pH > 8). EtOAc was then added and organic layer washed with saturated $NaHCO_3$, saturated NaCl, H_2O, dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to yield a clear oil (61 mg, 68%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.43 – 7.38 (m, 2H, Ar H), 7.37 – 7.32 (m, 2H, Ar H), 7.29 – 7.23 (m, 1H, Ar H), 6.18 – 6.14 (m, 1H, CH), 3.55 (dd, *J* = 5.9, 2.8 Hz, 2H, PhC=CH*CH$_2$*), 3.13 (t, *J* = 5.7 Hz, 2H, PhCCH$_2$*CH$_2$*), 2.48 (ddq, *J* = 5.6, 4.4, 2.7 Hz, 2H, PhC*CH$_2$*). **Mass Spectrum** (ESI): *m/z* 160.2 $[M+H]^+$.

2,2-diphenyl-4-(4-phenyl-5,6-dihydropyridin-1(2H)-yl)butanenitrile (12)

According to method B, 4-bromo-2,2-diphenylbutanenitrile (65 mg, 217 μmol) was treated with 4-phenyl-1,2,3,6-tetrahydropyridine **(24)** (35 mg, 217 μmol). The crude product was purified by column chromatography using a gradient solvent system (100% DCM – 2% MeOH in DCM) to afford a yellow oil (72 mg, 49%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.47 – 7.21 (m, 15H, Ar H), 6.07 – 6.03 (m, 1H, CH), 3.22 (ad, *J* = 2.4 Hz, 2H, PhC=CH*CH$_2$*), 2.83 – 2.72 (m, 4H, PhCCH$_2$*CH$_2$*, N*CH$_2$*CH$_2$C), 2.71 – 2.55 (m, 4H, PhC*CH$_2$*, NCH$_2$*CH$_2$*C). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 140.76, 140.00, 135.16, 128.99, 128.29, 127.99, 127.06, 126.78, 124.97, 122.07, 121.48, 54.54, 53.45, 50.63, 50.07, 37.07, 28.10. **Mass Spectrum** (ESI): *m/z* 379.1 $[M+H]^+$. (HR-ESI) Found: $[M+H]^+$, 379.2186. $C_{27}H_{26}N_2$ requires $[M+H]^+$, 379.2169.

4-(4-hydroxy-4-phenylpiperidin-1-yl)-2,2-diphenylbutanenitrile (13)[32]

According to method A, 4-bromo-2,2-diphenylbutanenitrile (2.00 g, 6.66 mmol) was treated with 4-phenylpiperidin-4-ol (1.18 g, 6.66 mmol). 100 mg of the crude product was purified by RP-HPLC to afford 66mg of a beige solid. **Mp:** 218–220 °C (lit. 221–223 °C). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.45 - 7.26 (m, 15H, Ar H), 3.56 (t, 2H, *J* = 11.4 Hz, N*CH$_2$*CH$_2$C), 3.27 (t, 2H, *J* = 11.0 Hz, NCH$_2$*CH$_2$*C), 3.11-3.00 (m, 4H, 4 × CH_{pip}), 2.91-2.79 (m, 4H, 4 × CH_{pip}). **^{13}C NMR** (600 MHz, $CDCl_3$) δ 145.3, 134.0, 129.5, 128.9, 128.7, 128.1, 126.4, 124.2, 121.1, 69.2, 54.2, 49.8, 49.4, 35.4, 33.3. **Mass Spectrum** (ESI): *m/z* 397.5 $[M+H]^+$.

4-(4-hydroxypiperidin-1-yl)-2,2-diphenylbutanenitrile (14)

4-bromo-2,2-diphenylbutanenitrile (2.14 g, 7.12 mmol), potassium iodide (1.17 g, 7.12 mmol) and DIPEA (4.13 ml, 23.7 mmol) were added to 30 ml of DMF and stirred at RT for 10 mins. 4-hydroxypiperidine (800 mg, 7.91 mmol) was then added to the reaction which was heated overnight at 90°C. DMF was then removed *in vacuo* and residue dissolved in DCM (40 ml) which was washed with saturated $NaHCO_3$, H_2O, saturated NaCl, dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography using a gradient solvent system (100% DCM – 10% MeOH in DCM) to afford yellow oil (1.84 g, 73% yield). This was then recrystallized in a 60:40 mixture of H_2O and MeOH to afford a clear solid (824 mg, 33%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.54 – 7.49 (m, 4H, Ar H), 7.44 – 7.39 (m, 4H, Ar H), 7.38 – 7.32 (m, 2H, Ar H), 4.26 (s, 1H, OH), 3.46 – 3.24 (m, 2H, 2 × CH_{pip}), 3.23 – 3.15 (m, 3H, N*CH$_2$*CH$_2$C, CH), 3.11 – 3.02 (m, 2H, NCH$_2$*CH$_2$*C), 2.56 (s, 2H, 2 × CH_{pip}), 1.90 (ad, *J* = 13.3 Hz, 2H, 2 × CH_{pip}), 1.63 (as, 2H, 2 × CH_{pip}). **Mass Spectrum** (ESI): *m/z* 321.2 $[M+H]^+$.

4-(3-hydroxypiperidin-1-yl)-2,2-diphenylbutanenitrile (15)

According to method B, 4-bromo-2,2-diphenylbutanenitrile (100 mg, 333 μmol) was treated with 3-hydroxypiperidine (37 mg, 366 μmol). The crude product was purified by column chromatography using a gradient solvent system (100% DCM – 10% MeOH in DCM) to yield a yellow oil which was then further purified using preparative TLC using a solvent system of 10% MeOH in DCM to afford yellow oil (45 mg, 42%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.45 – 7.12 (m, 10H, Ar H), 3.79 – 3.65 (m, 1H, OH*CH*), 2.57 – 2.36 (m, 6H, 2 × CH_{pip}, N*CH*$_2$$CH_2C, NCH_2*CH*_2$C), 2.20 – 2.06 (m, 2H, 2 × CH_{pip}), 1.76 – 1.64 (m, 1H, 1 × CH_{pip}), 1.53 – 1.36 (m, 3H, 3 × CH_{pip}). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 140.08, 128.94, 127.97, 126.81, 122.32, 66.09, 60.53, 54.68, 53.85, 49.97, 36.59, 31.53, 21.32. **Mass Spectrum** (ESI): *m/z* 321.2 $[M+H]^+$. (HR-ESI) Found: $[M+H]^+$, 321.1969. $C_{21}H_{24}N_2O$ requires [M+H]+, 321.1961.

1-(3,3-diphenylpropyl)piperidin-4-ol (16)[33]

According to the method B, (3-bromopropane-1,1-diyl)dibenzene (118 mg, 430 μmol) was treated with 4-hydroxypiperidine (100mg, 478 μmol). The crude product was purified by column chromatography using a gradient solvent system (100% hexane – 66% EtOAc in hexane) to afford a white solid (97 mg. 58%). **Mp:** 118–120 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.23 – 7.14 (m, 8H, Ar H), 7.13 – 7.06 (m, 2H, Ar H), 3.95 – 3.84 (m, 1H, OH*CH*), 3.61 (as, 1H, diphenyl-CH), 2.76 – 2.59 (m, 2H, 2 × CH_{pip}), 2.28 – 2.15 (m, 4H, N*CH*$_2$$CH_2C, NCH_2*CH*_2$C), 2.11 – 1.95 (m, 2H, 2 × CH_{pip}), 1.82 (ad, *J* = 9.3 Hz, 2H, 2 × CH_{pip}), 1.57 – 1.48 (m, 2H, 2 × CH_{pip}). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 144.78, 128.45, 127.84, 126.17, 67.89, 56.75, 51.05, 49.19, 34.40, 32.90. **Mass Spectrum** (ESI): *m/z* 296.5 $[M+H]^+$. (HR-ESI) Found: $[M+H]^+$, 296.2021. $C_{20}H_{25}NO$ requires $[M+H]^+$, 296.2009.

1-(3-phenylpropyl)piperidin-4-ol (17)

According to method B, (3-bromopropyl)benzene (92 μl, 719 μmol) was treated with 4-hydroxypiperidine (80 mg, 790 μmol). The crude product was purified by column chromatography using a gradient solvent system (100% Hexane – 100% $CHCl_3$ – 10% MeOH in $CHCl_3$). Fractions containing the product were then reduced *in vacuo*, $CHCl_3$ added, filtered and subsequently concentrated *in vacuo* again to afford a yellow oil (91 mg, 58%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.37 – 7.12 (m, 5H, Ar H), 3.84 – 3.63 (m, 1H, CH), 2.81 (bs, 2H, 2 × CH_{pip}), 2.74 – 2.58 (m, 2H, N*CH*$_2$$CH_2$$CH_2$), 2.51 – 2.34 (m, 2H, NCH_2CH_2*CH*$_2$), 2.33 – 2.12 (m, 2H, 2 × CH_{pip}), 1.91 (ad, *J* = 26.3 Hz, 4H, NCH_2*CH*$_2$$CH_2$, 2 × CH_{pip}), 1.63 (ad, *J* = 8.5 Hz, 2H, 2 × CH_{pip}). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 141.91, 128.38, 128.34, 125.83, 67.47, 57.82, 50.87, 34.11, 33.69, 28.49. **Mass Spectrum** (ESI): *m/z* 220.2 $[M+H]^+$. (HR-ESI) Found: $[M+H]^+$, 220.1693. $C_{14}H_{21}NO$ requires [M+H]+, 220.1696.

4-(4-methylpiperidin-1-yl)-2,2-diphenylbutanenitrile (18)[34]

According to method A, 4-bromo-2,2-diphenylbutanenitrile (1.00 g, 3.33 mmol) was treated with 4-methylpiperidine (394 μl, 3.33 mmol). 100 mg of the crude product was purified by RP-HPLC to afford 48 mg of a yellow oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.48 – 7.27 (m, 10H, Ar H), 3.49 – 3.32 (m, 4H, 4 × CH_{pip}), 2.98 (t, *J* = 12.8 Hz, 2H, N*CH*$_2$$CH_2$C), 2.96 (t, *J* = 11.2 Hz, 2H, NCH_2*CH*$_2$C), 1.86 (m, 1H, NCH_2CH_2*CH*), 1.84 – 1.70 (m, 4H, 4 × CH_{pip}), 1.26 (s, 3H, CH_3). **^{13}C NMR** (600 MHz, $CDCl_3$) δ 138.7, 137.6, 132.4, 130.1, 121.3, 51.6, 42.6, 38.3, 37.4, 35.6, 30.9, 26.6. **Mass Spectrum** (ESI): *m/z* 319.7 $[M+H]^+$.

4-(4-methoxypiperidin-1-yl)-2,2-diphenylbutanenitrile (19)

4-(4-hydroxypiperidin-1-yl)-2,2-diphenylbutanenitrile **(14)** (40 mg, 125 μmol) was dissolved in DMF (10 ml) containing NaH (9 mg, 375 μmol) and stirred for room

temperature for 30 mins. MeI (9 μl, 144 μmol) was then added dropwise and reaction stirred under N_2 at room temperature overnight. DMF was then removed *in vacuo* and residue dissolved in DCM (40 ml) and washed with saturated $NaHCO_3$, H_2O, saturated NaCl, dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography using a gradient solvent system (100% DCM – 5% MeOH in DCM) to afford yellow oil (36 mg, 86%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.36 – 7.19 (m, 10H, Ar H), 3.25 (s, 3H, OCH_3), 3.17 – 3.09 (m, 1H, CH), 2.76 – 2.29 (m, 6H, 2 × CH_{pip}, N$CH_2$$CH_2$C, NCH_2CH_2C), 2.23 – 1.98 (m, 2H, 2 × CH_{pip}), 1.93 – 1.76 (m, 2H, 2 × CH_{pip}), 1.52 (as, 2H, 2 × CH_{pip}). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 139.94, 128.98, 128.96, 128.04, 126.77, 53.16, 50.51, 47.50, 41.06, 37.10, 32.05. **Mass Spectrum** (ESI): *m/z* 335.2 $[M+H]^+$. (HR-ESI) Found: $[M+H]^+$, 335.2125. $C_{22}H_{26}N_2O$ requires $[M+H]^+$, 335.2118.

4-(4-aminopiperidin-1-yl)-2,2-diphenylbutanenitrile (20)

Tert-butyl 1-(3-cyano-3,3-diphenylpropyl)piperidin-4-ylcarbamate **(21)** (250 mg, 596 μl) added to 1:1 mixture of TFA/DCM (10 ml) and stirred for 30 mins. TFA/DCM was then removed *in vacuo*. The resulting residue was then dissolved in DCM and washed with 2M NaOH, saturated NaCl, H_2O, dried with anhydrous Na_2SO_4 and filtered. The solvent was then concentrated *in vacuo* to afford a clear oil (155 mg, 91%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.42 – 7.26 (m, 10H, Ar H), 2.88 – 2.77 (m, 2H, 2 × CH_{pip}), 2.68 – 2.57 (m, 3H, 2 × CH_{pip}, CH), 2.47 – 2.38 (m, 2H, N$CH_2$$CH_2$C), 2.08 – 1.96 (m, 2H, NCH_2CH_2C), 1.85 – 1.73 (m, 2H, 2 × CH_{pip}), 1.41 – 1.30 (m, 2H, 2 × CH_{pip}). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 140.05, 128.91, 127.92, 126.79, 122.09, 54.71, 52.75, 50.04, 48.61, 36.86, 35.85. **Mass Spectrum** (ESI): *m/z* 320.3 $[M+H]^+$. (HR-ESI) Found: $[M+H]^+$, 320.2135. $C_{21}H_{25}N_3$ requires $[M+H]^+$, 320.2121.

Tert-butyl piperidin-4-ylcarbamate (25)[35]

Tert-butyl 1-benzylpiperidin-4-ylcarbamate (500 mg, 1.72 mmol) was dissolved in methanol (10 ml) and stirred. 36% aqueous HCl (169 μl, 1.72 mmol) was added followed by $Pd(OH)_2$/C (525 mg, 689 μmol). Oxygen was then removed under reduced pressure and H_2 added to the system (via a balloon) and stirred overnight. The reaction mixture was then filtered through celite and fitrate reduced *in vacuo*. DCM was then added and washed with 2M NaOH, H_2O, saturated NaCl, dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to yield a yellow oil (321 mg, 94%). **^{1}H NMR** (300 MHz, MeOH) δ 3.63 – 3.46 (m, 1H, CH), 3.34, (s, NH), 3.23 (ad, J = 12.9 Hz, 2H, 2 × CH_{pip}), 2.97 – 2.71 (m, 2H, 2 × CH_{pip}), 2.08 – 1.09 (m, 2H, 2 × CH_{pip}), 1.61 – 1.40 (m, 11H, 2 × CH_{pip}, 3 × Boc CH_3). **Mass Spectrum** (ESI): *m/z* 201.2 $[M+H]^+$.

Tert-butyl 1-(3-cyano-3,3-diphenylpropyl)piperidin-4-ylcarbamate (21)

According to method A without the use of DIPEA, 4-bromo-2,2-diphenylbutanenitrile (320 mg, 1.07 mmol) was treated with tert-butyl piperidin-4-ylcarbamate (**25**) (256 mg, 1.28 mmol) and K_2CO_3 (442 mg, 3.20 mmol). The crude product was purified by column chromatography using a gradient solvent system (100% DCM – 10% MeOH in DCM) to afford a yellow oil (252 mg, 56%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.41 – 7.11 (m, 10H, Ar H), 3.36 (dd, J = 20.6, 9.8 Hz, 1H, CH), 2.70 (ad, J = 11.8 Hz, 2H, 2 × CH_{pip}), 2.62 – 2.41 (m, 2H, N$CH_2$$CH_2$C), 2.41 – 2.27 (m, 2H, NCH_2CH_2C), 1.99 (dd, J = 16.1, 6.3 Hz, 2H, 2 × CH_{pip}), 1.82 (ad, J = 11.4 Hz, 2H, 2 × CH_{pip}), 1.46 – 1.20 (m, 11H, 2 × CH_{pip}, 3 × Boc CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.16, 140.02, 128.92, 127.94, 126.79, 122.08, 79.27, 54.65, 52.55, 49.99, 47.59, 36.86, 32.49, 28.41. **Mass Spectrum** (ESI): *m/z* 420.2 $[M+H]^+$.

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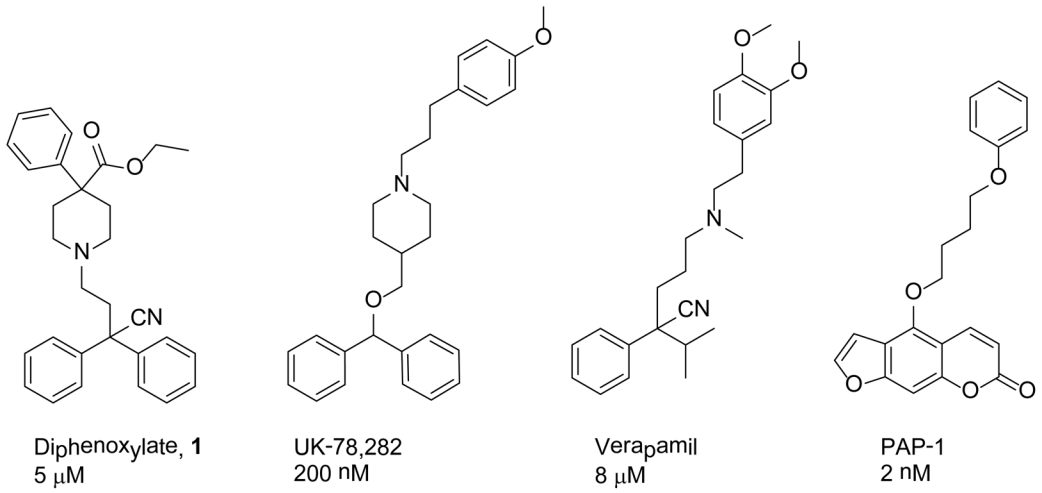
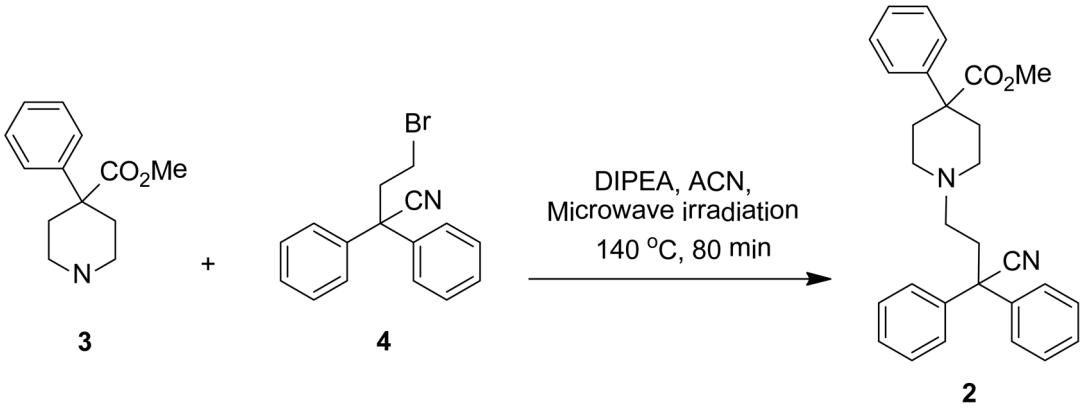


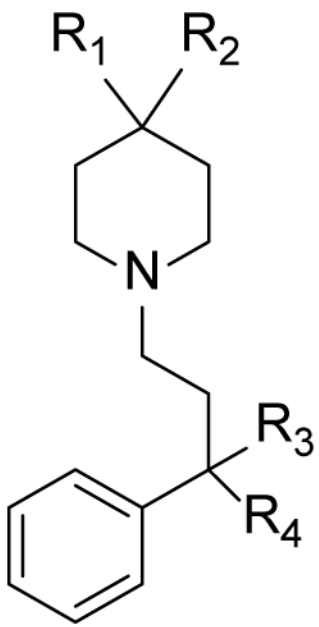
Figure 1.
Structures and Kv1.3 blocking activity of diphenoxylate (**1**), UK-78,282, verapamil and PAP-1.



Scheme 1.
Synthesis of diphenoxylate analogue **2**

Table 1

Kv1.3 blockade



Compound	R_1	R_2	R_3	R_4	Kv1.3 (IC_{50} μM)
Diphenoxylate, **1**	$-C_6H_5$	$-C{=}OOC_2H_5$	-CN	$-C_6H_5$	5.0[12]
2	$-C_6H_5$	$-C{=}OOCH_3$	-CN	$-C_6H_5$	2.52 ± 0.15
5	$-C_6H_5$	-H	-CN	$-C_6H_5$	2.61 ± 0.11
6	-H	$-C{=}OOC_2H_5$	-CN	$-C_6H_5$	230 ± 7.5
7	$-C_6H_5$	$-C{=}OOC_2H_5$	-H	$-C_6H_5$	0.90 ± 0.10
8	-H	-H	-CN	$-C_6H_5$	20.5 ± 1.6
9	$-C_6H_5$	$-C{=}OOC_2H_5$	-H	-H	0.80 ± 0.10
10[a]					1.86 ± 0.03
11[a]					1.27 ± 0.10

Compound	R_1	R_2	R_3	R_4	Kv1.3 (IC_{50} μM)
12[a]					4.20 ± 0.80
13	$-C_6H_5$	-OH	-CN	$-C_6H_5$	1.70 ± 0.20
14	-H	-OH	-CN	$-C_6H_5$	0.75 ± 0.05
15[a]					> 100
16	-H	-OH	-H	$-C_6H_5$	~75.0
17	-H	-OH	-H	-H	> 100
18	-H	$-CH_3$	-CN	$-C_6H_5$	5.80 ± 0.3
19	-H	$-OCH_3$	-CN	$-C_6H_5$	> 100
20	-H	$-NH_2$	-CN	$-C_6H_5$	> 100
21	-H	-NHBoc	-CN	$-C_6H_5$	~25.0

[a]