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Synthesis and in vivo evaluation of 3-substituted gababutins

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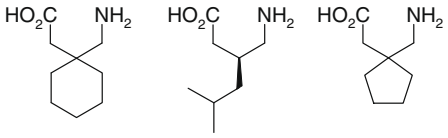
ABSTRACT

A range of 3-alkylated five-membered ring derivatives of Gabapentin were synthesized and several were found to have good levels of potency against the α2δ calcium subunit of a voltage-gated calcium channel. Two compounds were profiled in in vivo models of pain and anxiety.

Gabapentin (Neurontin®) (1)[1] was launched as an add-on therapy for epilepsy in 1994. Subsequently, positive effects in preclinical models of neuropathic pain[2,3] and anxiety[4,5] have been reported and its efficacy against neuropathic pain has been demonstrated clinically in humans.[6] Pregabalin[7,8] (Lyrica®) (2), has superior potency and pharmokinetics[9] to gabapentin and has been approved for the management of neuropathic pain associated with diabetic peripheral neuropathy and post-herpetic neuralgia.



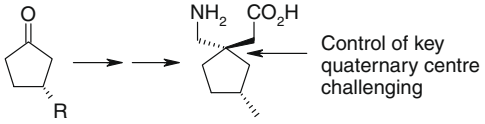
(1) Gabapentin IC_{50}=140nM

(2) Pregabalin IC_{50}=80nM

(3) Gababutin IC_{50}=420nM

The binding of both (**1**) and (**2**) to a high affinity binding site, located on the $\alpha_2\delta$ subunit of a voltage-gated calcium channel, has been reported[10] and it is thought that this site may be involved in the mediation of the pharmacological actions of both gabapentin and pregabalin.[11] Gabapentin and pregabalin bind to this $\alpha_2\delta$ subunit with IC_{50} values of 140 nM and 80 nM, respectively. A number of groups have carried out SAR studies around $\alpha_2\delta$ ligands.[12] In our search for potent and efficacious analogues of gabapentin, we have investigated a range of five-membered ring analogues which we have called gababutins. Gababutin (**3**) has a binding affinity of 420 nM at the gabapentin binding site and we hypothesised that the gababutin five-membered ring is not optimally filling the available space in the binding pocket. We reasoned that appending a variety of alkyl groups to the gababutin ring would allow us to probe this binding pocket, with the aim of generating compounds of similar or better potency to gabapentin. For reasons of synthetic expediency we chose to append substituents to the 3-position of the gababutin ring.

The synthesis of 3-alkyl gababutins presents a number of challenges with the key difficulty being controlling the stereochemistry of the quaternary centre carrying the aminomethyl group.



Single enantiomer required

Additionally, the 3-alkyl cyclopentanones required as starting materials for the syntheses are not easy to obtain commercially: few are available as racemates, and only 3-methyl cyclopentanone is available in enantiomerically pure form as the (*R*)-(+)-isomer. For this reason, initial work focused on the 3-methyl gababutin.

Our initial synthetic strategy is shown in Scheme 1. Ketone (**4**) was converted to α,β-unsaturated ester (**5**) via a Horner–Emmons reaction. Subsequent nitromethane anion addition gave an

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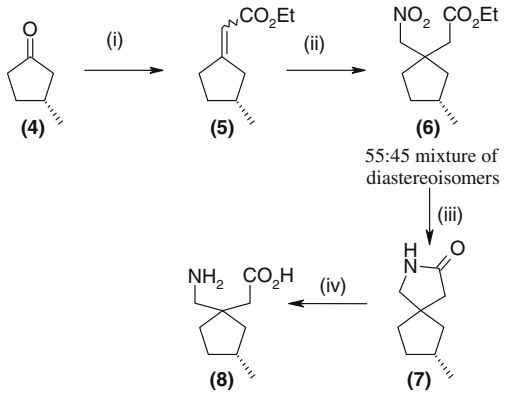


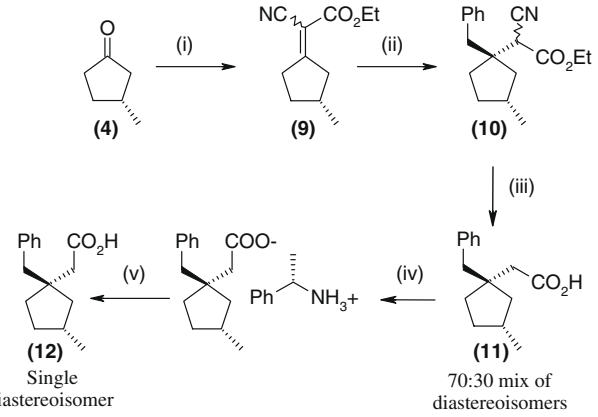
Scheme 1. Reagents and conditions: (i) triethylphosphonoacetate, NaH, THF, 0 °C to rt (95%); (ii) $MeNO_2$, TBAF, THF, reflux (65%); (iii) H_2, Ni, MeOH; (iv) 6 N HCl, 1,4-dioxane, reflux (69% from nitroester).

approximately 1:1 mixture of diastereoisomeric nitroesters (**6**) with the remote methyl group exerting no control over the face of addition under the reaction conditions. Hydrogenation to the lactam (**7**) and then acid hydrolysis gave a mixture of diastereoisomers of the final amino acid (**8**).

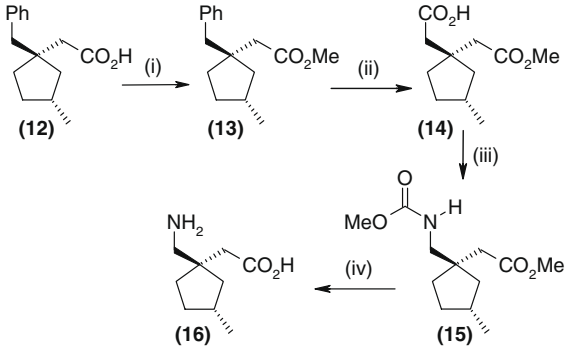
To synthesise the individual diastereoisomers of 3-methyl gabapentin (**8**) we synthesized benzyl acid (**12**) as a single diastereoisomer (Scheme 2).

Knoevenagel condensation of malonitrile onto ketone (**4**) generated the unsaturated cyanoester (**9**) with no apparent geometric control. Low temperature addition of benzylmagnesium chloride to the cyanoester gave a mixture of diastereoisomeric benzyl cyanoesters (**10**) which on hydrolysis gave a 7:3 mixture of diastereoisomers of benzyl acid (**11**) with the dominant diastereoisomer forming as a result of the bulky benzyl group coming in on the opposite face to the methyl group on the cyclopentyl ring. The (*S*)-(−)-α-methylbenzylamine salt of this acid could be recrystallised to give the major product as a single diastereoisomer. Acid treatment of the salt gave benzyl acid (**12**) as a single diastereoisomer (the relative stereochemistry being confirmed by NOE studies). Benzyl acid (**12**) could be converted into either diastereoisomer of the final 3-methyl gabapentin by varying the order of reactions.

To get the diastereoisomer where the aminomethyl group is on the opposite face to the methyl group (**16**) (Scheme 3), acid (**12**) was converted to the methyl ester (**13**) and then the phenyl ring



Scheme 2. Reagents and conditions: (i) $NCCH_2CO_2Et$, NH_4OAc, AcOH, toluene, reflux (97%); (ii) BnMgCl, THF, −78 °C (94%); (iii) KOH, ethylene glycol, 160 °C (77%); (iv) (*s*)-(−)-α-methylbenzylamine, EtOAc, 0 °C; 2 × recrystallisation (40%); (v) dilute HCl (91%).

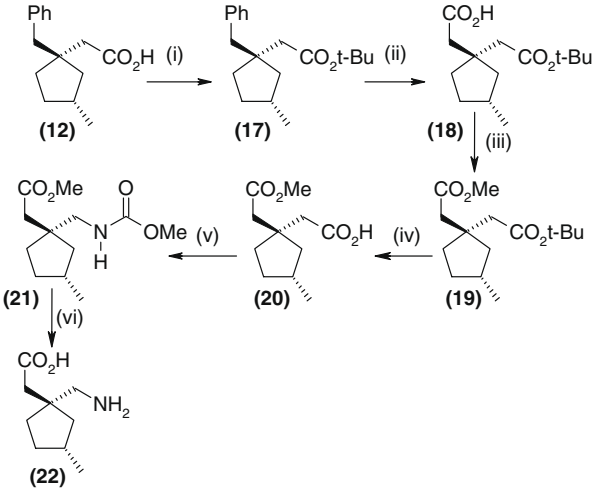


Scheme 3. Reagents and conditions: (i) $TMSCHN_2$, toluene (100%); (ii) $RuCl_3$, $NaIO_4$, CCl_4, MeCN, H_2O (82%); (iii) DPPA, NEt_3, toluene, reflux; MeOH, toluene, reflux (47% from acid); (iv) 6 N HCl, 1,4-dioxane, reflux (85%).

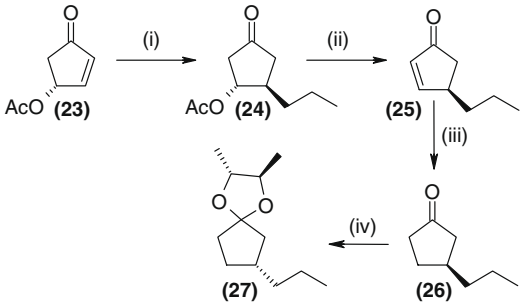
was oxidised to a carboxylic acid with ruthenium tetroxide generated from catalytic ruthenium trichloride and sodium periodate giving (**14**).[13] The resulting acid was converted to a carbamate via a Curtius rearrangement and hydrolysis of this carbamate gave the amino acid (**16**) (the relative stereochemistry was confirmed by NOE studies; chiral HPLC showed the enantiomeric excess to be 98%).

To form the opposite diastereoisomer, the order of events was changed (Scheme 4). In this case, benzyl acid (**12**) was converted to *t*-butyl ester (**17**) followed by oxidation of the phenyl ring to an acid (**18**). Conversion of the acid to methyl ester (**19**) was followed by deprotection of the *t*-butyl ester to give acid (**20**). Curtius rearrangement gave carbamate (**21**) and finally, acidic hydrolysis gave the target amino acid (**22**) (the relative stereochemistry being confirmed by NOE studies; enantiomeric excess of 98% was established by chiral HPLC).

To investigate the impact of varying the 3-alkyl group on potency of the gabapentins, we needed access to a range of enantiomerically pure 3-alkyl cyclopentanones. To achieve this, we used 3-acetoxy cyclopent-2-en-1-one. Routes to both enantiomers of this compound are known in the literature.[14–16] Conjugate addition of an alkyl zincate reagent to the acetoxy cyclopentenone (made with enantiomeric excess of >99%) followed by elimination and hydrogenation gave us 3-alkyl cyclopentanones without loss of stereochemical integrity (Scheme 5).



Scheme 4. Reagents and conditions: (i) oxalyl chloride, DMF, DCM; *t*-BuOH, DIPEA, DCM (88%); (ii) $RuCl_3$, $NaIO_4$, CCl_4, MeCN, H_2O (73%); (iii) $TMSCHN_2$, toluene (92%); (iv) TFA, DCM (98%); (v) DPPA, NEt_3, toluene, reflux; MeOH, toluene, reflux (63%); (vi) 6 N HCl, 1,4-dioxane, reflux (60%).



Scheme 5. Reagents and conditions: (i) *n*-PrMgCl, Me_2Zn, THF, −78 °C; (ii) DBU, Et_2O, −40 °C (68% over two steps); (iii) H_2, 10% Pd/C, EtOAc (88%); (iv) (2*R*,3*R*)-(−)-2,3-butanediol, *p*-TSA, benzene, reflux (85%).

For example, conjugate addition of the *n*-propyl zincate (generated from *n*-propyl magnesium chloride and dimethylzinc) occurred exclusively on the least hindered face of the cyclopentenone (the acetoxy group forcing the *n*-propyl group onto the opposite face) to generate propyl cyclopentanone (**24**). Low temperature elimination with DBU followed by hydrogenation occurred without any epimerisation to give the 3-propyl cyclopentanone (**26**) in >98% enantiomeric excess (as determined by chiral gas chromatography or by derivatisation to acetals such as (**27**) and examination of ^{13}C NMR spectra).

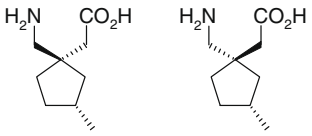
As well as using 3-acetoxy cyclopent-2-en-1-one to synthesise the chiral 3-propyl cyclopentanone, we tested out the use of the chiral cyclopentadienone synthon, ketodicyclopentadiene (**28**)[17] with the aim of obtaining benzyl acid derivative (**34**) as a single diastereoisomer (Scheme 6). Conjugate addition of propylzincate to ketone (**28**) gave the desired propyl ketone (**29**) as a single diastereoisomer. Knoevenagel condensation was followed by benzyl Grignard addition to unsaturated cyanoester (**30**). Following basic hydrolysis, benzyl acid (**32**) was obtained as a single diastereoisomer. The retro Diels–Alder reaction occurred without epimerisation to give (**33**) which was hydrogenated to give (**34**) as a single diastereoisomer.

Initially, we synthesized the alkylated gababutins as equimolar mixtures of all four possible isomers and determined their binding affinities (a radioligand binding assay incorporating [^{3}H]gabapentin at the $\alpha_2\delta$ subunit of a calcium channel was utilised as previously described[10]) (Table 1). The 3-methyl, ethyl and *n*-propyl substituents increased binding affinity relative to the starting gababutin, while larger groups and dialkyl substitution were detrimental showing that space in the binding pocket is relatively tight.

The impact of the chirality of the 3-alkyl stereocentre on binding was now examined (Table 2). For ease of synthesis, while the 3-alkyl stereocentre was set, the quaternary stereogenic centre was undefined and compounds of type A and B were approximately 1:1 mixtures of diastereoisomers.

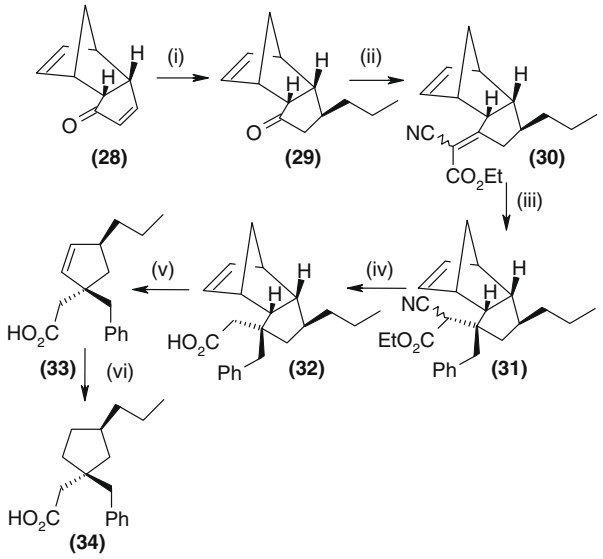
The stereochemistry at the 3-position was critical for activity with compounds of type A, with the (*R*)-configuration at the 3-alkyl position, having good binding affinities and compounds of type B, with an (*S*)-configuration at the 3-alkyl position, binding weakly.

To assess the impact of the quaternary stereogenic centre on binding affinity, the single diastereoisomers of the 3-(*R*)-methylgababutin were synthesized.



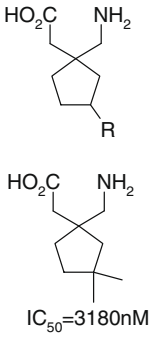
Both compounds (**16**) and (**22**) had good binding affinities at the gabapentin binding site and so were evaluated in in vivo models of pain and anxiety.

Firstly, both compounds were evaluated in the carageenan induced thermal hyperalgesia model of pain (Fig. 1). In this model, the pro-inflammatory agent carrageenan was administered via intraplantar injection into the paw of a rat. Within two hours of injection, the paw became inflamed and hyperalgesia had been induced. An infra-red light source was shone on the paw and the time it took the animal to withdraw its paw from the heat-source was measured. Before treatment, baseline measurements were taken giving a reading of 10 s to paw withdrawal. After 2 h, once the



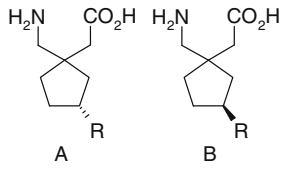
Scheme 6. Reagents and conditions: (i) *n*-PrMgCl, Me_2Zn, THF, −78 °C (98%); (ii) $NCCH_2CO_2Et$, NH_4OAc, AcOH, benzene, reflux (75%); (iii) BnMgCl, Me_2Zn, THF, −78 °C; (iv) KOH, ethylene glycol, 160 °C (75% over two steps); (v) Ph_2O, Et_2O, 215 °C (58%); (vi) H_2, PtO_2, EtOAc, 30 °C, 60 psi (99%).

Table 1



R	IC_{50} (nM)
H	420
Me	82
Et	55
n-Pr	69
n-Bu	690
i-Bu	760
t-Bu	>10,000
Ph	5060
$PhCH_2$	9280
$PhCH_2CH_2$	6230

Table 2



R	Compound	IC_{50} (nM)
Me	A	88
Et	A	53
n-Pr	A	29
Me	B	1300
Et	B	706
n-Pr	B	1200

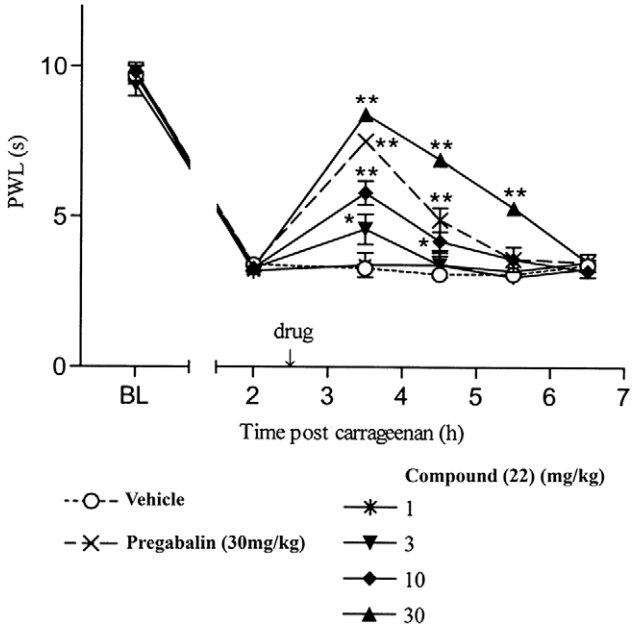


Figure 1. * = *P* <0.05, ** = *P* <0.01, significantly different compared to vehicle.

hyperalgesia had fully formed the time to paw withdrawal had reduced to 2.5 s. At this point the compound to be assessed was dosed orally and any reversal of the hyperalgesia was assessed by measuring the time to paw withdrawal.

Compound (**22**) showed good levels of efficacy in this model with a 30 mg/kg dose reversing the hyperalgesia and giving a similar effect to that of pregabalin. Compound (**16**) showed no effect in this model on oral dosing but did reverse the hyperalgesia on *intrathecal* dosing (direct into the spinal cord). This data suggested that compound (**16**) did not penetrate the blood–brain barrier. Gabapentin itself is a substrate for the Large amino acid transporter (System-L amino acid transporter)[11] and it has been assumed that it is this transporter that conveys these highly polar compounds into the CNS. It is possible that different diastereoisomers have different affinities for the transporter and this could explain the difference seen between the two compounds in the CITH model. However, this hypothesis was undermined by the data for compound (**16**) from the water-lick (Vogel) conflict model of anxiety (Fig. 2). In this model, a group of rats were dosed orally with compound and were then placed in a cage containing a drinking tube from which they received a shock each time they drank. The mean number of shock episodes received per rat over 10 min was measured at different doses. The more anxiety that the animals feel, the less they drink and the less shocks they receive. For an effective compound, as the dose of compound increases then the number of shocks received by the animals should also increase. The minimum effective dose (MED) for activity for pregabalin in this model is 10 mg/Kg. Compound (**16**) had an MED of 3 mg/Kg while compound (**22**) proved to have poor efficacy in this model having an MED of 30 mg/Kg.

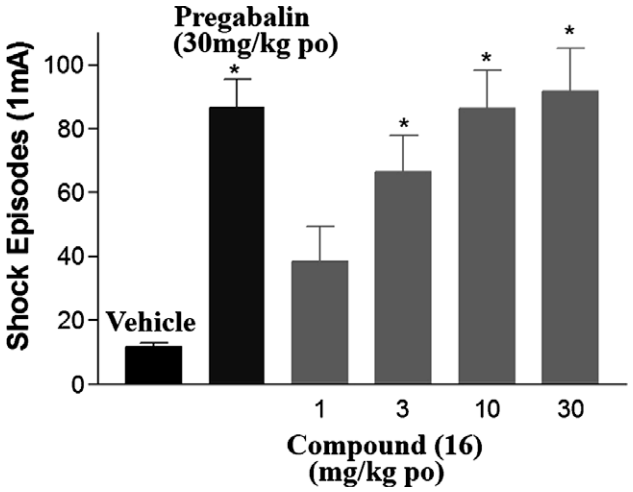


Figure 2. * = P <0.05, significantly different compared to vehicle.

The difference between the two compounds is surprising and shows that binding affinity alone is not a good indicator of in vivo activity. The fact that (**16**) is only effective in the CITH model on *intrathecal* dosing but effective in the Vogel conflict model on oral dosing shows that there are some subtle effects at work and that pain and anxiety effects may be mediated differently. Notwithstanding, we have identified one compound with similar efficacy to pregabalin in an in vivo model of pain and one compound with superior efficacy to pregabalin in a model of anxiety.

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