Bioorganic & Medicinal Chemistry Letters 18 (2008) 5609–5613

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and synthesis of 6-phenylnicotinamide derivatives as antagonists of TRPV1

Susan M. Westaway [a,*], Mervyn Thompson [a], Harshad K. Rami [a], Geoffrey Stemp [a], Leontine S. Trouw [a], Darren J. Mitchell [a], Jon T. Seal [a], Stephen J. Medhurst [a], Sarah C. Lappin [a], James Biggs [a], James Wright [a], Sandra Arpino [c], Jeffrey C. Jerman [b], Jennifer E. Cryan [c], Vicky Holland [b], Kim Y. Winborn [b], Tanya Coleman [a], Alexander J. Stevens [a], John B. Davis [a], Martin J. Gunthorpe [a]

[a] Neurology CEDD, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK
[b] Molecular Discovery Research, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK
[c] Molecular Discovery Research, GlaxoSmithKline, Medicines Research Centre, Gunnels Wood Road, Stevenage, Herts SG1 2NY, UK

ARTICLE INFO

Article history:
Received 15 July 2008
Revised 26 August 2008
Accepted 28 August 2008
Available online 31 August 2008

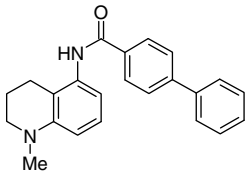
Keywords:
TRPV1 antagonist
Vanilloid
SB-782443

ABSTRACT

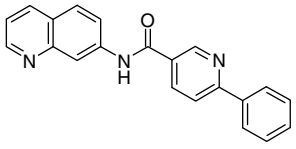
6-Phenylnicotinamide (**2**) was previously identified as a potent TRPV1 antagonist with activity in an in vivo model of inflammatory pain. Optimization of this lead through modification of both the biaryl and heteroaryl components has resulted in the discovery of 6-(4-fluorophenyl)-2-methyl-*N*-(2-methylbenzothiazol-5-yl)nicotinamide (**32**; SB-782443) which possesses an excellent overall profile and has been progressed into pre-clinical development.

The TRPV1 (transient receptor potential vanilloid type 1) ion channel is an exciting target for the discovery of novel analgesics. TRPV1 is a non-selective ion channel which is primarily, although not exclusively, expressed on peripheral sensory neurons.[1] Activation of TRPV1 may occur through exposure to noxious heat (>42 °C) and low pH. Exogenous compounds such as the 'vanilloids' capsaicin and resiniferatoxin (RTX) are well-known agonists of TRPV1. A variety of endogenous substances such as the cannabinoid anandamide and inflammatory mediators such as arachidonic acid metabolites from the lipoxygenase pathway can also directly activate and/or sensitise the channel to activation by other mediators. Other substances such as inflammatory prostaglandins and bradykinin indirectly lead to sensitisation of TRPV1 through phosphorylation of the channel. Thus TRPV1 may be seen as an integrator of many noxious stimuli which lead to the sensation of pain. Early efforts in this area focused on TRPV1 agonists such as capsaicin and RTX as analgesic agents through the desensitisation/denervation approach and various formulations of capsaicin are either marketed or are currently under development.[2] However this approach is often hindered by the pain and discomfort experienced on initial treatment.[2] More recently, there has been an explosion in the discovery and development of TRPV1 antagonists for the treatment of osteoarthritic, neuropathic, post-operative and dental pain[3] and several compounds (SB-705498, AMG-517, NGD-8243/MK-2295, GRC-6211, AZD-1386 and ABT-102) are known to have entered clinical trials.[3,4]

In an earlier letter, we reported on the SAR studies around HTS hit (**1**), which led to the identification of the *N*-quinolin-7-yl-6-phenylnicotinamide (**2**).[5]



1



2

This compound possessed excellent potency at human and rat TRPV1 receptors and a favourable in vitro DMPK profile. However, the low intrinsic clearance observed in liver microsomes (CL_i 3.1 (human), 2.2 (rat), 3.9 (guinea pig) mL/min/g tissue) did not translate into acceptable in vivo stability in the rat (CL_b 33 mL/min/kg, $T_{½}$ 0.36 h at 1 mg/kg iv). Furthermore, (**2**) showed only modest activity in the guinea pig model of Freunds Complete Adjuvant (FCA)-induced inflammatory pain and was less efficacious than expected

* Corresponding author at present address: II CEDD, GlaxoSmithKline, Medicines Research Centre, Gunnels Wood Road, Stevenage, Herts SG1 2NY, UK. Tel.: +44 (0) 1438 763469.
E-mail address: sue.m.westaway@gsk.com (S.M. Westaway).

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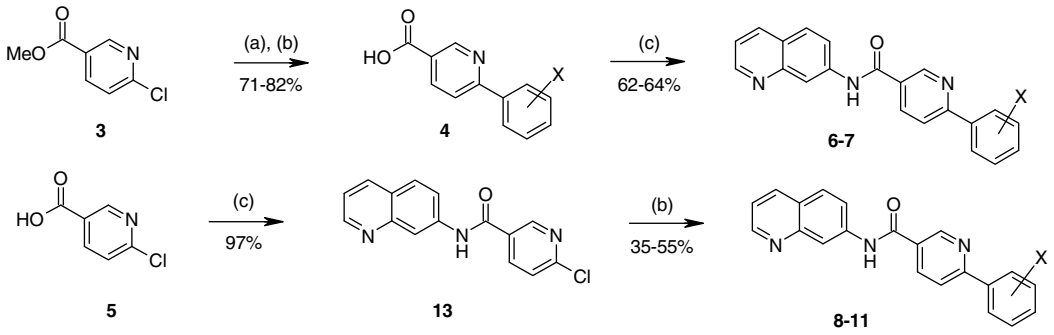
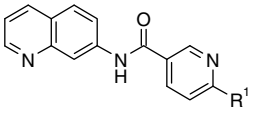
doi:10.1016/j.bmcl.2008.08.105



Scheme 1. Reagents and conditions: (a) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, 1,2-DME, H_2O, 80–85 °C; (b) LiOH, H_2O, 1,4-dioxane, rt; (c) 7-aminoquinoline[5], EDCI·HCl, DMAP, DCM, rt.

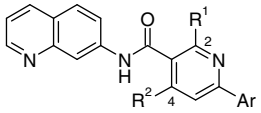
Table 1
Antagonist activity[9] of nicotinamide derivatives **2** and **6–13** at human TRPV1 expressed in a 1321N1 cell line[7] against capsaicin in FLIPR (Fluoresence Imaging Plate Reader) assay



Compound[8]	R^1	hTRPV1[a] pK_b
2	Ph	8.7
6	2-FPh	8.7
7	3-FPh	8.6
8	4-FPh	8.2
9	4-ClPh	7.8
10	4-CF_3Ph	7.3
11	4-CNPh	<6.6
12	4-CN	<6.7
13	4-Cl	<6.7

[a] Standard error of mean (SEM) for all pK_b values ⩽0.1.

Table 2
Antagonist activity[9] of methylnicotinamides at human TRPV1 expressed in 1321N1 cell line[7] against capsaicin (FLIPR)



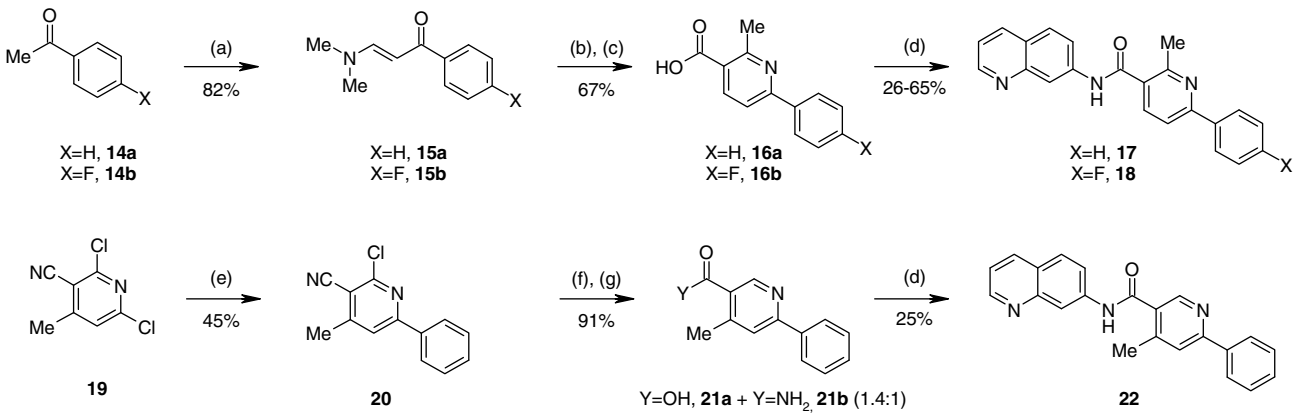
Compound[8]	R^1	R^2	Ar	hTRPV1[a] pK_b
17	Me	H	Ph	9.0
18	Me	H	4-FPh	8.5
22	H	Me	Ph	6.7

[a] SEM ⩽0.1 for (**17**) and (**18**), SEM = 0.3 for (**22**).

based on its high level of in vitro potency. In this letter, we report on further optimisation of this lead series targeting, in particular, an improved in vivo profile. This work has resulted in the identification of a compound which showed excellent levels of activity in the guinea pig FCA model on oral dosing.

Initial efforts focused on modification of the terminal phenyl ring of (**2**). Substituted analogues (**6**) and (**7**) were prepared from methyl 6-chloronicotinate (**3**) and the appropriate boronic acid using Suzuki–Miyaura coupling conditions[6] followed by hydrolysis and standard EDCI-mediated amide coupling. Alternatively, compounds (**8**)–(**11**) were prepared directly from chloro-nicotinamide (**13**) as shown in Scheme 1.

As illustrated in Table 1, the introduction of a fluoro-substituent into the 2- or 3-positions, (**6**) and (**7**), was well tolerated giving compounds with potency similar to that of the unsubstituted parent (**2**). The 4-fluoro substituted compound (**8**) showed ~3-fold lower potency at TRPV1 with pK_b 8.2 but following determination of in vitro ADME properties it was found that this compound possessed the best overall in vitro profile in this series with an acceptable P450 inhibition profile (IC_{50} >10 μM at five major human isoforms), and low intrinsic clearance in human and rat liver microsomes (CL_i 1.7 (human), 1.1 (rat) mL/min/g tissue). However, in guinea pig microsomes, (**8**) proved to be a high-clearance compound (CL_i 42 mL/min/g). Replacement of 4-F with 4-Cl, 4-CF_3 or 4-CN (**9**)–(**11**) was detrimental to potency and complete



Scheme 2. Reagents and conditions: (a) Dimethylformamide dimethylacetal, PhMe, 110 °C; (b) ethyl acetoacetate, NH_4OAc, AcOH, 120 °C; (c) NaOH, H_2O, MeOH, rt; (d) 7-aminoquinoline[5], EDCI·HCl, DMAP, DCM, rt; (e) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, 1,2-DME, H_2O, 85 °C; (f) H_2, 10% Pd/C, Et_3N, DMF, rt, 1 atm; (g) KOH, EtOH, H_2O, rt.

replacement of the terminal phenyl ring with smaller groups such as cyano (**12**) or chloro (**13**) also resulted in reduced levels of TRPV1 antagonist activity.

To investigate the effect of altering the conformation around the biaryl amide linker, the 2- and 4-methylnicotinamide analogues (**17**), (**18**) and (**22**) were prepared (Scheme 2). The 2-methylnicotinic acid intermediates (**16a**) and (**16b**) were readily synthesised using literature methods.[10] The 4-methyl analogue (**21a**) was prepared from the commercially available 2,6-dichoro-4-methylnicotinonitrile (**19**). A regioselective Suzuki–Miyaura coupling reaction followed by reductive removal of the remaining 2-chlorine atom, then aqueous hydrolysis of the nitrile gave a mixture of the desired acid (**21a**) and the primary amide (**21b**). This mixture was reacted with 7-aminoquinoline[5] under standard amide coupling conditions and the unreacted amide (**21b**) was removed on purification of the final product (**22**).

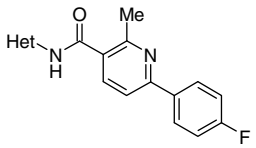
Interestingly, it was found that the presence of a methyl in the 2-position was beneficial to TRPV1 antagonist potency, compounds (**17**) and (**18**), whereas the 4-methylnicotinamide (**22**) was much less active (Table 2). The reduced potency seen with (**22**) may be due to the 4-methyl group causing a conformational change resulting in the pyridyl nitrogen in (**22**) occupying an alternative and less favourable position in the active site when compared to the 2-methyl analogue (**17**) and unsubstituted analogue (**2**).

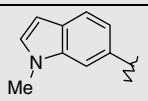
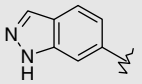
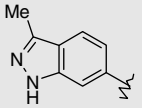
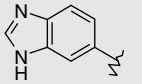
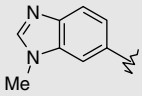
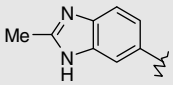
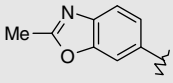
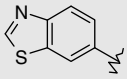
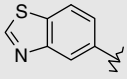
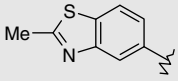
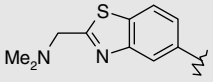
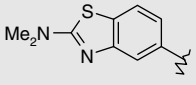
The 2-methylnicotinamide (**18**) was tested in a guinea pig model of FCA-induced inflammatory pain[11] and showed 42% reversal of hyperalgesia at a dose of 5 mg/kg ip. Although this represented a significant improvement over the activity of (**2**) in the same in vivo model (32% reversal at 30 mg/kg sc), the P450 profile of (**18**) was not ideal with the IC_{50} value for inhibition of CYP 3A4 being less than our preferred target of higher than 10 μM.

A series of [5,6] heterocyclic analogues was then evaluated as alternatives to the quinoline. Compounds (**23**)–(**34**) were prepared in acceptable yield by standard EDCI-mediated amide coupling of acid (**16b**) to a range of amines.[12] Many of the compounds showed interesting levels of antagonist activity (Table 3) although potency in this series was reduced by at least 10-fold compared to that seen in the quinoline series. Indazole (**24**) gave acceptable potency at TRPV1 but showed significant inhibition of a number of P450 isoforms. In an attempt to improve the P450 profile of (**24**), a methyl group was introduced into the 3-position of the indazole, (**25**) but, unfortunately this resulted in reduced TRPV1 potency. This same trend for reduction in TRPV1 potency on introduction of methyl substituents was also seen in the benzimidazole series of compounds (**26**)–(**28**). The importance of the position of the heterocyclic nitrogen atom was demonstrated by the results observed with benzoxazole (**29**) and benzothiazoles (**30**)–(**32**); the 6-substituted benzoxazole (**29**) and benzothiazole (**30**) were considerably less active than the corresponding 5-substituted benzothiazoles (**31**) and (**32**). The introduction of more polar functionality onto the benzothiazole was also not well tolerated (**33**) and (**34**).

The benzothiazole analogue (**32**) was of particular interest as it combined both acceptable potency and an attractive in vitro

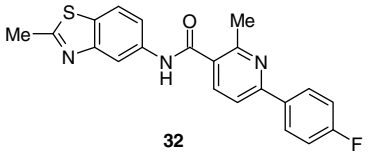
Table 3
Antagonist activity[9] of 2-methyl-6-(4-fluorophenyl)nicotinamides at human TRPV1 expressed in a 1321N1 cell line[7] against capsaicin (FLIPR)



Compound[8]	Het	hTRPV1[a] pK_b
23		<6.5
24		7.5
25		6.9
26		7.2
27		6.7
28		6.0
29		<6.7
30		6.7
31		7.5
32		7.4
33		<6.7
34		<5.8

[a] SEM ⩽0.1 for all compounds.

Table 4
Antagonist activity of (**32**) at human, rat and guinea pig TRPV1 expressed in a HEK293 cell line[7] against capsaicin and acid (pH 5.3) (FLIPR)



32

Agonist	TRPV1 potency[a]		
	Human	Guinea pig	Rat
Capsaicin pK_b	7.2	7.4	7.0
Acid (pH 5.3) pIC_{50}	7.4	7.2	7.0

[a] Data given are pK_b against capsaicin activation ($n = 6$) and functional pIC_{50} against acid activation ($n = 3$). SEM for all data ⩽0.1.

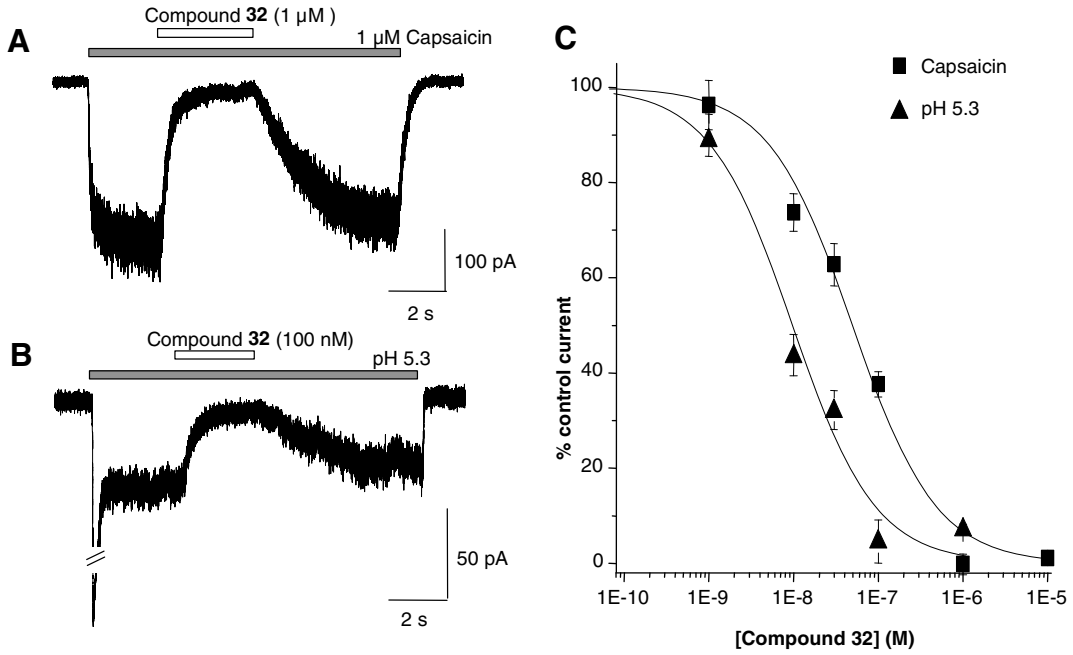


Figure 1. Inhibition of human TRPV1 receptors by (**32**). Whole-cell patch-clamp recordings were made from HEK293 cells expressing hTRPV1. (A) Capsaicin-gated hTRPV1-mediated currents (grey bar) were inhibited by co-application of 1 μM (**32**) (white bar; 100 ± 2% inhibition; $n = 7$). (B) Acid-mediated activation of hTRPV1 was achieved by the application of an extracellular solution of reduced pH (pH 5.3, grey bar). Note that the response shown contains a fast desensitising ASIC (acid-sensing ion channel) response followed by a sustained TRPV1 response.[17] Subsequent co-application of 100 nM (**32**) (white bar) caused inhibition of the TRPV1 acid-gated current (96 ± 5% block, $n = 4$). (C) Pooled data ($n = 3$–7) were used to define the concentration–response relationship for the effects of (**32**) versus both capsaicin and acid yielding IC_{50}s of 49 ± 5 and 9 ± 2 nM, respectively.

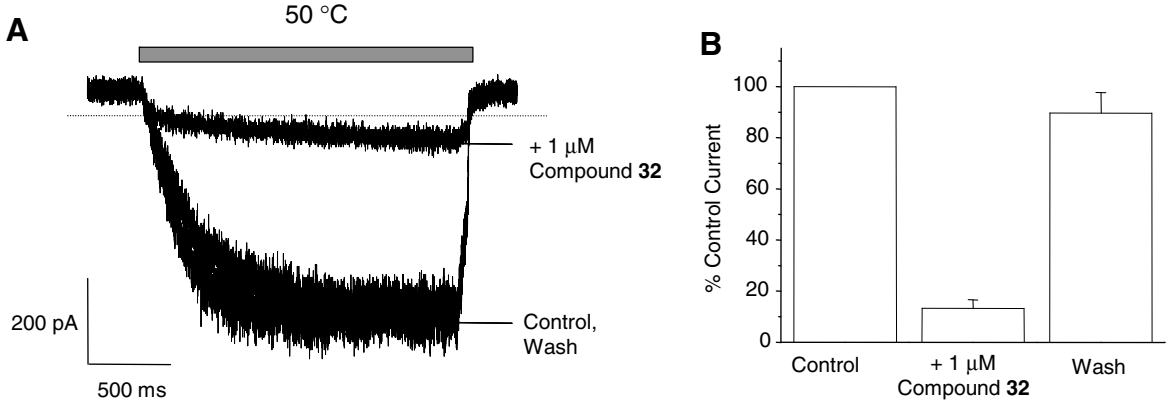


Figure 2. Inhibition of the heat-mediated activation of human TRPV1 receptors by (**32**). (A) Whole-cell currents evoked by noxious heat (50 °C, applied for the duration indicated by the bar) were recorded from hTRPV1-expressing HEK293 cells using the patch-clamp technique. The dotted line indicates the level of non-specific background currents that were subtracted before calculation of the degree of blockade. (B) Heat-activated currents were reversibly inhibited by 1 μM (**32**) (87 ± 3% inhibition relative to control, $n = 3$).

DMPK profile [CL_i 2.2 (human), 4.4 (rat), 2.7 (guinea pig) mL/min/g tissue; P450 IC_{50} >18 μM at five major human isoforms, all data $n = 4$]. Comparison of the antagonist activity of (**32**) against both capsaicin- and acid (pH 5.3)-induced activation at human, rat and guinea pig TRPV1 receptors expressed in a HEK293 cell line revealed that (**32**) possessed similar levels of potency across species, Table 4.

The antagonistic properties of (**32**) at hTRPV1 were also assessed using patch-clamp electrophysiology.[7,13] Compound (**32**) gave an IC_{50} of 49 ± 5 nM versus capsaicin activation (Fig. 1A and C), in good agreement with its effect in the FLIPR. Further experiments demonstrated that (**32**) was also a potent and reversible antagonist of the acid (pH 5.3)-mediated activation of the receptor with an IC_{50} of 9 ± 2 nM (Fig. 1B and C). The activity of (**32**) was also confirmed versus heat-mediated activation of TRPV1 where it showed 87 ± 3% inhibition at a concentration of 1 μM (Fig. 2). These data confirm the multi-modal nature of this antagonist and interestingly, showed enhanced potency of (**32**) versus the acid-mediated activation of the receptor.

The requirement for TRPV1 antagonists to show activity against multiple modes of activation of the channel in order to possess in vivo pain model activity has been discussed in literature[11,14] and our results from testing of (**32**) are consistent with this hypothesis. On dosing at 5 mg/kg ip in the guinea pig model of FCA-induced inflammatory pain, (**32**) showed 48% reversal of mechanical hyperalgesia.

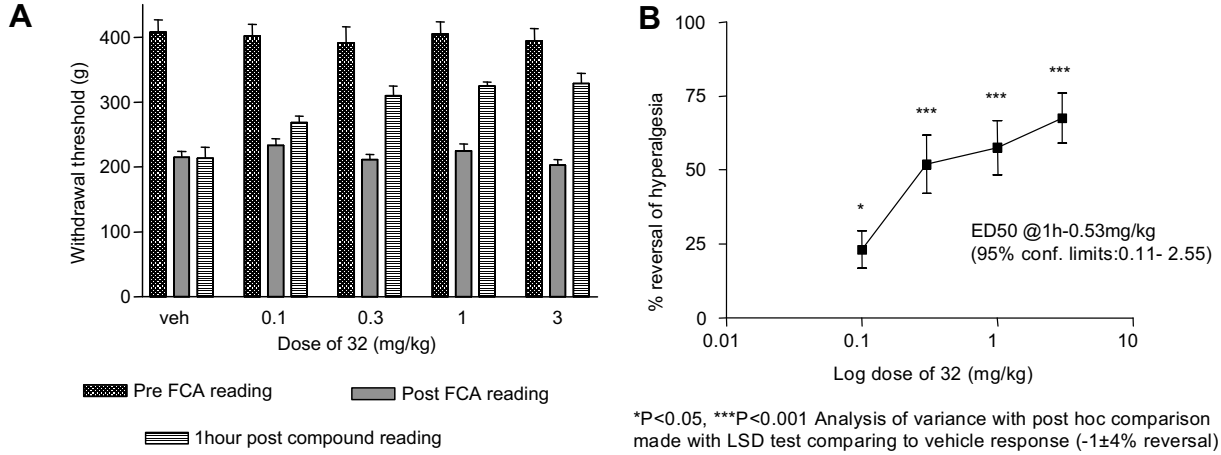


Figure 3. Effects of oral doses of compound (**32**) in the guinea pig model of FCA-induced inflammatory pain. (A) Paw withdrawal thresholds 1-h post-dose of compound (**32**). (B) Dose–response curve showing ED_{50} = 0.53 mg/kg.

In addition, (**32**) possessed an acceptable level of oral bioavailability in the guinea pig (F_{po} 24%), therefore a full dose–response experiment in the FCA model with oral administration was undertaken. As shown in Figure 3, (**32**) gave an excellent response with an ED_{50} of 0.53 mg/kg and maximum reversal of 68 ± 9%, when assessed 1 h post-dose.

In summary, further lead optimisation around *N*-quinolinyl nicotinamide (**2**) has led to the development of benzothiazolyl analogue (**32**), SB-782443.[15] The combination of the attractive in vitro activity, selectivity[16] and DMPK profiles of SB-782443 with a highly promising level of oral activity in an in vivo disease model of inflammatory pain has resulted in the progression of SB-782443 into pre-clinical development.

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8. All new compounds gave satisfactory ^{1}H NMR and/or LC–MS data.
9. pK_b = functional potency of a competitive antagonist (also known as functional pK_i or fpK_i). Mean pK_b values from at least three experiments (n) in all cases except: compounds **23**, **25** and **30** where n = 2.
10. The corresponding ethyl esters of (**16a**) and (**16b**) were prepared using methods similar to those described in Al-Saleh, B.; Abdelkhalik, M. M.; Eltoukhy, Afaf M.; Elnagdi, M. H. *J. Heterocycl. Chem.* **2002**, *39*, 1035.
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15. *Characterising data for* (***32***): ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.67 (1H, s), 8.42 (1H, d, *J* 2.0 Hz), 8.22 (2H, dd, *J* 8.8, 5.2 Hz), 8.03 (1H, d, *J* 8.0 Hz), 7.99 (1H, d, *J* 8.4 Hz), 7.97 (1H, d, *J* 8.0 Hz) 7.71 (1H, dd, *J* 8.8, 2.0 Hz), 7.36 (2H, t, *J* 8.8 Hz), 2.80 (3H, s), 2.68 (3H, s). Mp (DSC) 215 °C. LCMS (ESI^+) 378 (MH^+), purity >98% (UV detection—averaged signal from 210 to 350 nm; 5-min runtime; solvent system—from 3% organic (0.05% formic acid in MeCN)/97% aqueous (0.05% formic acid in water) to 97% organic/3% aqueous, Waters Atlantis column (dimensions 4.6 mm × 50 mm, stationary phase particle size 3 μm; flow-rate 3 mL/min).
16. Compound (**32**) was selective against a variety of ion channels, receptors and enzymes through external (CEREP, ⩽20% inhibition at 1 μM) and in-house screening (pIC_{50}/pK_i <5.7).
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