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Chiral tetrahydroquinoline derivatives as potent anti-hyperalgesic agents in animal models of sustained inflammation and chronic neuropathic pain

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Abstract—Chiral tetrahydroquinoline derivatives have been prepared by an asymmetric Mannich-type condensation reaction using commercially available vinyloxyethylsilane and a *N*-arylimino *R*-(+)-*t*-butyl lactate ester, in the presence of a catalytic amount of metal triflates as Lewis acids. This synthetic approach gave rise to the target aldehyde intermediate in moderate facial diastereoselectivity and in high chemical yield. This efficient route enabled to scale up the synthesis of an orally bioavailable glycine antagonist showing outstanding in vivo anti-hyperalgesic activity in different animal models of sustained inflammation and chronic neuropathic pain.

In the last decades the instrumental role of the glutamate both in the physiology and pathophysiology of the central nervous system (CNS) has been widely investigated.[1–5] Nowadays, it is widely accepted that the increased excitability of spinal cord neurons (*wind-up*) caused by the massive depolarization induced mainly by glutamate is associated with the development and maintenance of pain hypersensitivity (hyperalgesia and allodynia).[6–14] This pathological status occurs when peripheral tissues and/or nerves are severely damaged (e.g., neoplastic infiltration, inflammation, etc.).[15] Antagonists of the glycine binding site associated to the NMDA receptor,[16–21] restoring the baseline level of nociceptive transmission, have been hypothesized to be effective for the treatment of the neuropathic pain.[22,23] In particular, the indole-2-carboxylate derivative

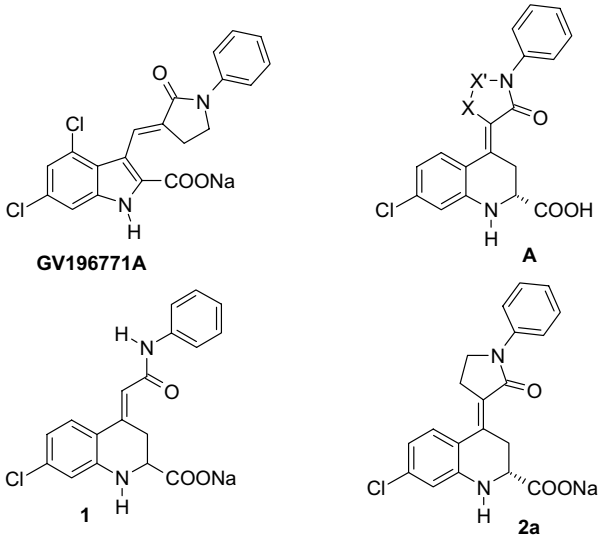

Figure 1. GV196771A and some tetrahydroquinoline (THQs) derivatives.

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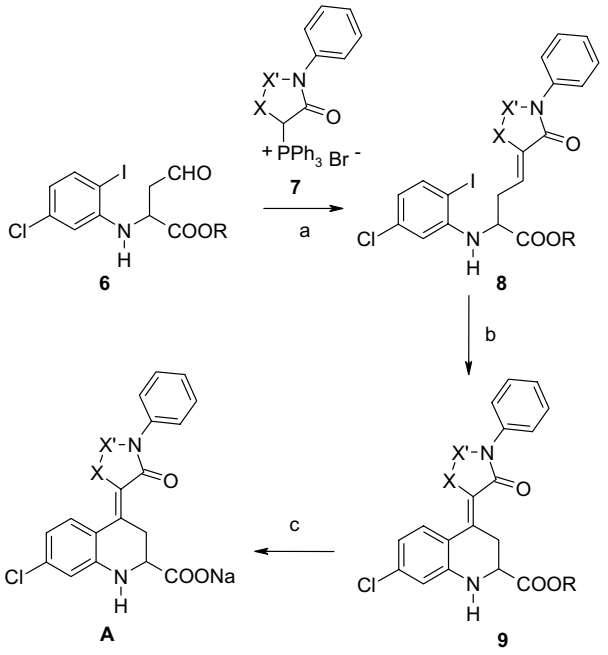
GV196771A, shown in Figure 1, was identified as a potent hyperalgesic agent in different animal models of chronic pain.[24,25]

As part of a wide research programme aimed towards the identification of chemically diverse, orally bioavailable glycine antagonists, we focus our chemical strategy on the appropriate exploration of the tetrahydroquinoline (THQ) template **A**, as depicted in Figure 1. We discovered in fact, from the preliminary characterization of a selected number of THQ derivatives available in-house from the exploration made in the glycine antagonists/stroke project,[26] that this chemical series offered significant advantages over the corresponding 2-carboxyindoles in terms of oral bioavailability. In particular, the racemate derivative **1**, shown in Figure 1, when administered at 1 mg/kg dose iv and 3 mg/kg po, both in rats and dogs, exhibited good oral bioavailability ($F = 35\%$ and 40%, respectively). To optimize further the pharmacokinetics of this chemical series, we managed to remove the H-bond donor present in the amide group bridging the amide nitrogen and the *exendo* double bond using a suitable X–X′ spacer (see compounds **2**–**5** in Table 1).

As far as the synthesis of these new THQ derivatives is concerned, as shown in Scheme 1, the known[27] aldehyde intermediate **6** (R = Et or Bn) was transformed in high yield into the desired α,β-unsaturated intermediates **8**. The following Heck-*type* cyclization reaction enabled to get the cyclized compounds **9**, with complete control of the stereochemistry of the double bond. Finally, the hydrolysis of the ester gave the title THQs **2**–**5**.

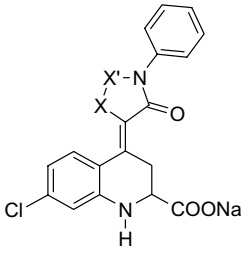
These compounds were initially characterized in terms of in vitro binding studies in rat cerebral cortex membranes as described by Kishimoto.[28] As shown in Table 1, the first compound synthesized, the γ-lactam derivative **2**, inhibited [^{3}H]glycine binding in a concentration-dependent fashion; the resulting pK_i was 7.02, a value not far from the in vitro potency observed for the 'open' analogue **1** (pK_i = 7.42). Notably, both the enlargement of the lactam ring and the replacement of the methylene group by a carbonyl group resulted in a significant loss of the in vitro activity (compounds **3** and **4**, pK_i = 5.71 and 5.64, respectively). Conversely, the hydantoin derivative **5** was the most potent compound belonging to this sub-series, accounting for additional binding interactions with the receptor site. However, despite the higher in vitro activity of compound **5** with respect to the γ-lactam derivative **2**, due to the outstanding preliminary in vivo pharmacokinetics in rats ($F = 76\%$) of the latter compound, priority was given to the preparation and the biological characterization of the R enantiomer **2a**, shown in Figure 1, expected to be the most active based on the previous exploration of the THQ template.[26]

This enantiomer was initially synthesized using the route previously described,[29] in which the key step, as shown in Scheme 2, was represented by the diastereoselective addition of an allyl metal derivative to the chiral *N*-aryl

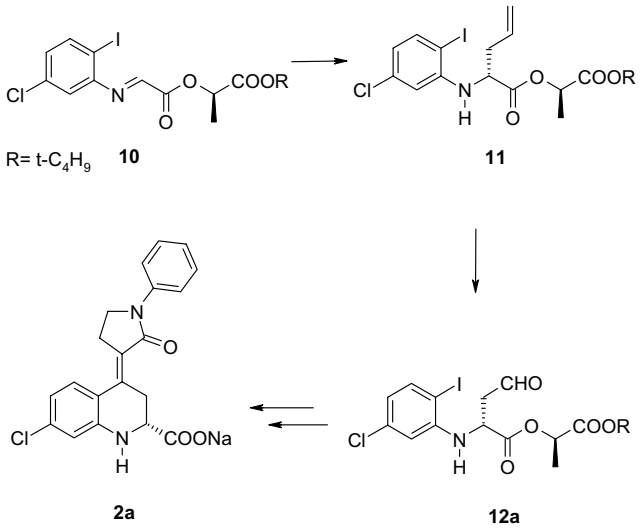


Scheme 1. Synthesis of racemic THQs. Reagents and conditions: (a) **7**, DBU, CH_3CN, −20 °C to rt 1–3 h; (b) $Pd(PPh_3)_4$, TEA, DMF, 110 °C, 1–3 h; (c) NaOH, IMS, 1 h, rt or BCl_3, CH_2Cl_2, −20 °C, 30 min.

Table 1. Bridged THQ derivatives



Compound	X	X′	pK_i
1	—	—	7.42
2	CH_2	CH_2	7.02
3	CH_2	$(CH_2)_2$	5.71
4	CH_2	C=O	5.64
5	NH	C=O	7.80

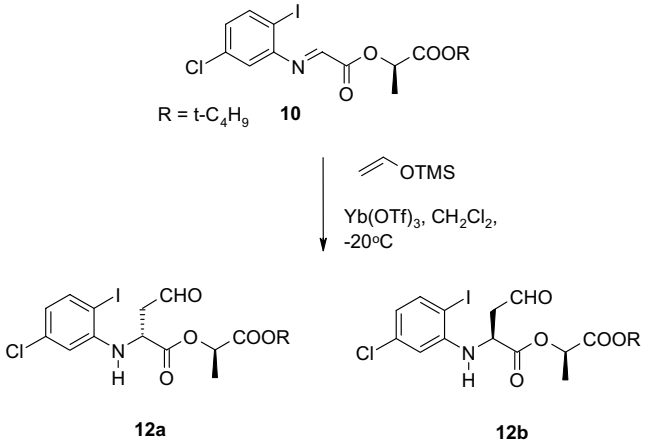


Scheme 2. Diastereoselective synthesis of THQs.

aldimine **10**, to afford the desired *N*-aryl allyl glycine derivative **11** as a single diastereoisomer. Then, the ozonolysis reaction enabled to get the key aldehyde intermediate **12a** in high yield, which was then efficiently transformed into the final compounds by stereoselective Heck-type intramolecular cyclization reaction.

Although this synthetic approach was suitable to prepare the **2a** in multigrams scale, we reasoned on the possibility to get the chiral aldehyde intermediate **12a** directly from the aldimine **10**, exploiting a Mannich-type condensation reaction using the commercially available vinyloxytrimethylsilane and a suitable Lewis acid. If successful this modified synthesis, shown in Scheme 3, would have enabled to reduce the number of synthetic steps, avoiding, at the same time, the potentially hazardous ozonolysis reaction, particularly when run in large scale.

The desired asymmetric induction in the formation of the α-amino acid-*type* stereogenic centre could have



Scheme 3. Addition reaction of the vinyloxytrimethylsilane to the aldimine derivative.

been induced, once again, by the presence of the distal stereogenic centre present in the *R*-(+)-lactate ester. In case of partial facial diastereoselectivity in the addition reaction, the two aldehyde derivatives **12a** and **12b** would have been separated by flash chromatography.

Despite the large number of investigations made in the last decades on the Mannich-type reactions of silyl enolethers and various kinds of aldimines, both in anhydrous and aqueous medium,[30] surprisingly the use of vinyloxytrimethylsilane has never been reported.

The proposed reaction was run as follows: the aldimine intermediate **10** was dissolved in CH_2Cl_2 and reacted at −20 °C with vinyloxytrimethylsilane (1 equiv), in the presence of a catalytic amount of $Yb(TfO)_3$ (0.2 equiv). The reaction mixture was stirred at −20 °C for 1 h. Then, the HPLC analysis has shown the presence of a mixture of the diastereoisomers **12a** and **12 b** (ratio 85:15 by ^1H NMR). After aqueous workup, the crude residue obtained was purified by flash chromatography to give rise to the target compound **12a** in 55% overall yield from the starting aniline derivative.

Notably, no reaction was observed in the presence of other Lewis acids, namely BF_3, $SnCl_4$, $TiCl_4$, ZnI_2 and $CeCl_3$. Conversely, the reaction was run successfully with $Sc(OTf)_3$ obtaining **12a** and **12b** in comparable ratio and yield with respect to the reaction performed in the presence of $Yb(TfO)_3$.[31,32]

The preferential formation of the R-type stereogenic centre was explained, as shown in Figure 2, by the intramolecular addition of the vinyloxytrimethylsilane to the Re face of the 'open chain' or 'chelated' aldimine complex of type **I** and **II**, respectively.

Intermediate **12a**, after smooth purification by flash chromatography, was efficiently transformed into the desired compounds **2a** in three steps, as previously reported.[29] This THQ derivative inhibited [^{3}H]glycine binding in a concentration-dependent fashion (pK_i = 7.22). Moreover, high receptor selectivity over seventy different receptors was observed (pK_i < 5), including both the AMPA and kainate. Based upon this encouraging preliminary profile, compound **2a** was characterized in terms of ability to suppress the wind up[33] in isolated neonatal rats spinal cord. This effect is considered as an index of the capability to abolish the central sensitization of

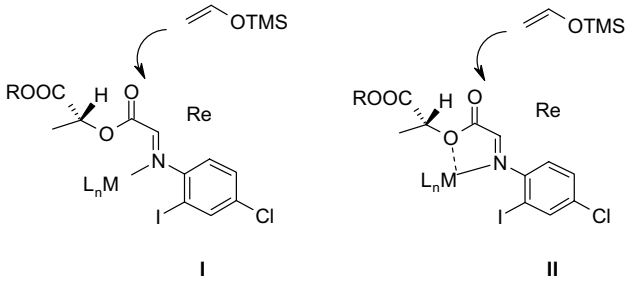


Figure 2. Proposed models of the transition state.

Table 2. Comparative in vitro and in vivo profiles

Compound	pK_i	CCI, rats ED_{50} (mg/kg)	Formalin, mice ED_{50} (mg/kg)	
			EP	LP
2a	7.22	0.14 (0.05–0.9)	—	0.14 (0.08–0.3)
GV196771A	7.56	2.95 (1.5–8.4)	—	0.6 (0.1–2.1)
MK-801	—	—	—	0.1[a]
Morphine[b]	—	—	0.73 (0.2–1.4)	0.56 (0.2–1.6)

[a] 50% reduction of the licking time at 0.1 mg/kg dose given ip 10 min prior to formalin.
[b] ip, 30 min prior to formalin.

spinal cord neurons, the event responsible for the onset of chronic pain. Notably, the superfusion of isolated neonatal rat spinal cord with a 10 μM solution of compound **2a** attenuated significantly the amplitude of the wind up; this effect was qualitatively and quantitatively similar to GV196771A, D-AP_5, a commercially available competitive NMDA antagonist, and morphine.

The in vivo pharmacokinetics was then evaluated both in rats and dogs, at 1 mg/kg dose iv and 3 mg/kg po. A higher oral bioavailability with respect to compound **1** was seen in both species (F = 85% and 64%, respectively), confirming the proposed working hypothesis. In addition, low plasma clearance (Cl = 3.5 and 1.4 ml/min/kg, respectively) and moderate elimination half-life ($\tau_{1/2}$ = 1.4 and 2.4 h, respectively) were observed. Notably, the α-amino acid-*type* stereogenic centre was stable in vivo: no traces of the opposite enantiomer were recorded in all of the samples tested.

Based upon both its excellent in vitro profile and pharmacokinetics, this compound was profiled up in an animal model of acute and sustained inflammatory pain (formalin test in mice) and in a model of chronic neuropathic pain (chronic constriction injury in rats) (see Table 2).[34]

The results obtained are summarized in Table 2. In the formalin model in mice, when compound **2a** was administered po at 0.03, 0.1 and 0.3 mg/kg dose, 1 h prior to formalin injection into the left hind paw, similarly to GV196771A, no suppression of the early phase (EP) of the nociceptive behaviour was observed at all doses. Conversely, a dose-dependent reduction of the paw-licking time was seen in the late phase (LP) response (ED_{50} = 0.14 mg/kg), with a 4–5 times increase in potency with respect to our reference compound GV196771A (ED_{50} = 0.6 mg/kg, po).

The analgesic activity observed in the LP was found to be comparable to the activity observed with the non-competitive NMDA antagonist MK-801 (50% reduction of the licking time at 0.1 mg/kg, ip), further confirming the hypothesis that the NMDA receptor is involved in the sustained nociceptive transmission. Conversely, as expected, morphine was found to be active, both in the EP (ED_{50} = 0.73 mg/kg, ip) and in the LP (ED_{50} = 0.56 mg/kg, ip). In particular, as far as the activity in latter phase is concerned, it is worth stressing that, although the routes of administration of morphine and the compound **2a** were different (ip and po, respectively) similar potency was observed at comparable doses.

In the chronic constriction injury (CCI), a model of chronic pain in hyperalgesic rats, the oral administration of compound **2a** at 0.1, 0.3 and 1 mg/kg dose produced a dose-related reduction in thermal hyperalgesia of the ligated paw. By measuring the analgesic effect at 1 h after treatment, an ED_{50} of 0.14 mg/kg po was estimated. Notably, this anti-hyperalgesic activity lasted up to 8 h at 1 mg/kg dose. This effect was highly specific since the withdrawal latencies of the non-operated paw were unaffected by the described treatment. As shown in Table 2, this compound was found to be 20 times more potent than our 'gold standard' GV196771A (ED_{50} = 2.95 mg/kg, po).[35]

These results confirmed once again that the glycine antagonists are potent anti-hyperalgesic agents in animal models of sustained inflammatory pain, able to prevent the establishment of central sensitization restoring the baseline level of excitability of the spinal cord neurons.

In conclusion, a new orally bioavailable glycine antagonist has been identified as an in vitro and in vivo potent anti-hyperalgesic agent. The outstanding in vivo profile observed further reinforces the hypothesis on the instrumental role of NMDA receptor in the onset and maintenance of central sensitization, as biological target for the discovery of innovative drugs able to treat chronic neuropathic pain.

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