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THE ANTIINFLUENZA ACTIVITY OF PYRROLO[2,3-*d*]PYRIMIDINES

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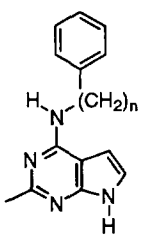
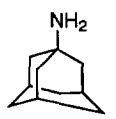
Abstract. From a group of pyrrolo[2,3-*d*]pyrimidine compounds that have been screened against influenza virus, one derivative, 4-(3-piperidinyl benzylamino)-2-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**9**), has shown promising activity against both the A and B strains. The compound had activity comparable to amantadine, but was inactive when given orally. 4-(Substitutedphenyl ethylamino)-2-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidines showed no improved activity.

Introduction. Random antiviral screening of varied chemical classes in the Burroughs Wellcome registry has uncovered interesting activity against influenza virus in the 2-methyl-4-substituted pyrrolo[2,3-*d*]pyrimidine series, which prompted us to synthesize a variety of these compounds.

Chart 1



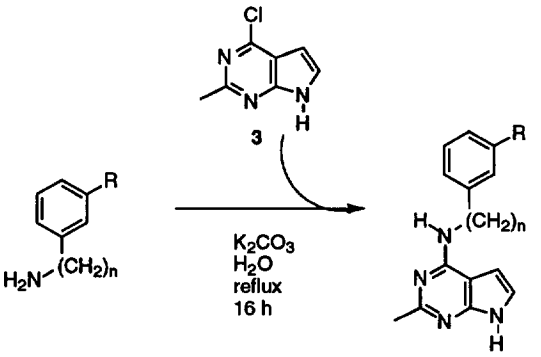
1, n = 1
2, n = 2



Amantadine

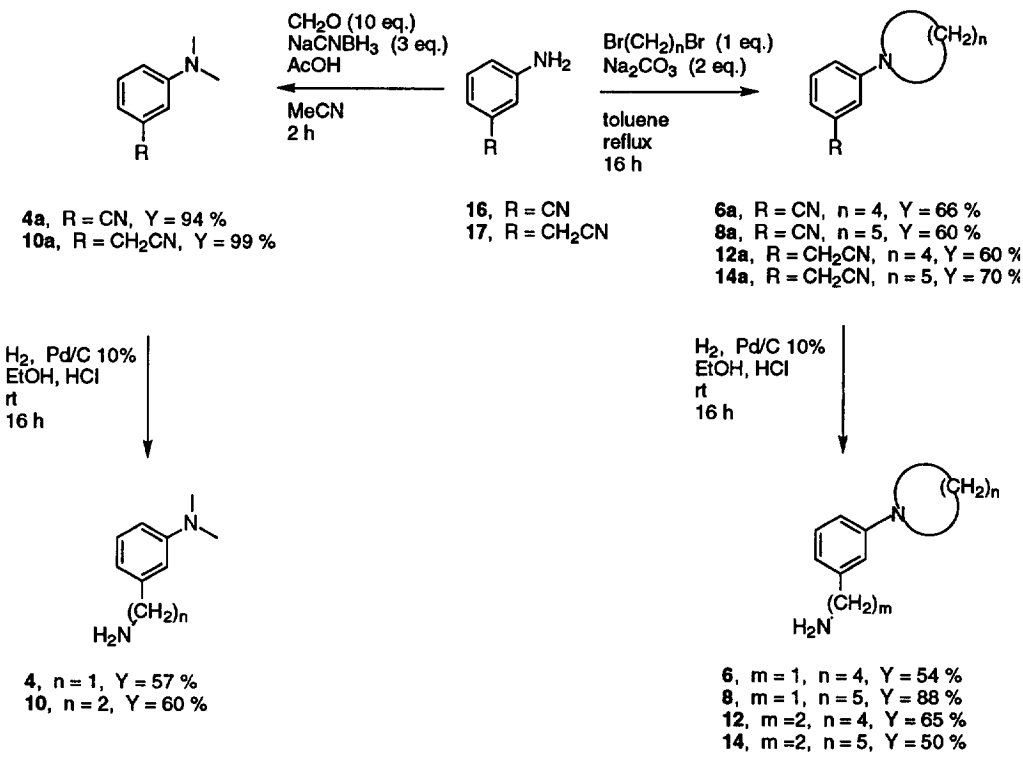
Chemistry. The substituted 4-benzylamino-2-methylpyrrolo[2,3-*d*]pyrimidines[1] were prepared by displacement of the chloro group of 4-chloro-2-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine[2] (**3**) in refluxing water with the requisite benzylamine in the presence of K_2CO_3 (Scheme 1). The benzylamines were obtained in multistep syntheses, as illustrated in Scheme 2. The (3-dimethylamino) benzylamine **4** was obtained in two steps from 3-amino benzonitrile (**16**) by treating this material with CH_2O and $NaCNBH_3$ in MeCN[3] followed by reduction of the resulting nitrile under hydrogenation conditions. The 2-(3-dimethylaminophenyl)ethylamino compound **10** was obtained in a similar way from **17**. The pyrrolidine and piperidine derivatives **6**, **8**, **12**, and **14** were obtained from the corresponding nitriles (**16** or **17**). Treatment of either **16** or **17** with 1,4-dibromobutane or 1,5-dibromopentane in the presence of base afforded the corresponding pyrrolidine and piperidine nitrile derivatives (**6a**, **8a**, **12a**, and **14a**). Hydrogenation of these intermediates afforded the desired amines in reasonable yields.

Scheme 1



4	R = $N(CH_3)_2$, n = 1	**5**, Y = 71 %
6	R = pyrrolidinyl, n = 1	**7**, Y = 82 %
8	R = piperidinyl, n = 1	**9**, Y = 95 %
10	R = $N(CH_3)_2$, n = 2	**11**, Y = 66 %
12	R = pyrrolidinyl, n = 2	**13**, Y = 62 %
14	R = piperidinyl, n = 2	**15**, Y = 50 %

Scheme 2



Results and Discussion. Of the compounds screened to date in vitro[4] against influenza, the most active compound was **9** (Table 1), which showed inhibition of both the A and B viral strains. This compound had comparable activity to amantadine,[5] the drug currently used for influenza A infections. However, administered by the oral route to mice,[6] **9** had no antiviral effect. Since one of the reasons for this difference may have been extensive metabolism by the oral route, we examined the effect of incubation of **9** with S9 rat liver extracts for four hours at 37 °C.[7] The HPLC pattern of the supernatant indicated several metabolites were formed. Possible structural assignments, as determined by GC/MS analyses, include hydroxylation of the piperidino group in one or more sites and oxidative cleavage of the piperidino ring. LD_{50} determinations (mice) of **9** gave values of >500 mg/kg by both oral and ip routes.

From the results in Table 1 it can be seen that compound **2**, with an extended carbon chain, had better activity than **1**. Also, all the meta substituted analogues of **1** (**5**, **7**, and **9**) showed much improved antiviral activity. To determine whether the combination of an extended carbon chain with a properly substituted aromatic ring would further increase anti-influenza activity, we prepared analogues of **2** with the corresponding meta-substituted aromatic ring: **11**, **13**, and **15**. Although these compounds showed some activity, the improvement was not as dramatic as in the case of the benzyl analogues of **1**.

Table 1. Influenza A/B *in vitro* testing results.

	IC_{50} (μM)	
#	A	B
Amantadine	1.25	
1	>100	
5	18	<100
7	4.8	6.4
9	2.3	5.1
2	38.4	
11	55.0	
13	23.9	
15	28.6	

Conclusions. Compound **9** is the most active anti-influenza derivative of this limited series, having activity against both A and B strains in vitro. Activity in vivo was not confirmed in a murine species, possibly due to extensive first pass metabolism. Since a suspension of **9** in aqueous methyl cellulose (0.25%) was used in the animal testing, alternate formulations of **9** in solution may improve absorption and efficacy.

Acknowledgments. The authors wish to thank the NMR and MS staff of the Bioanalytical Sciences Division for obtaining spectra and interpretation and to P. Chandrasurin and J. Cichetti for S9 metabolism and LC/MS studies.

References and Notes.

1. Compounds **5**, **7**, **9**, **11**, **13**, and **15** were fully characterized by CHN analysis, ^{1}H-NMR and mass spectra. Melting points are: **5**: 192-196 °C, **7**: 218-221 °C, **9**: 193-195 °C, **11**: 160-162 °C, **13**: 170-173 °C, **15**: 180-182 °C.

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6. Compound **9** was administered by oral gavage to a group of 5 male 20 g Balb/c mice in three doses of 50 mg/Kg. Animals were dosed 4 h before aerosol challenge with A/Sweden/50 and 4 and 20 h after challenge. A positive control group were given 50 mg/Kg of Amantadine, and a negative control group were untreated. All animals were killed at 24 h and the lunge removed for virus titration. Compound **9** had no antiviral effect.

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