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Synthesis and Nicotinic Binding of Novel Phenyl Derivatives of UB-165. Identifying Factors Associated with α7 Selectivity

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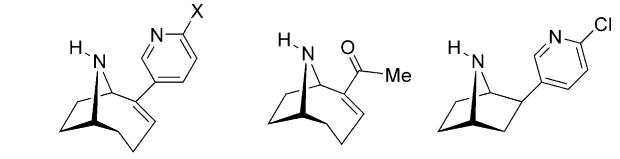
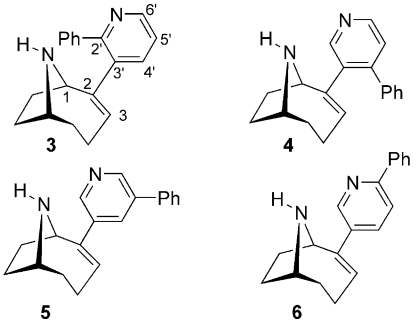
Abstract—Four racemic phenyl-substituted analogues **3**–**6** of the potent nicotinic agonist UB-165 **1** have been synthesised and evaluated against the $\alpha_4\beta_2$, $\alpha_3\beta_4$, and α_7 neuronal nicotinic receptors. The 2′-phenyl derivative **3** shows no activity at these major receptor subtypes, while the 4′-phenyl analogue **4** shows an enhanced level of α_7 selectivity as compared to UB-165 and deschloro UB-165 **2**. These results are discussed within the context of recent pharmacophore models.

Neuronal nicotinic acetylcholine receptors (nAChRs) are intimately involved in a diverse range of important CNS functions and are also linked to a variety of clinically significant disease states.[1–6] A role for α4-containing nAChRs in the control of pain has recently provided further incentive for the characterisation of individual receptors via subtype selective ligands.[7] Such molecular entities would facilitate elucidation of the relationship between receptor subtypes and function and, in turn, generate useful leads for drug discovery.[8,9] UB-165 **1**, a hybrid ligand based on combining structural elements derived from anatoxin-a and epibatidine, is a potent nicotinic agonist but shows (like anatoxin-a and epibatidine) limited discrimination between $\alpha_4\beta_2$, $\alpha_3\beta_4$ and α_7 receptor subtypes.[10,11]

Recently, UB-165 **1** and the deschloro variant 2 have been the subject of two independent structure–activity relationship and pharmacophore studies, with the 2′- and 4′-pyridyl analogues, together with a series of diazine variants, having been prepared and evaluated.[12,13] It is clear that the 3′-pyridyl moiety (as in **1** and **2**), either alone or embedded within a diazine unit, is critical for binding and agonist activity. Further substitution of the 3′-pyridyl unit of **1** or **2** has not, however, been examined, but additional interactions associated with this region of the ligand would provide an important opportunity to achieve enhanced subtype selectivity. In this paper, we disclose the synthesis of a series of racemic phenyl-substituted 3′-pyridyl derivatives **3**–**6** of UB-165, and the ability of these novel ligands to bind to $\alpha_4\beta_2$, $\alpha_3\beta_4$ and α_7 nAChR subtypes is defined. The results obtained reinforce some recent suggestions relating to nicotinic pharmacophores and point towards structural factors that may contribute to selectivity for the α_7 subtype.





The 2′-phenyl, 4′-phenyl and 6′-phenyl analogues **3**, **4**, and **6**, respectively, were prepared by means of a key Negishi coupling reaction using the versatile enol triflate

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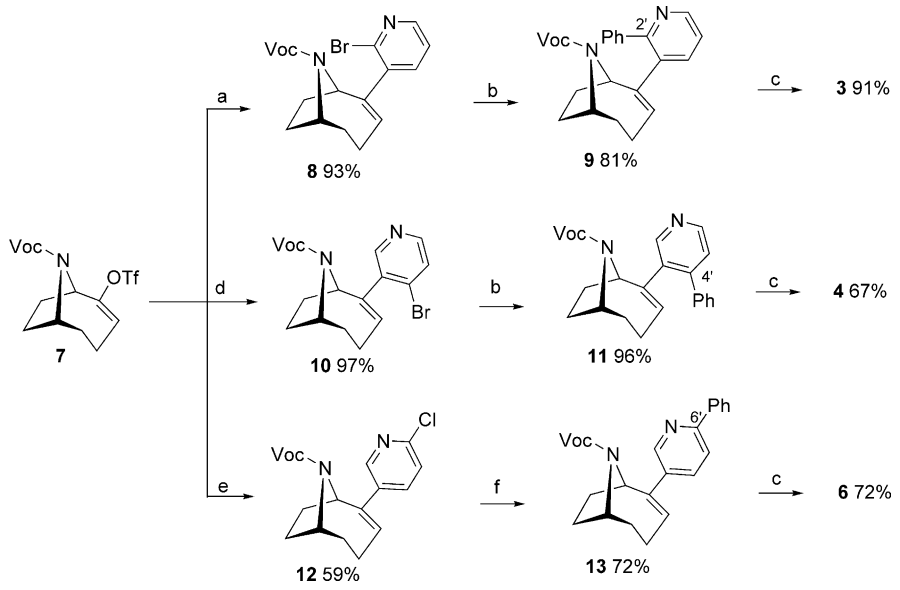


Scheme 1. Reagents and conditions: (a) (i) 2-bromopyridine, LDA, THF, −78 °C, 30 min; (ii) $ZnCl_2$, −78 °C to rt; (iii) $Pd(PPh_3)_4$, **7**, reflux; (b) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, PhMe, EtOH, reflux; (c) (i) concd HCl, 1,4-dioxane, water, reflux; (ii) Boc_2O, Et_3N, THF, water; (iii) 2 M HCl, 1,4-dioxane; (d) (i) 4-bromopyridine, LDA, THF, −78 °C, 30 min; (ii) $ZnCl_2$, −78 °C to rt; (iii) $Pd(PPh_3)_4$, **7**, reflux; (e) (i) 2-chloro-5-iodopyridine, BuLi, THF, −78 °C; (ii) $ZnCl_2$, −78 °C to rt; (iii) $Pd(PPh_3)_4$, **7**, reflux; (f) $PhB(OH)_2$, $Pd(PPh_3)_4$, DME, K_2CO_3, reflux.

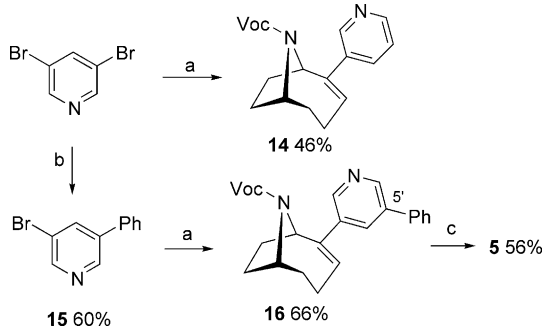
7 as outlined in Scheme 1.[10] The basic strategy followed is illustrated for the preparation of the 2′-phenyl analogue **3**. Halide-directed lithiation[14] of 2-bromopyridine followed by transmetallation with $ZnCl_2$ and Negishi cross coupling with enol triflate **7** gave the 2′-bromopyridyl derivative **8** in 93% yield.[15] Reaction of **8** with phenylboronic acid under Suzuki conditions gave the 2′-phenylpyridyl derivative **9** in 81% yield, and removal of the vinyloxycarbonyl (Voc) protecting group was then carried out using a three step deprotection strategy[10,12] (via the Boc intermediate) to give the target compound **3** as the hydrochloride salt, in 91% yield.

Application of the directed deprotonation-transmetallation strategy[16] to 4-bromopyridine, Negishi coupling with **7**, subsequent Suzuki coupling of **10** and deprotection of adduct **11** gave the 4′-phenylpyridyl analogue **4**. The synthesis of **6** involved iodine-lithium exchange of 2-chloro-5-iodopyridine, followed by transmetallation with $ZnCl_2$ and Negishi coupling to give the 6′-chloro-3′-pyridyl adduct **12** in 59% yield. A modified Suzuki coupling with **12** to phenylboronic acid gave the *N*-Voc derivative **13** which was deprotected (as above) to give the 6′-phenylpyridyl analogue **6**. This chemistry all proceeds efficiently and the multistep deprotection protocol (*N*-Voc to *N*-Boc to *N*-H) was used because we have found it most convenient to purify at the *N*-Boc stage. In our hands, *N*-Boc cleavage is cleaner and more efficient than *N*-Voc cleavage.

We had intended to prepare the 5-phenyl analogue **5** via monometallation and Negishi coupling of 3,5-dibromopyridine with enol triflate **7**, then Suzuki coupling to incorporate the phenyl moiety. However, bromine–lithium exchange of 3,5-dibromopyridine followed by transmetallation with $ZnCl_2$ and coupling with the enol triflate **7** gave only the desbromo coupled product **14** in 46% yield (Scheme 2). This problem was circumvented by incorporation of the phenyl moiety in the first step to give **15** in 60% yield. Conversion of **15** to the corresponding organozinc species followed by reaction with enol triflate **7** under Negishi conditions gave the desired adduct **16** in 66% yield. Deprotection of **16** (as described above) gave the 5′-phenylpyridyl analogue **5** in 56% yield over three steps.

The four phenyl-substituted UB-165 analogues **3–6** (as their hydrochloride salts) were evaluated for binding to three major nAChR subtypes present in the CNS and the peripheral nervous system. These comprised the rat brain $\alpha_4\beta_2$ and α_7 subtypes which were labelled by [^{3}H]nicotine and [^{3}H]MLA, respectively, and the rat $\alpha_3\beta_4$ nAChR expressed in a stably transfected cell line and labelled by [^{3}H]epibatidine.[12,17] The results of these binding studies along with those for UB-165 **1** and the deschloro variant **2** are shown in Table 1.

It should also be appreciated that loss of the chlorine has little effect on the affinity of UB-165 for these receptors; UB-165 **1** and the deschloro analogue **2**



Scheme 2. Reagents and conditions: (a) (i) BuLi, THF, −78 °C, 30 min; (ii) $ZnCl_2$, −78 °C to rt; (iii) $Pd(PPh_3)_4$, **7**, reflux; (b) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, PhMe, EtOH, reflux; (c) (i) concd HCl, 1,4-dioxane, water, reflux; (ii) Boc_2O, Et_3N, THF, water; (iii) 2 M HCl, 1,4-dioxane.

Table 1. Binding affinities for UB-165 and analogues (note compounds **1**–**6** are racemic)[a]

Compd	nAChR subtype		
	K_i (nM)		
	$\alpha_4\beta_2$	$\alpha_3\beta_4$	α_7
UB-165 **1**	0.27	6.5	2760
Deschloro UB-165 **2**	0.43	22.5	89
2′-Ph **3**	> 50,000	> 50,000	> 50,000
4′-Ph **4**	234	1990	927
5′-Ph **5**	4.8	468	15,980
6′-Ph **6**	21.4	56.3	1306

[a]Values are the means from at least 3 independent assays.

(which is more closely related to ligands **3**–**6**) have similar profiles. Four significant observations can be made regarding the data for the phenyl-substituted ligands shown in Table 1. Firstly, incorporation of a phenyl substituent at the 2′-position (as in **3**) results in a complete loss of nicotinic potency against all three receptors subtypes. Secondly, steric bulk associated with the phenyl substituent does reduce potency compared with **2**, but clearly also makes an impact on selectivity. Thirdly, phenyl substitution at the 5′- and 6′-positions (**5** and **6**, respectively) results in profiles that are qualitatively similar to deschloro UB-165 **2**: potency at $\alpha_4\beta_2 > \alpha_3\beta_4 > \alpha_7$. However, it should be pointed out that **6** in particular is less capable than deschloro UB-165 **2** of discriminating between these three receptor subtypes.

The final and, in our view, most interesting observation relates to the 4′-phenyl analogue **4**. This ligand, while substantially (2 orders of magnitude) less potent than UB-165 **1** and deschloro UB-165 **2** at $\alpha_4\beta_2$ and $\alpha_3\beta_4$ subtypes, retains a comparatively high potency at the α_7 receptor. Analogue **4** is only 8.5 times more potent at $\alpha_4\beta_2$ than at $\alpha_3\beta_4$, whereas deschloro UB-165 is 52 times more potent, respectively. However, at α_7 **4** is only four times less potent than at $\alpha_4\beta_2$, whereas deschloro UB-165 is 200 times less potent at α_7. Interestingly, UB-165 **1** shows a binding differential $> 10^4$ times between these two receptor subtypes.

Nicotinic pharmacophores continue to attract significant interest, with important contributions being made from a number of groups.[6,18–22] Given our earlier studies,[12] it is pertinent to consider these biological results alongside the pharmacophore model recently described by Tønder et al.,[18,19] the key conformational features of which are shown in schematic form in Figure 1.

In this model, which was developed for the $\alpha_4\beta_2$* nAChR, the bioactive conformation of deschoro UB-165 **2** has the C(1)–C(2)–C(3′)–C(2′) in a cisoid arrangement with the pyridyl nitrogen (N(1′)) above the C(1)–C(2)–C(3) plane; the C(1)–C(2)–C(3′)–C(2′) dihedral angle is approximately 55° (cf. Fig. 1).[23] In this conformation, the 2′-phenyl substitutent in **3** (a ligand which completely lacks nicotinic activity) is located in very close proximity to the N(9) ammonium center. This

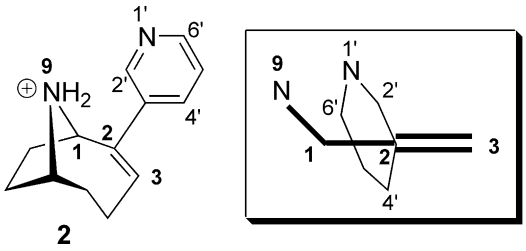


Figure 1. Schematic representation of the $\alpha_4\beta_2$* nAChR pharmacophore. The structure in the box is a view down the C(2)-C(3′) bond.

arrangement might be expected to inhibit the interaction of this key pharmacophore component with its associated receptor binding site and this would explain the lack of nicotinic activity associated with **3**. Phenyl substitution at the 5′- and 6′-positions does not have a major impact on subtype selectivity although as noted above, some moderation of binding potency is associated with substitution of these sites (see Table 1). The 4′-phenyl derivative **4** is well able to attain the conformation associated with the Tønder pharmacophore model and the substituent does not impact on the ability of the N(9) ammonium center to interact with the receptor. The retention of affinity at α_7 (but not $\alpha_4\beta_2$ and $\alpha_3\beta_4$) that is observed in this case may indicate that the 4′-phenyl substituent of **4** occupies a region of space relative to the other key components of the ligand structure that could usefully be exploited for enhancing selectivity for this nAChR subtype, and studies to test this hypothesis are underway.[24]

In conclusion, we have synthesised the four possible phenyl-substituted variants of UB-165. Results of binding studies are consistent with the recent suggestion that the conformation represented in Figure 1 is important for an effective nicotinic ligand and this is further supported by the observation that the 2′-phenyl isomer **3** shows the most dramatic loss in potency. The 4′-phenyl derivative **4** shows enhanced α_7 selectivity and this may provide a means of refining and extending current models to derive a useful template specific for α_7 ligands.

Acknowledgements

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References and Notes

1. Role, L. W.; Berg, D. K. *Neuron*. **1996**, *16*, 1077.
2. Lindstrom, J. *Mol. Neurobiol*. **1997**, *15*, 193.
3. Arneric, S. P., Brioni, J. D., Eds. *Neuronal Nicotinic Receptors*. John Wiley and Sons: New York, 1999.
4. Jones, S.; Sudweeks, S.; Yakel, J. L. *Trends Neurosci*. **1999**, *22*, 555.
5. Paterson, D.; Nordberg, A. *Prog. Neurobiol*. **2000**, *61*, 75.
6. Curtis, L.; Chiodini, F.; Spang, J. E.; Bertrand, S.; Patt, J. T.; Westera, G.; Bertrand, D. *Eur. J. Pharmacol*. **2000**, *393*, 155.

7. Bannon, A. W.; Decker, M. W.; Holladay, M. W.; Curzon, P.; Donnelly-Roberts, D.; Puttfarcken, P. S.; Bitner, R. S.; Diaz, A.; Dickenson, A. H.; Porsolt, R. D.; Williams, M.; Arneric, S. P. *Science* **1998**, *279*, 77.
8. Holladay, M. W.; Dart, M. J.; Lynch, J. K. *J. Med. Chem.* **1997**, *40*, 4169.
9. Lloyd, G. K.; Williams, M. *J. Pharmacol. Exp. Ther.* **2000**, *292*, 461.
10. Wright, E.; Gallagher, T.; Sharples, C. G. V.; Wonnacott, S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2867.
11. Sharples, C. G. V.; Kaiser, S.; Soliakov, L.; Marks, M. J.; Collins, A. C.; Washburn, M.; Wright, E.; Spencer, J. A.; Gallagher, T.; Whiteaker, P.; Wonnacott, S. *J. Neurosci.* **2000**, *20*, 2783.
12. Sharples, C. G. V.; Karig, G.; Simpson, G. L.; Spencer, J. A.; Wright, E.; Millar, N. S.; Wonnacott, S.; Gallagher, T. *J. Med. Chem.* **2002**, *45*, 3235.
13. Gohlke, H.; Gundisch, D.; Schwarz, S.; Seitz, G.; Tilotta, M. C.; Wegge, T. *J. Med. Chem.* **2002**, *45*, 1064.
14. Gribble, G. W.; Saulnier, M. G. *Tetrahedron Lett.* **1980**, *21*, 4137.
15. Karig, G.; Spencer, J. A.; Gallagher, T. *Org. Lett.* **2001**, *3*, 835.
16. Karig, G.; Thasana, N.; Gallagher, T. *Synlett.* **2002**, 808.
17. Lewis, T. M.; Harkness, P. C.; Sivilotti, L. G.; Colquhoun, D.; Millar, N. S. *J. Physiol.* **1997**, *505*, 299.
18. Tønder, J. E.; Olesen, P. H.; Hansen, J. B.; Begtrup, M.; Pettersson, I *J. Comput.-Aided Mol. Des.* **2001**, *15*, 247.
19. Tønder, J. E.; Olesen, P. H. *Curr. Med. Chem.* **2001**, *8*, 651.
20. Dukat, M.; Damaj, M. I.; Glassco, W.; Dumas, D.; May, E. L.; Martin, B. R.; Glennon, R. A. *Med. Chem. Res.* **1993**, *4*, 131.
21. Glennon, R. A.; Herndon, J. A.; Dukat, M. *Med. Chem. Res.* **1994**, *6*, 465.
22. Meyer, M. D.; Decker, M. W.; Rueter, L. E.; Anderson, D. J.; Dart, M. J.; Kim, K. H.; Sullivan, J. P.; Williams, M. *Eur. J. Pharmacol.* **2000**, *393*, 171.
23. The conformations associated with ligands **3**–**6** are based on the computational methods and results obtained earlier.[12]
24. The quinoline analogue of **2**, which was prepared by essentially the same strategy as shown in Scheme 1, shows binding affinity values (nM) of 140, 2535 and $>50{,}000$ for $\alpha_4\beta_2$, $\alpha_3\beta_4$, and α_7, respectively. This would suggest that too much rigidity associated with substituents in the 5′ and 6′-region blocks α_7 recognition. Glennon[25] has reported binding data for 6-phenyl nicotine and the corresponding homoazanicotine analogue (cf. ligand **6**), both of which show low affinity for the α4β2 nAChR. The corresponding quinolines show somewhat increased levels of potency.
25. Ferretti, G.; Dukat, M.; Giannella, M.; Piergentili, A.; Pigini, M.; Quaglia, W.; Damaj, M. I.; Martin, B. R.; Glennon, R. A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 733.