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# 4-ALKYL- AND 3,4-DIALKYL-1,2,3,4-TETRAHYDRO-8-PYRIDONO[5,6-*g*]QUINOLINES: POTENT, NONSTEROIDAL ANDROGEN RECEPTOR AGONISTS

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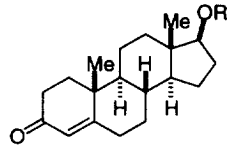
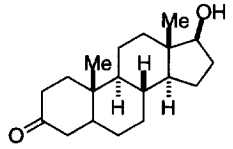
**Abstract:** A series of human androgen receptor (hAR) agonists based on 4-alkyl-; 4,4-dialkyl-; and 3,4-dialkyl-1,2,3,4-tetrahydro-8-pyridono[5,6-*g*]quinoline was synthesized and evaluated in competitive receptor binding assays and an androgen receptor cotransfection assay in a mammalian cell background. A number of compounds in this series demonstrated activity equal to or better than dihydrotestosterone in both assays and represent a novel class of compounds for use in androgen replacement therapy. 

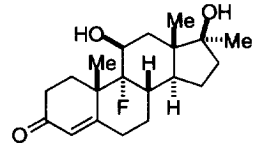
**Introduction.** The androgens testosterone (**T**) and dihydrotestosterone (**DHT**) play important roles in male sexual development and function,[5] and musculo-skeletal growth.[5,6] Since **T** has poor oral pharmacokinetic (PK) properties, androgen deficiency is frequently treated with transdermal T patches, or intramuscular (IM) injections of **T** esters (**1**). However, patches frequently cause severe skin irritation, and IM injections result in severe fluctuations in **T** blood levels.




R = H, Testosterone (**T**)
R = C(O)$CH_2$cyclopentyl (**1**)



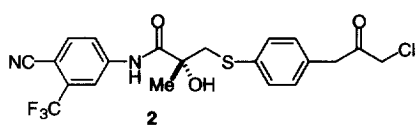

Dihydrotestosterone (**DHT**)




Fluoxymesterone

**Figure 1. Steroidal Androgen Agonists**

Alkylation of androgens at C-17, such as in fluoxymesterone (Figure 1), results in AR agonists with a PK profile suitable for oral administration, but compounds in this class are associated with liver toxicity.[7] This suggests that an androgen suitable for oral dosing and with an acceptable therapeutic index represents an unmet clinical need.

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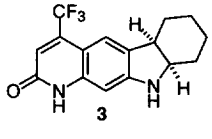


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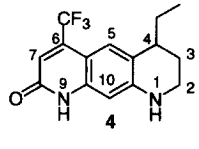


**4**

**Figure 2. Nonsteroidal Androgen Agonists**


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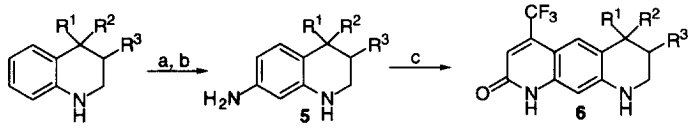
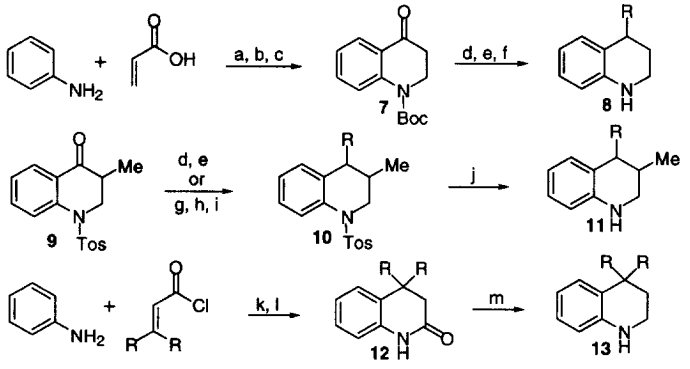
Inroads to nonsteroidal androgen receptor agonists have been rare. Recently, compounds with full efficacy in cotransfection assays were reported (**2**, Figure 2).[8] AR agonists based on 4-(trifluoromethyl)-2(1*H*)-pyrrolidino[3,2-*g*]quinolinone (**3**, Figure 2)[9] and 1,2,3,4-tetrahydro-6-(trifluoromethyl)-**8**-pyridono[5,6-*g*]quinoline (**4**)[10] have shown excellent potency in cotransfection and binding assays. In addition, **4** demonstrates oral in vivo activity (suppression of leutinizing hormone in a castrated rat),[11] indicating that clinical opportunities may be close at hand. In this Letter, we report that the 4-alkyl-, and 3,4-dialkyl analogues based on **4** show excellent potency and efficacy in cell-based in vitro assays.

**Chemistry.** The appropriately substituted 1,2,3,4-tetrahydroquinolines were conveniently manipulated to the desired targets as shown in Scheme 1. A standard protocol of nitration, catalytic hydrogenation, and modified Knorr cyclization with ethyl 4,4,4-trifluoroacetoacetate afforded the desired 4-alkyl-1,2,3,4-tetrahydro-6-(trifluoromethyl)-8-pyridono[5,6-*g*]quinolines (**6**) in 33-40% overall yields for the three steps.[12]




**Scheme 1.** (a) 90% $HNO_3$, $H_2SO_4$, 0°C; (b) $H_2$, Pd-C, EtOH; (c) ethyl 4,4,4-trifluoroacetoacetate, $ZnCl_2$, EtOH, reflux.

The nitrogen positions can be systematically functionalized. Treatment of **4** with excess paraformaldehyde and $NaBH_3CN$ in AcOH afforded the *N*-1-methyl derivative **4a**. Treatment of **4** with stoichiometric NaH and MeI in THF afforded the *N*-9 methyl derivative **4b**, while treatment with excess reagents led to the 1,9-dimethyl derivative **4c**. The *N*-1-formyl derivative **4d** was prepared by treatment of **4** with $Ac_2O$ in formic acid.

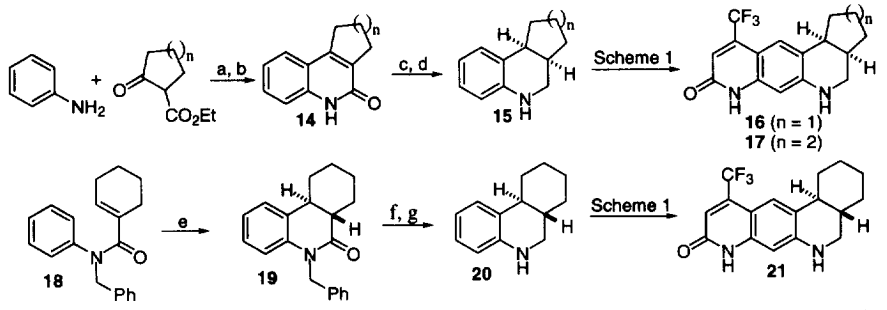



**Scheme 2.** (a) toluene, 100° C; (b) polyphosphoric acid, 100 °C; (c) $Boc_2O$, DMAP, THF; (d) RMgBr, RLi, or $RCeCl_2$, THF, 0° C; (e) $H_2$, Pd/C, TFA (cat.), EtOAc, EtOH; (f) TFA; (g) vinyl magnesium bromide, THF (h) $H_2$, Pd/C (i) $Et_3SiH$, TFA, reflux; (j) conc. HCl/HOAc, 100 °C; (k) $Et_3N$, $CH_2Cl_2$; (l) $AlCl_3$, $CH_2Cl_2$, reflux; (m) borane-dimethylsulfide complex, THF, reflux.

4-Substituted tetrahydroquinolines could be obtained by the route shown in Scheme 2. Michael addition of aniline to acrylic acid, followed by cyclization with polyphosphoric acid and subsequent Boc-protection afforded 4-quinolone **7** in 30% overall yield. Grignard, organolithium, or organocerium addition to the ketone followed by benzylic alcohol reduction ($H_2$, Pd-C, cat. TFA) and Boc hydrolysis (TFA) afforded tetrahydroquinolines **8**, which in turn were carried on to the desired targets as described in Scheme 1. All compounds were prepared and evaluated as racemates.

The 3,4-dialkylated derivatives were prepared in a similar manner (Scheme 2). Grignard or organolithium addition and reduction of **9**[13] afforded **10** as a mixture of diastereomers. Alternatively, the 4-ethyl version of **10** was prepared by the addition of vinyl magnesium bromide, hydrogenation, and reduction with $Et_3SiH$/TFA. The sulfonamide was cleaved under acidic conditions (HCl/HOAc) to afford **11**, which was carried on as described above to afford 3,4-dialkyl-1,2,3,4-tetrahydro-6-(trifluoromethyl)-8-pyridono[5,6-*g*]quinolines.[14] The desired compounds were obtained as a mixture of diastereomers, and were separated by reverse phase HPLC (MeOH:water, ODS semiprep column). Assignment of relative configuration was determined by NOE difference experiments.

The 4,4-disubstituted derivatives **13** were constructed by amide formation of aniline and a 3,3-dialkylated acryloyl chloride, followed by a Friedel-Crafts alkylation mediated by $AlCl_3$ to afford quinolone **12** (Scheme 2). Borane reduction led to **13**, then manipulation of **13** as described above led to the target compounds **6**.




**Scheme 3.** (a) neat, 130 °C; (b) conc. $H_2SO_4$, 100 °C; (c) $POCl_3$, reflux; (d) $NiCl_2$ hexahydrate, $NaBH_4$, MeOH; (e) hv, ether; (f) LAH, ether, 0 °C to reflux; (g) $H_2$, $Pd(OH)_2$, EtOH, 50°C.

Tetracyclic analogues were obtained by a Knorr cyclization[15] of aniline onto a cycloalkanonecarboxylate afforded **14** in low yields (7-10%). Transformation to the chloropyridine ($POCl_3$) followed by exhaustive reduction ($NiCl_2/NaBH_4$)[16] afforded the *cis*-fused compounds **15**. The preparation of the *trans*-3,4-cyclohexano-derivative was realized by photocycloaddition of **18** to afford **19**.[17] Reduction of the amide with LAH, followed by debenzylation [$Pd(OH)_2$, $H_2$] led to **20**. Conversion to the target compounds for both tetrahydroquinoline derivatives proceeded uneventfully as described above.

**Results and Discussion:** The compounds were evaluated in cotransfection assays with hAR in a mammalian cell background (CV-1) as previously described.[10,12] Whole-cell binding studies were performed in mammalian (COS) cells using $^{3}$H-**DHT** as a standard in competition experiments. Modifications at the 4-position of **6** indicated that this region has a strong impact on agonist activity. A study of carbon chains indicate that ethyl (**4**, $EC_{50}$ = 4 nM, 100% efficacy, $K_i$ = 7 nM) and isopropyl (**6d**, $EC_{50}$ = 3 nM, 81% efficacy, $K_i$ = 13 nM) were optimal for agonist activity (Table 1). Smaller substituents, such as methyl (**6b**), or longer ones, such as propyl (**6c**) and isobutyl (**6e**), resulted in compounds with reduced potency and efficacy.

**Table 1. hAR Cotransfection and Binding Data**

| | | | | hAR Cotransfection Assay in CV-1 Cells[a,d] | | | | hAR Binding[a,e] |
|---|---|---|---|---|---|---|---|---|
| | | | | Agonist[b] | | Antagonist[b] | | |
| # | $R^1$ | $R^2$ | $R^3$ | Eff(%) | $EC_{50}$ (nM)[c] | Eff(%) | $IC_{50}$ (nM)[c] | $K_i$ (nM) |
| DHT | - | - | - | 100 ± 0 | 6 ± 1 | -- | -- | 2 ± 0.4 |
| **4** | - | - | - | 100 ± 7 | 4 ± 1 | 20 ± 10 | 4580 ± 2030 | 7 ± 1 |
| **4a** | *N*-1-methyl | | | 74 ± 8 | 158 ± 34 | -- | -- | 630 ± 150 |
| **4b** | *N*-9-methyl | | | -- | -- | 45 ± 11 | 20 ± 1 | 9 ± 1 |
| **4c** | 1,9-dimethyl | | | -- | -- | 83 ± 1 | 216 ± 61 | 263 ± 53 |
| **4d** | *N*-1-formyl | | | 72 ± 20* | 137 ± 36 | -- | -- | -- |
| **6a** | H | H | H | 35 ± 2 | 317 ± 131 | 44 ± 7 | 12 ± 5 | 19 ± 5* |
| **6b** | Me | H | H | 48 ± 2 | 15 ± 6 | 16 ± 8 | 259 ± 104 | 9 ± 3 |
| **6c** | Pr | H | H | 63 ± 7 | 19 ± 4 | -- | -- | 29 ± 9 |
| **6d** | *i*-Pr | H | H | 81 ± 5 | 3 ± 0 | -- | -- | 13 ± 3 |
| **6e** | *i*-Bu | H | H | 61 ± 7 | 25 ± 5 | -- | -- | 22 |
| **6f** | Ar[f] | H | H | -- | -- | 66 ± 5 | 251 ± 67 | -- |
| **6g** | Me | Me | H | 36 ± 8 | 68 ± 5 | 39 ± 9 | 16 ± 3 | 12 ± 4* |
| **6h** | Et | Et | H | -- | -- | 73 ± 2 | 73 ± 6 | 58 |
| **6i** | Me | H | *trans*-Me | 92 ± 12 | 10 ± 2 | -- | -- | 16 ± 5 |
| **6j** | Me | H | *cis*-Me | 62 ± 11 | 22 ± 3 | -- | -- | 8 ± 2 |
| **6k** | Et | H | *trans*-Me | 106 ± 10 | 7 ± 2 | 13 ± 8 | 2710 ± 580 | 8 |
| **6l** | Et | H | *cis*-Me | 89 ± 8 | 7 ± 3 | -- | -- | 27 |
| **6m** | TFP[g] | H | *trans*-Me | 12 ± 7 | 13 ± 8 | 72 ± 5 | 61 ± 18 | 2 |
| **6n** | TFP | H | *cis*-Me | 22 ± 4 | 82 ± 5 | 47 ± 9 | 63 ± 30 | -- |
| **6o** | *i*-Bu | H | *trans*-Me | 25 ± 5 | 56 ± 10 | 55 ± 4 | 29 ± 18 | 72 |
| **16** | *-cis*-$(CH_2)_3$- | | | 56 ± 3 | 9 ± 3 | -- | -- | 8 |
| **17** | *-cis*-$(CH_2)_4$- | | | 107 ± 6 | 3 ± 1 | -- | -- | 2 |
| **21** | *-trans*-$(CH_2)_4$- | | | 86 ± 7 | 2 ± 0 | -- | -- | 15 |

[a] Values with standard errors (SEM) represent the mean value of at least three separate experiments with triplicate determinations; values without standard deviation represent a single experiment and values with * represent the mean value of two experiments with standard deviation. [b] Agonist efficacies were compared to that of dihydrotestosterone (100%) and antagonist efficacies were determined as a function (%) of maximal inhibition of dihydrotestosterone ($EC_{50}$). [c] All $EC_{50}$ and $IC_{50}$ values were determined from full dose-response curves ranging from $10^{-12}$ to $10^{-5}$. [d] A "--" indicates an efficacy < 10% or a potency >10000 nM. [e] The radioligand used in the competitive binding assay was $^{3}$H-DHT. A "--" indicates a $K_i$ > 1000. [f] Ar = 3,5-bis-(trifluoromethyl)phenyl. [g] TFP = 3,3,3-trifluoropropyl.

The *N*-1-methyl- and *N*-1-formyl derivatives (**4a** and **4d**, respectively) showed dramatically reduced agonist activity. Methylation at *N*-9 resulted in total loss of agonist activity, as both the *N*-9-methyl derivative **4b** and the 1,9-dimethyl derivative **4c** were antagonists. Consequently, the free N-H derivatives were generally pursued. To address whether geminal substitution at the 4-position could be beneficial, the 4,4-diethyl derivative **6h** was prepared and was shown to be an antagonist ($IC_{50}$ = 73 nM, 70% efficacy). The corresponding dimethyl derivative **6g** showed modest partial agonist activity, but neither compound demonstrated the potency of the monosubstituted derivatives. All of the compounds in this series, with the exception of the three analogues functionalized at *N*-1 (**4a**, **4b**, and **4d**) exhibited good to excellent binding affinity for AR (Table 1).

The 3,4-disubstituted derivatives generated a panel of potent AR agonists. In the acyclic cases, the 4-ethyl-3-methyl analogues **6k** (*trans*) and **6l** (*cis*) showed excellent activity ($EC_{50}$ = 7 nM, 106% efficacy and 7 nM, 89% efficacy, respectively). Interestingly, the *trans*-3,4-dimethyl derivative **6i** ($EC_{50}$ 10 nM, 92% efficacy) is a potent, full agonist, whereas the 4-methyl derivative **6b** has partial agonist activity, which indicates that the 3-position can synergize with the 4-substituent to boost agonist activity. Both the *cis*- and *trans*-3-methyl-4-(3,3,3-trifluoropropyl)-derivative (**6n** and **6m**, respectively) were devoid of significant agonist activity, but were moderately active as antagonists.

Three tetracyclic analogues were evaluated. Both the *cis*- and *trans*-3,4-cyclohexyl analogs (**17** and **21**, respectively) are very potent and efficacious agonists. The activity of these analogues is consistent with **4**, **6k** and **6l**, which suggests a binding pocket which accomodates rings or short side chains at the 3- and 4-position. The comparable activity of the *cis*- and *trans*-analogues implies that this region of the pharmacophore could be further optimized by additional SAR investigations.

**Conclusion:** A series of compounds based on 4-alkyl and 3,4-dialkyl-1,2,3,4-tetrahydro-6-(trifluoromethyl)-8-pyridono[5,6-*g*]quinoline were shown to be potent androgen receptor agonists using cell-based assays. The SAR about this series demonstrates that excellent AR agonist potential for these analogs reside in the 3,4-disubstituted analogs, particularly with short alkyl substituents or cyclic derivatives. These compounds show AR agonist activities comparable to DHT in cotransfection and binding assays and show promising potential as nonsteroidal agonists for androgen replacement therapy.

**References and Notes**

1. Present Address: The R. W. Johnson Pharmaceutical Research Institute, 3535 General Atomics Court, San Diego, CA 92121
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4. Present Address: Vertex Pharmaceuticals, Medicinal Chemistry, Cambridge, MA 02139.
5. Bagatelli, C.J.; Bremner, W. J. *N. Engl. J. Med.* **1996**, *334*, 707.
6. Bhasin, S.; Storer, T. W.; Berman, N; Yarasheski, K. E.; Clevenger, B; Phillips, J; Lee, W, P.; Bunnel, T.J.; Casaburi, R. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 407.

7. (a) Ishak, K. G.; Zimmerman, H. J. *Sem. Liver Dis.* **1987**, *7*, 230; (b) Heywood, R.; Chesterman, H.; Ball, S. A; Wadsworth, P. F. *Toxicology* **1977**, *7*, 357.
8. Dalton, J. T.; Mukherjee, A.; Zhu, Z.; Kirkovsky, L.; Miller, D. D. *Biochem. Biophys. Res. Comm.* **1998**, *244*, 1.
9. Edwards, J. P.; West, S. J.; Pooley, C. L. F.; Marschke, K. B.; Farmer, L. J.; Jones, T. K. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 745.
10. Hamann, L. G.; Mani, N. S.; Davis, R. L.; Wang, X. N.; Marschke, K. B.; Jones, T. K. *J. Med. Chem* **1999**, *42*, 210.
11. Negro-Vilar, A; Ojeda, S. R.; McCann, S. M. *Endocrinology*, **1973**, *93*, 729.
12. Hamann, L. G.; Higuchi, R. I.; Zhi, L.; Edwards, J. P.; Wang, X.-N.; Marschke, K. B.; Kong, J. W.; Farmer, L. J.; Jones, T. K. *J. Med. Chem.* **1998**, *41*, 623.
13. Mori, M.; Uesaka, N.; Shibasaki, M. *J. Chem. Soc. Chem. Commun.* **1990**, 1222.
14. Preparation of **6i** and **6j** from **9**: A solution of **9** (1.37 g, 4.34 mmol) in 15 mL THF was added to a 1.0 M solution of vinylmagnesium bromide (13 mmol) in THF at 0 °C and warmed to rt over 0.5 h. The reaction was quenched with 40 mL water, neutralized with 10% $NaHSO_4$, extracted with ether (3 x 30 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (10-25% EtOAc/hexane gradient) afforded the desired allyl alcohol (1.33 g, 89% yield) as a yellow oil. This material was taken up in 20 mL EtOAc and treated with 10% Pd on carbon (0.1 g) for 0.75 h under a hydrogen atmosphere. The reaction mixture was filtered through Celite and concentrated to afford **10** (R = Et, 1.30 g, 97% crude yield) as a yellow gum, which was carried on without further purification. The crude tertiary alcohol (1.30 g, 3.76 mmol) was treated with $Et_3SiH$ (3.01 mL, 18.8 mmol) and TFA (1.45 mL, 18.8 mmol) in 20 mL refluxing 1,2-dichloroethane for 1 h. The reaction was quenched with 100 mL water, neutralized with saturated $NaHCO_3$, extracted with chloroform (2 x 100 mL), dried ($MgSO_4$) and concentrated to afford 1.46 g of a brown oil. A 0.9 g portion of the crude was purified by column chromatography (5-10% EtOAc/hexane gradient) to give the desired diastereomeric alkanes (1.5:1 *trans:cis*) (0.62 g, 81% yield) as a yellow oil. **10** (0.55 g, 1.67 mmol) was treated with 20 ml of a (3:1) AcOH/HCl mixture and heated to 100 °C for 20 h. The reaction was cooled and poured into 150 mL water. The mixture was neutralized with $K_2CO_3$ and extracted with ether (3 x 100 mL) and dried ($MgSO_4$). Removal of solvent and column chromatography gave the desired tetrahydroquinolines (0.155 g, 53% yield) as a yellow oil. This material was converted to the Knorr substrate **11** according to the general nitration and hydrogenation procedure in 60% overall yield as previously described.[12] **11** (0.050 g, 0.26 mmol) was treated with $ZnCl_2$ (0.054 g, 0.39 mmol), ethyl-4,4,4-trifluoroacetoacetate (0.042 mL, 0.29 mmol), and 4 Å molecular sieves (~0.06 g) in 3 mL benzene. The reaction mixture was cooled to rt, and TsOH (0.013 g, 0.07 mmol) in 1 mL EtOH was added, and the mixture heated at reflux for 10 h. The reaction mixture was quenched with 25 mL water, extracted with EtOAc (3 x 20 mL) and dried ($MgSO_4$). Removal of solvent and column chromatography (50–100% EtOAc/hexane gradient,) afforded a 1.5:1 *trans:cis* mixture of the desired 2-pyridones (0.046 g, 56% yield). These isomers were separated by reverse-phase HPLC (ODS column, 5μm, 10 x 250 mm, 3 mL/min, 75% MeOH:$H_2O$). **6i** (*trans*): $t_R$ 19 min (HPLC); $^1$H NMR (400 MHz, $CDCl_3$) 11.83 (br s, 1H), 7.34 (br s, 1H), 6.66 (s, 1H), 6.37 (s, 1H), 4.55 (br s, 1H), 3.50 (dd, 1H, $J$ = 11.8, 3.7), 3.03 (d, 1H, $J$ = 11.7), 2.41–2.34 (m, 1H), 2.10–2.01 (m, 1H), 1.65–1.49 (m, 2H), 1.01–0.90 (m, 6H). **6j** (*cis*): $t_R$ 23 min (HPLC); $^1$H NMR (400 MHz, $CDCl_3$) 11.54 (br s, 1H), 7.30 (br s, 1H), 6.64 (s, 1H), 6.31 (s, 1H), 4.57 (br s, 1H), 3.35–3.27 (m, 1H), 3.15 (dd, 1H, $J$ = 10.5, 10.5), 2.57–2.50 (m, 1H), 2.21–2.13 (m, 1H), 1.67–1.57 (m, 1H), 1.43–1.32 (m, 1H), 1.02 (d, 3H, $J$ = 6.1), 0.96 (t, 3H, $J$ = 7.6).
15. (a) Kelly, T. R.; Field, J. A.; Li, Q. *Tetrahedron Lett.* **1988**, *29*, 3545. (b) Gurnos, J. In *Quinolines Part I.*; Gurnos, J., Ed.; John Wiley and Sons: New York, 1977; Ch. 2, p 93.
16. Nose, A.; Kudo, T. *Chem. Pharm. Bull.* **1988**, *36*, 1529.
17. Ninomiya, I.; Yamauchi, S.; Kiguchi, T.; Shinohara, A.; Naito, T. *J. Chem. Soc. Perkin Trans. 1* **1974**, 1747.