Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1207–1214

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Structure–Activity Relationships of Some Opiate Glycosides

Andrew V. Stachulski,[a,*] Feodor Scheinmann,[a] John R. Ferguson,[a] Jayne L. Law,[a] Keith W. Lumbard,[a] Peter Hopkins,[a] Naina Patel,[b] Simon Clarke,[b] Anna Gloyne[b] and Simon P. Joel[b]

[a]*Ultrafine UFC Ltd, Synergy House, Guildhall Close, Manchester Science Park, Manchester M15 6SY, UK*
[b]*Barry Reed Oncology Laboratory, Department of Medical Oncology, St. Bartholomew's Hospital, West Smithfield, London EC1A 7BE, UK*

Received 13 June 2002; revised 2 September 2002; accepted 13 November 2002

Dedicated to Professor Sir Alan Battersby in acknowledgement of his seminal work on morphine biosynthesis

Abstract—A number of analogues of morphine-6-glucuronide **1** have been prepared and evaluated as potential analgesic agents by competitive μ-receptor binding assay and in vivo antinociceptive activity. The analogues show variation in the nature of the carbohydrate residue, the *N*-substituent, the *O*(3)-substituent and saturation of the 7,8-double bond compared to **1**. In general, only the 6β-glucoside or β-glucuronide carbohydrate residues showed potent agonism; other modified carbohydrates were less active or exhibited potential antagonism. Variations in *N*-substituent led to either reduced agonism (N–H) or potential antagonism [*N*-allyl, *N*-(cyclopropyl)methyl]; a polar *N*-substituent, carboxymethyl, failed to bind. Saturation of the 7,8-double bond led to increased agonism compared to the parent compound in all three examples studied.

Introduction

We report the synthesis, receptor binding and in vivo antinociceptive activity of a number of analogues of morphine-6-β-D-glucuronide (M6G)**1**. M6G was first synthesised by Yoshimura and co-workers in 1968[1] and a series of papers later disclosed that this substance, far from being merely a detoxifying metabolite, actually exhibited superior analgesic activity to morphine **2** itself on a mg/mg basis.[2,3] Additionally, several studies report a reduction in typical opioid side effects such as nausea, vomiting and respiratory depression after M6G compared to morphine,[3] possibly related to a different receptor affinity profile.[4]

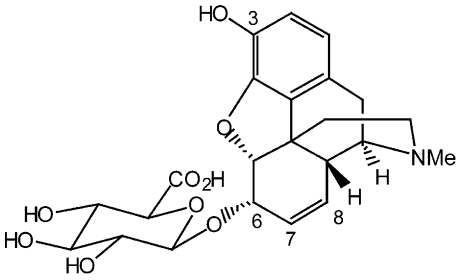
It was necessary for reasons of toxicity to devise a new process for the preparation and in vivo evaluation of **1**, not employing a heavy metal catalyst.[1] We have described[5] metal-free, Lewis acid-catalysed procedures for the conjugation of appropriate glucuronic acid derivatives to 3-protected morphines which satisfy this condition as well as enzyme-catalysed methods; the condensation of a tri-isobutyryl-protected trichloroacetimidate with a 3-acyl morphine is particularly efficient.[6,7]

In this paper we describe the syntheses of a number of analogues of **1**, compounds **3–19**, many of them new compounds, together with their biological evaluation by competitive binding assays and in vivo antinociceptive activity using the hotplate method.[8] The analogues involve carbohydrate variants (**3–11**), a codeine analogue (**12**), *N*-substituent variants (**13–16**) and 7,8-dihydro variants (**17–19**).

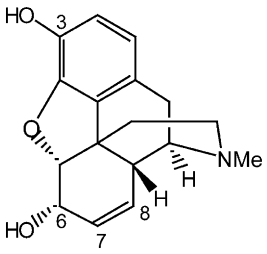
A number of recent reports have described the loss of morphine and M6G induced analgesia in μ opioid (MOP) receptor deficient mice, but the retention of morphine analgesia in κ or δ receptor knockout mice.[9] μ2 Mediated morphine side effects (respiratory depression and GI transit) are also absent in MOP receptor deficient mice, emphasing the importance of the MOP in mediating morphine and M6G effects. Moreover, although the relationship between the pharmacologically defined μ1 and μ2 receptors and the cloned MOP receptor, or splice variants of the MOP receptor, is yet to be defined, it is known that general MOP receptor antagonists (i.e., β-funaltrexamine) block all known morphine and M6G

*Corresponding author at present address: Stylacats Lecturer, Robert Robinson Laboratories, Department of Chemistry, University of Liverpool, Liverpool L69 7ZD, UK. Fax: +44-151-794-3588; e-mail: stachuls@liverpool.ac.uk

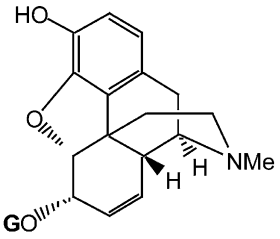
0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00056-8

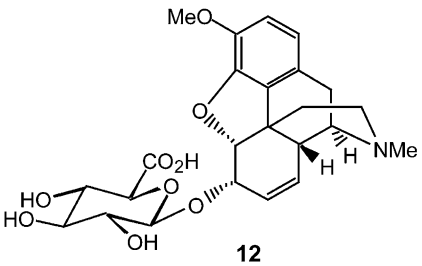


1

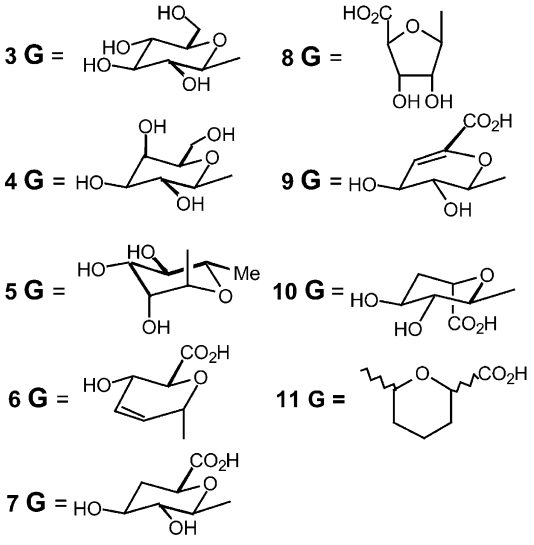


2



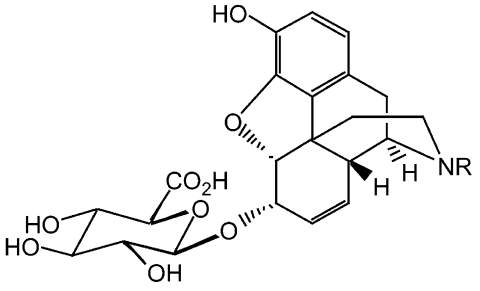


12

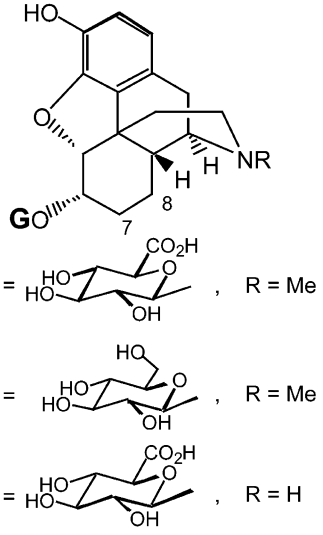


3 to 11: Carbohydrate variants of 1.

12: Codeine analogue



13 R = H
14 R = $CH_2.CH=CH_2$
15 R = CH_2CO_2H
16 R = CH_2*cyclo* -C_3H_5



17 G = , R = Me
18 G = , R = Me
19 G = , R = H

13 to 16: N-substituent variants of 1

17 to 19 : 7,8-dihydro variants of 1, 3 and 13

induced effects, whereas side effects (respiratory depression and inhibition of GI transit) are not blocked by specific μ1 antagonists (i.e., naloxonazine).[10] At the time the present study was initiated, antagonists against the putative M6G receptor (i.e., 3-*O*-methylnaltrexone) had not been reported.[11] The receptor binding studies reported herein therefore focus on μ-receptor binding, at both μ1 (spinal analgesia) and μ2 (supraspinal analgesia and side effects) sites to investigate whether these new compounds interact more specifically with the μ1 receptor.

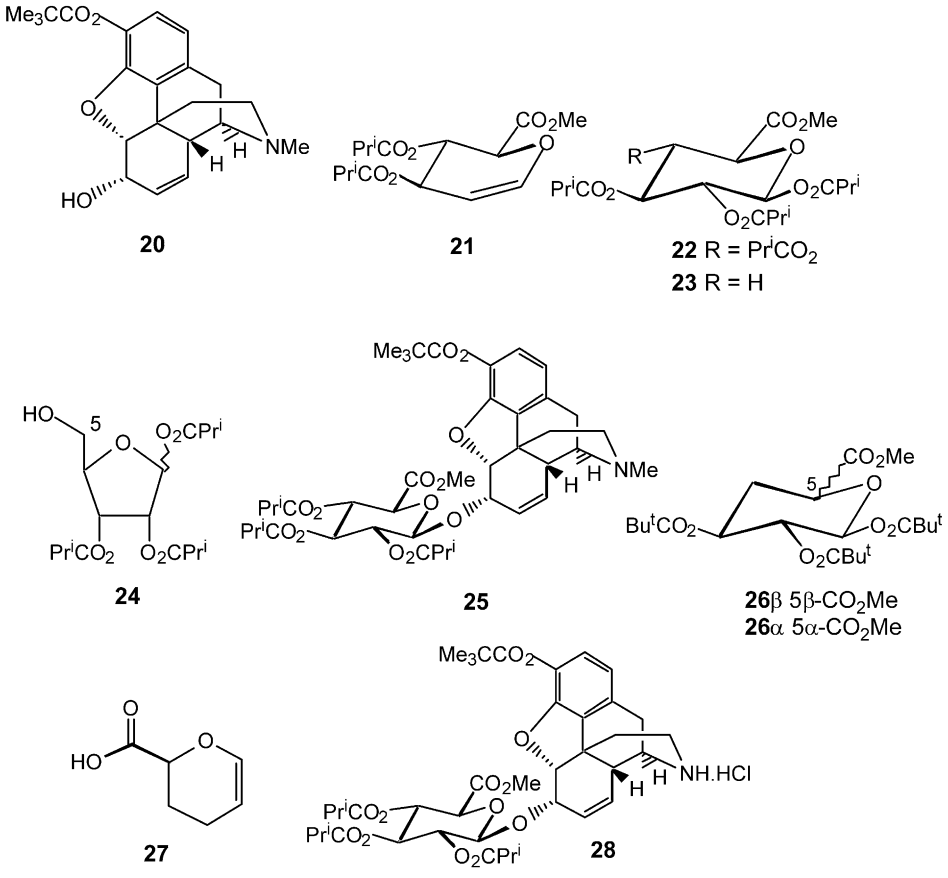
Chemical synthesis of analogues[12]

Morphine was obtained from the McFarlane-Smith Company (Edinburgh) as morphine sulfate and conventionally converted to the free base form. Other chemicals were obtained from standard chemical suppliers or synthesised as noted in references below.

Carbohydrate analogues. Considering first the non-uronic acid sugars, the 6β-glucoside **3** was prepared essentially according to the literature procedure[13] and the 6β-galactoside **4** was prepared similarly, both from the appropriate bromosugar. The 6α-rhamnoside **5** was obtained by direct coupling of rhamnopyranose α-tetraacetate to 3-pivaloyl morphine **20** (prepared by two-phase acylation of morphine with pivaloyl chloride under Schotten–Baumann conditions) mediated by $CF_3SO_3SiMe_3$ followed by base hydrolysis of the esters.

Moving to the modified glucuronide analogues, the $\Delta_{2,3}$-unsaturated analogue **6** was prepared by a Ferrier-type reaction of 3-pivaloyl morphine **20** with glycal **21**, (obtained via Zn elimination from the anomeric bromosugar, as for the known[14] diacetate) followed by ester hydrolysis: the α-stereochemistry depicted is that expected for this type of glycosidation.[15] 4-Deoxy analogue **7** was obtained from β-tetraisobutyrate **22**[5,16] by a sequence of elimination using DBU followed by hydrogenation of the intermediate $\Delta_{4,5}$-ester; the major 5β-isomer **23** crystallised and was coupled to **20** followed by deprotection to give **7**. The ribose analogue **8** was prepared via a sequence of tritylation of D-ribose[17] followed by acylation and detritylation to give intermediate **24**. Following oxidation to the 5-carboxylic acid and esterification, the fully protected sugar was coupled as an α/β-mixture to **16**; subsequent deprotection gave **8** as a *single* (β-)anomer.

The above mentioned DBU elimination was inefficient when applied to the fully protected ester **25**. To prepare the $\Delta_{4,5}$-unsaturated analogue **9** the fully protected intermediate **25**[5] was treated with a strong base [$KN(SiMe_3)_2$, −10° C], which effected elimination in 70% yield, then ester hydrolysis delivered **9**. The C(5)-inverted analogue **10** was obtained from the 4-deoxy-sugar **26**β, prepared analogously to **23**; radical bromination at C(5),[18,19] followed by Zn–AcOH reduction, gave mainly the 5α-product **26**α, the α/β-ratio being more favourable (3:1) in the pivaloate series; separation of **26**α, coupling to **20** and deprotection afforded **10**. The carboxy-tetrahydropyranyl analogue **11**, lacking any hydroxy substitution, was obtained by direct acid-catalysed addition of **20** to the methyl ester of acid **27**, obtained



by methylation of the commercially available sodium salt. Hydrolysis of the esters produced **11** as a diastereoisomeric mixture.

***O*(3)- and *N*-variants.** Codeine-6-glucuronide **12** is now commercially available (Sigma-Aldrich Co. Ltd.) or it may be made by Yoshimura's procedure.[1]

The previously reported synthesis of nor-M6G **13**[20] was very low-yielding. Instead, *N*-demethylation of **25** using α-chloroethyl chloroformate[21] followed by warming in methanol, then ester hydrolysis, afforded **13** in about 60% overall yield. The intermediate secondary amine **28** in the latter sequence (isolated as its HCl salt) could be alkylated using allyl bromide (K_2CO_3, DMF) in excellent (93%) yield; deprotection afforded nalorphine-6-glucuronide **14**.[22]

Alternatively, alkylation using methyl bromoacetate or (chloromethyl)cyclopropane (KI catalysed in the latter case), followed by deprotection, afforded analogues **15** and **16**.

C(7)-C(8) saturated analogues. Finally, the 7,8-dihydro analogues **17**–**19** were obtained by hydrogenation of **1**, **3** and **13**, respectively; **18** was conveniently isolated as its succinate salt, while **17** and **19** were kept in their zwitterionic forms.

Biological Materials and Methods

μ Receptor affinities

Affinities for μ1 and μ2 receptors were determined from binding studies on membranes derived from rat brains, using a previously reported procedure.[4] The μ1 affinity of each compound was determined from the displacement of ^{3}H [D-Ala^2, D-Leu^5] enkephalin (DADL, 1 nM, Du Pont NEN Research Products, Hounslow, Middlesex, UK) after blocking δ receptors, to which DADL also binds, with excess [D-Pen^2, D-Pen^5] enkephalin (DPDPE, Peninsula Laboratories, San Carlos, CA, USA). μ2 Affinity was determined from the displacement of ^{3}H [D-Ala^2, (N-Me)Phe^4, Gly^5-ol] enkephalin (DAGO, 1 nM, Amersham International, Little Chalfont, Bucks, UK) after blocking μ1 receptors with excess [D-Ser^2, L-Leu^5, L-Thr^6] enkephalin (DSLET, Peninsula Laboratories). K_i values were determined from a minimum of six separate experiments using the IC_{50} data according to the formula;

$$K_i = \frac{IC_{50}}{1 + \frac{[\text{ligand}]}{K_d}}$$

Mouse antinociceptive assays

The antinociceptive activity of each compound was determined using the hot-plate method (55±0.5 °C) in male LACA mice (25–30 g). For all experiments the maximum hotplate exposure time was 30 s, and only mice with a control hotplate latency of <10 s were used. Compounds were administered by intravenous injection into a tail vein, with a minimum of six (different) mice studied at each of three dose levels. The starting dose was 2 mg/kg for each compound, with subsequent dose levels at 1 and 4 mg/kg, or 4 and 8 mg/kg, depending on the activity of the starting dose. Animals were tested prior to drug administration (time 0), and at 10, 20 and 40 min after drug administration. Antinociception at each time point was expressed as the% change from control, determined as;

$$\text{A (\%)} = \frac{(\text{test latency-control latency})}{(\text{30-control latency})} \times 100$$

As a measure of total antinociceptive activity across the study period the area under the antinociception effect–time curve (AUEC) was calculated using the trapezoidal method.

Biological Results

Receptor binding data and antinociceptive activity for each compound are shown in Table 1. The percentage change in hotplate latency with time for compounds with an AUEC of >1000 A%/min at the 4 mg/kg dose level is shown in Figure 1, and the relationship between receptor affinity and antinociceptive activity (AUEC) is shown for μ1 binding (Fig. 2A) and μ2 binding (Fig. 2B). There was a good relationship between increasing receptor affinity (decreasing K_i value) and increasing antinociceptive activity for both μ1 (supraspinal analgesia, Fig. 2A) and μ2 (spinal analgesia, Fig. 2B) receptors, with the exception of 5 compounds in the μ1 assay (5, 10, 11, 14 and 16) and 4 in the μ2 assay (4, 5, 14 and 16). There was also a good relationship between μ1 and μ2 receptor affinities, as shown in Figure 3. As expected, the compound modified at the 3-position (**12**, codeine 6-glucuronide) showed the lowest receptor affinity, followed by the *N*-carboxymethyl glucuronide **15**.

Discussion

The effects of *N*-substitution, *O*(3)-substitution and reduction of the 7,8-double bond on biological activity have been described for morphine itself.[23,24] At the start of our work, however, nothing was known concerning the effects of variation of the carbohydrate residue on the activity of **1**. It was of particular interest to discover that the nature of the carbohydrate had a profound influence on activity.

Carbohydrate variants

Considering first non-glucuronic acid variants, the known[13] β-glucoside **3** was the only compound which showed significant antinociceptive activity at the 2 and 4 mg/kg dose levels, along with a slightly higher affinity for the μ1 receptor compared to morphine and M6G (Table 1). In contrast, the rhamnoside **5** showed markedly higher μ1 affinity than morphine (K_i 0.17 vs 0.78 nM,

Table 1. Affinity of novel compounds for μ1 and μ2 receptors and their antinociceptive activity in a mouse hotplate model[a]

Compd No.	Receptor binding activity (K_i, nM±SEM)		Area under the antinociception effect time curve (AUEC, A%/min±SEM)		
	μ1 receptor	μ2 receptor	2 mg/kg	4 mg/kg	8 mg/kg
1 (M6G)	1.5±0.26	26±4.9	937 ±164	1359±246	—
2 (Morphine)	0.78±0.33	4.8±0.72	1030±251	1987±228	—
Sugar variants					
3	0.28±0.06	3.2±1.0	1130±253	2283±240	—
4	1.2±0.55	4.8±0.7	682±224	686±208	2397±233
5	0.17±0.05	6.2±1.2	421±77	250±213	—
6	3.2±0.41	178±48	491±339	130±169	851±252
7	1.9±0.36	57±13	18±458	365±312	1046±491
8	1.6±0.39	44±13	−56±129	797±412	596±389
9	1.4±0.25	48±10	651±193	759±353	701±371
10	0.60±0.17	172±29	35±221	80±198	380±205
11	0.51±0.07	168±20	−230±152	−26±120	478±439
3-Position variants					
12	85±12	5702±933	ND	ND	ND
N-Substituted variants					
13	0.98±0.18	37±7.6	393±197	1614±469	2487±363
14	0.20±0.03	10±2.3	−221±158	147±265	492±275
15	33±3.3	1622±264	−266±95	−45±106	322±386
16	0.06±0.01	3.5±0.6	126±113	599±139	266±167
7,8−Dihydro variants					
17	0.25±0.05	5.7±0.94	1667±405	1885±548	—
18	0.21±0.06	2.5±0.41	1490±337	2701±64	—
19	ND	ND	2196±395	2461±216	—

[a]M6G (1) and morphine (2) were included in both analyses. Data is the mean of at least five experiments throughout (±SEM) (ND, no data).

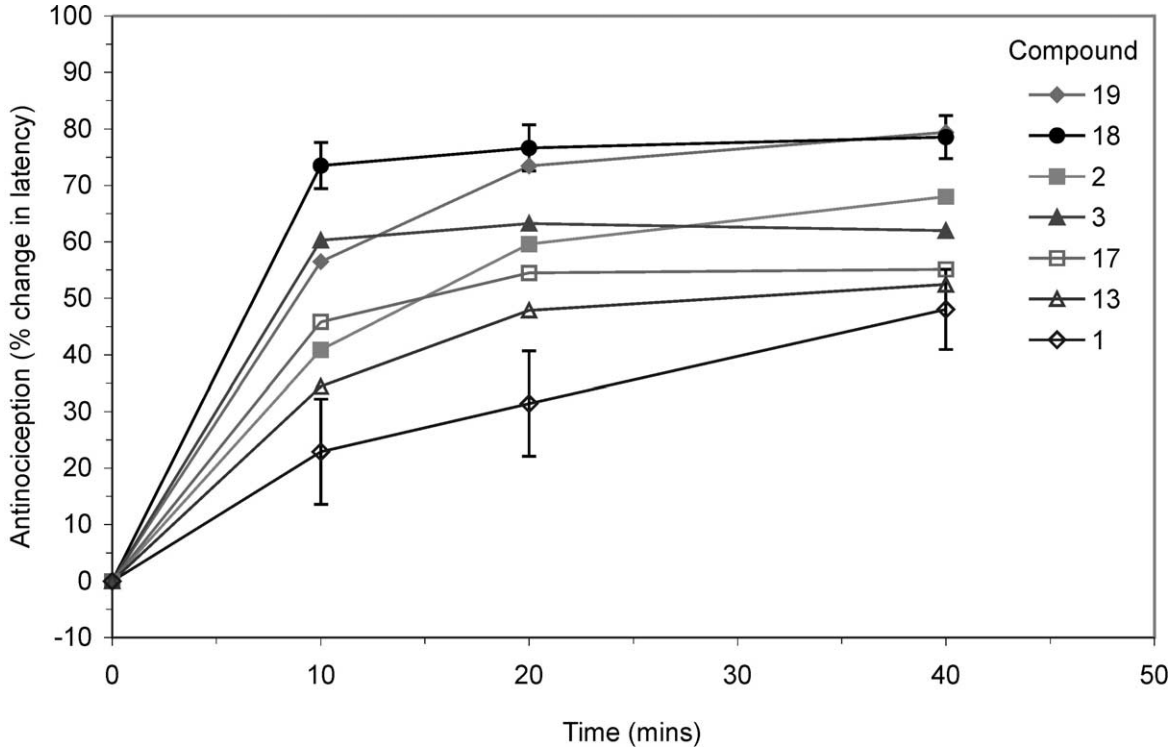


Figure 1. Antinociceptive activity of opiate glycosides with AUEC values of greater than 1000 A%/min at a dose of 4 mg/kg in the mouse hotplate model. Data are the mean of at least five experiments expressed relative to the response time of control mice with saline. For compounds **1** (M6G) and **18** the mean±SD are shown.

respectively), but almost no antinociceptive activity.[25] Compounds **5** and **10** were the only L-series sugars used in this study. The galactoside **4** had similar μ1 affinity to morphine and M6G, and showed some antinociceptive activity at 4 mg/kg but good activity at 8 mg/kg. None of these compounds (**3**–**5**) differed from morphine with regard to μ2 affinity. Analogues **6**–**11** were all acidic sugar residues featuring unsaturation, deoxygenation or altered ring size compared to **1**. Compounds **6**, **7**, **8**, **9** showed similar, or slightly lower μ1 affinities compared

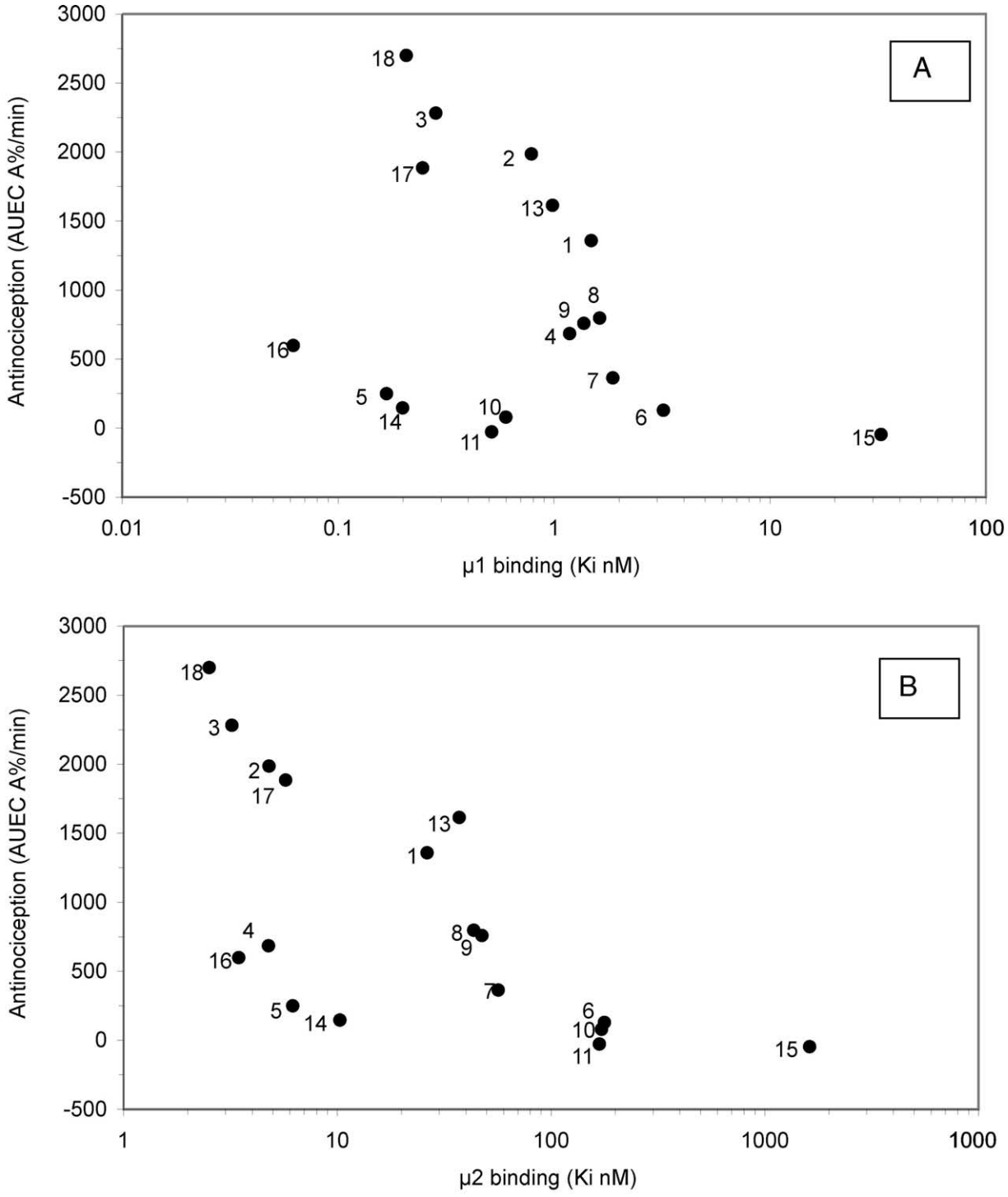


Figure 2. Relationship between receptor affinity (K_i, nM) and antinociceptive activity expressed as the AUEC for % change in hotplate latency from control for μ1 receptors (panel A) and μ2 receptors (panel B). Each compound is identified next to its data point, compound **12** excluded.

to morphine, but somewhat reduced antinociception. The μ2 affinities of these compounds (**6–9**) were also markedly reduced compared to morphine. Compound **9** was especially important to evaluate since small amounts of this derivative may be formed in the final hydrolysis step of the synthesis of **1**. In contrast, the C(5)-inverted analogue **10**, and the carboxy-tertrahydropyran **11** showed good μ1 affinity, but markedly reduced μ2 affinity, and had little or no antinociceptive activity. It should be noted that **11** is a diastereoisomeric mixture.

These carbohydrate variants therefore showed markedly differing μ1 and μ2 affinities compared to morphine, but only the glucoside **3,** which had improved μ1 affinity with similar μ2 affinity, retained the in vivo activity of morphine. It has previously been suggested that the reduced toxicity of M6G, which retains potent analgesic activity in vivo, is attributable to a decreased μ2 affinity compared to morphine.[4] The markedly decreased activity of compounds **6–11**, which all had substantially reduced μ2 affinities compared to **1** or **2**, was therefore

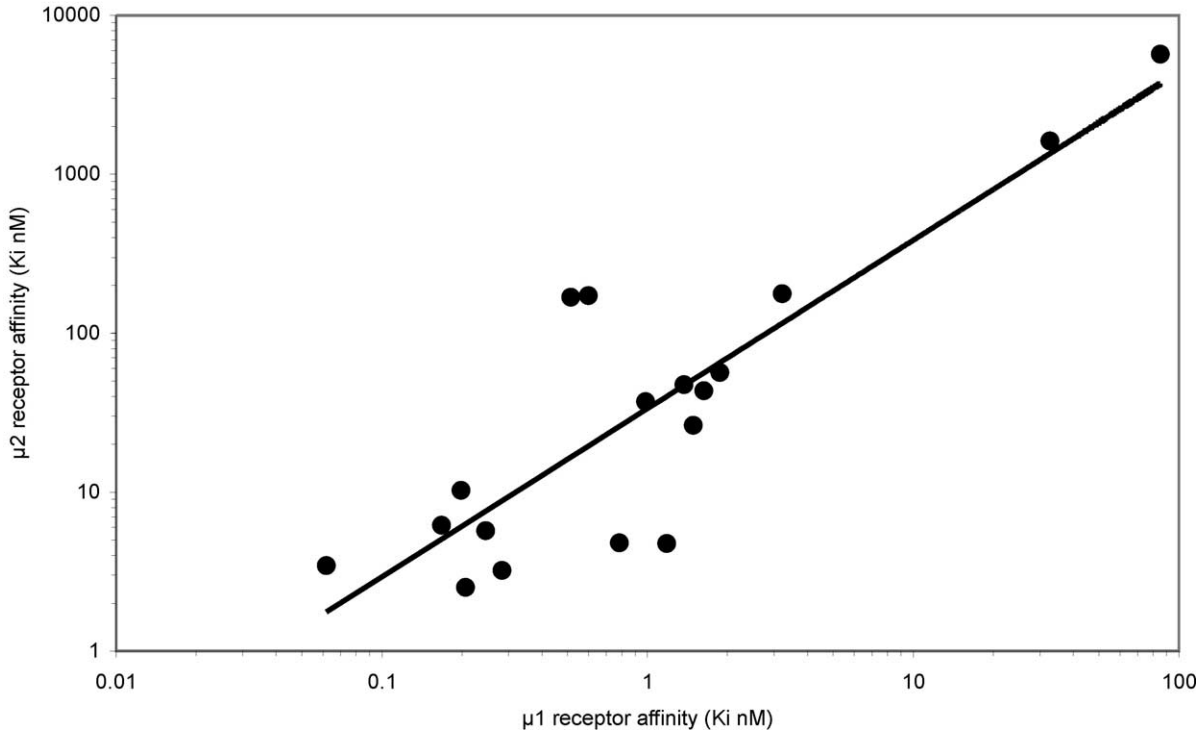


Figure 3. Relationship between μ1 and μ2 receptor affinity. Solid line is the best fit by linear regression analysis ($r = 0.86$).

disappointing. The lack of antinociceptive activity of several of these carbohydrate variants may have been due, at least in part, to poor penetration into the CNS, rapid metabolism or potential antagonistic activity, factors that were outside the remit of this study.

Codeine analogue

Compound **12**, codeine-6-glucuronide,[1] showed very low affinity for either receptor subtype, in keeping with the known requirement for a free phenolic group. Because of its low receptor affinity it was not tested in the in vivo model.

N-Substituted variants

Nor-M6G, compound **13**, has been reported before.[20] It has similar affinity for the μ1 receptor compared to morphine, but reduced μ2 affinity. Overall, the receptor binding of this compound was in line with that of M6G, and the in vivo activity was also similar to M6G. Compound **14**, nalorphine-6-glucuronide,[22] was strongly bound to both μ-receptor subtypes while showing no antinociception, in line with the known opioid antagonistic properties of nalorphine. Compound **15**, bearing a polar *N*-substituent (carboxymethyl) was inactive in vivo, and had very low affinity for both receptor subtypes. In contrast the *N*-cyclopropylmethyl analogue **16** showed the highest μ1 affinity of any compound studied, along with high μ2 affinity, but was also inactive in vivo. Although consistent with the known antagonistic activity of the corresponding *N*-substituted morphine variants, this assertion cannot be supported by the current data.

7,8-Dihydro analogues

This group of analogues showed the highest antinociceptive activity, in line with the known potency of 7,8-dihydromorphine.[23] Compound **17**, 7,8-dihydro M6G, showed high affinity for both receptor subtypes, particularly the μ1 site, and potent in vivo activity. Analogues **18** and **19**, namely 7,8-dihydro **3** and 7,8-dihydro **13**, respectively, were also more potent in vivo than their unsaturated counterparts.

In conclusion, these data show that modification of the carbohydrate at the 6-position of morphine has marked effects on binding to MOP receptor subtypes, and on the in vivo antinociceptive activity of the compounds. The data presented suggest a requirement for a glucuronide or glucoside moiety at the 6-position for potent in vivo activity, and saturation of the 7,8 double bond.

Acknowledgements

We are grateful to Zoe Gaffen, King's College, London for skilled experimental assistance.

References and Notes

1. Yoshimura, H.; Oguri, K.; Tsukamoto, H. *Chem. Pharm. Bull.* **1968**, *16*, 2114.
2. Osborne, R. J.; Joel, S. P.; Trew, D.; Slevin, M. *Lancet* **1988**, *i*, 828.
3. Osborne, R. J.; Thompson, P. I.; Joel, S. P.; Trew, D.; Patel, N.; Slevin, M. *Br. J. Clin. Pharmacol.* **1992**, *34*, 130.

4. Hucks, D.; Thompson, P. I.; McLoughlin, L.; Joel, S. P.; Patel, N.; Grossman, A.; Rees, L. H.; Slevin, M. L. *Br. J. Cancer* **1992**, *65*, 122.
5. Scheinmann, F.; Lumbard, K. W.; Brown, R. T.; Mayalarp, S. P. Process for Making Morphine-6-Glucuronide or Substituted Morphine-6-Glucuronide. International Patent, WO 93/3051, 1993.
6. Brown, R. T.; Carter, N. E.; Scheinmann, F.; Turner, N. J. *Tetrahedron Lett*. **1995**, *36*, 1117.
7. Jenkins, G. N.; Stachulski, A. V.; Scheinmann, F.; Turner, N. J. *Tetrahedron: Asymmetry* **2000**, *11*, 413.
8. Joel, S. P.; Stachulski, A. V.; Scheinmann, F. Morphine Derivatives. PCT 1998, GB 98/01071.
9. Kieffer, B. L. *Trends Pharmacol. Sci*. **1999**, *20*, 19.
10. Pasternak, G. W. *Trends Pharmacol. Sci*. **2001**, *22*, 67.
11. Walker, J. R.; King, M.; Izzo, E.; Koob, G. F.; Pasternak, G. W. *Eur. J. Pharmacol*. **1999**, *27*, 115.
12. All new compounds submitted for receptor binding and in vivo evaluation, namely nos. **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **15**, **16**, **18**, **19** were characterised by ^{1}H NMR spectroscopy at 300 MHz, mass spectroscopy (MS) and elemental analysis or high-resolution MS and were of at least 97% purity as measured by peak area on reverse-phase analytical HPLC (C_{18} μ-bondapak column, acetonitrile-water + 0.1% trifluoroacetic acid as the mobile phase).
13. Kovac, P.; Rice, K. C. *Heterocycles* **1995**, *41*, 697.
14. Wieczorek, E.; Theim, J. *Synlett*. **1998**, 467. Fehlhaber, H.-W.; Snatzke, G.; Vlahov, I. *Liebigs. Ann. Chem*. **1987**, 637.
15. Ferrier, R. J.; Prasad, N. *J. Chem. Soc. (C)* **1969**, 570.
16. Mayalarp, S. P. PhD thesis, University of Manchester, 1993.
17. Beigelman, L. N.; Mikhailov, S. N. *Carbohydr. Res*. **1990**, *203*, 324.
18. Blattner, R.; Ferrier, R. J.; Tyler, P. C. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1535.
19. Chiba, T.; Sinay, P. *Carbohydr. Res*. **1986**, *151*, 379.
20. Oguri, K.; Kuo, C. K.; Yoshimura, H. *Chem. Pharm. Bull*. **1989**, *37*, 955.
21. Olofson, R. A. *Pure Appl. Chem*. **1988**, *60*, 1715.
22. Yoshimura, H.; Mori, M.; Oguri, K. *Chem. Pharm. Bull*. **1970**, *18*, 2548.
23. Thorpe, D. A. *Anesth. Analg*. **1984**, *63*, 143.
24. Casy, A. F.; Parfitt, R. T. *'Opioid Analgesics'*; Plenum Press, 1986.
25. A direct competitive experiment against **1** and **2** would be necessary to prove antagonism for this and other suspected antagonists.