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UTILIZATION OF WIELAND FUROXAN SYNTHESIS FOR PREPARATION OF 4-ARYL-1,2,5-OXADIAZOLE-3-YL CARBAMATE DERIVATIVES HAVING POTENT ANTI-HIV ACTIVITY

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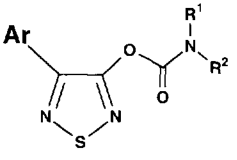
Abstract The classical Wieland furoxan synthesis was reinvestigated and this procedure was applied to the preparation of 4-aryl-1,2,5-oxadiazole-3-yl *N,N*-dialkylcarbamate derivatives, which were found to exhibit potent anti-HIV-1 activity. Copyright © 1996 Elsevier Science Ltd

Several structurally diverse anti-HIV chemotherapeutic agents belonging to the non-nucleoside family, such as HEPT,[1] nevirapine,[2] loviride,[3] and pyridone derivative,[4] have been found and developed as selective and potent inhibitors of HIV-1 reverse transcriptase (RT). We have recently found that 4-aryl-1,2,5-thiadiazole-3-yl *N,N*-dialkylcarbamate (TDA) derivatives (**1**) are also potent and specific inhibitors of HIV-1 replication in vitro.[5, 6] In general, rapid emergence of drug-resistant mutants as well as cross-resistant mutants among these non-nucleoside HIV-1 specific RT inhibitors have been observed. This prompted us to develop structurally modified TDA families, and furthermore, to improve the hydrophilicity of the TDA derivatives having a relatively high-binding property to human serum protein, we planned the synthesis of TDA analogs possessing the 1,2,5-oxadiazole nucleus (**2**). In 1903, Wieland reported the novel reaction of styrene with sodium nitrite to produce 3-phenyl-4-nitrofuroxan.[7] Recently, Kunai et al. reported utilization of this product for the synthesis of *E*-2-hydroxyimino-2-phenylacetonitrile.[8] We reinvestigated the features of this classical Wieland reaction and applied this procedure to the preparation of 4-aryl-1,2,5-oxadiazole-3-yl *N,N*-dialkylcarbamate derivatives (**2**), which were found to exhibit potent anti-HIV-1 activities. We now describe our preliminary results concerning the synthesis and anti-HIV activity of this new class of compounds.



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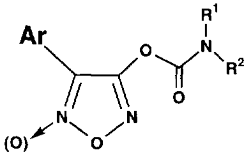
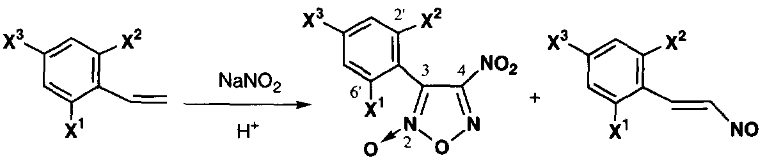


2

According to the reported procedure developed by Wieland,[7] styrene (**3**) was treated with sodium nitrite under acidic conditions (acetic acid and aqueous hydrochloric acid) to yield 3-phenyl-4-nitrofuroxan (**7**) (mp. 101.5-102°C, lit mp. 100-101.5°C) as the sole isolatable product in 51% yield.[9] Next, 2-chlorostyrene (**4**) and 2,6-dichlorostyrene (**5**) were chosen as substrates for the Wieland reaction, since the halogen atom(s) on the benzene ring in TDA derivatives had been found to be effectively enhance the antiviral activity.[5, 6] In case of 2-chlorostyrene, the desired furoxan (**8**) (mp. 60-64°C)[10] was obtained in 65% yield by employing the Wieland procedure. However, 2,6-dichlorostyrene afforded the furoxan derivative (**9**; mp. 125-126°C) in 16.4% yield together with a non-furoxanoid compound (**10**; mp. 64-65°C) in 28% yield. The ^{1}H-NMR spectrum [δ 8.25 (1H, d, J=14 Hz) and δ 7.85 (1H, d, J=14 Hz)] and other spectroscopic data of (**10**) revealed the structure of the major product to be *trans*-2-(2,6-dichlorophenyl)nitroethylene, which was confirmed by X-ray analysis.[11] 2,4,6-Trimethylstyrene (**6**) also gave the furoxan (**11**) and 2-nitrostyrene type compound (**12**) in 16% and 30% yields, respectively. These results indicated that the existence of substituents at both the C2' and C6' positions on the benzene ring caused the steric hindrance that interfered with the formation of the furoxan derivatives.



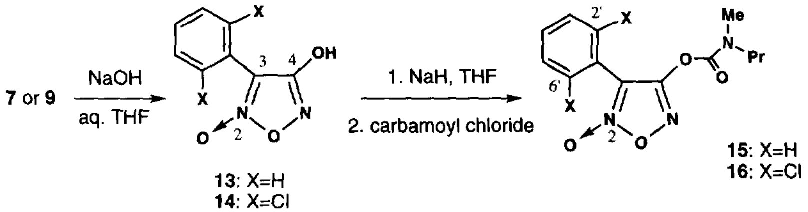
3: $X^1=X^2=X^3=H$
4: $X^1=Cl, X^2=X^3=H$
5: $X^1=X^2=Cl, X^3=H$
6: $X^1=X^2=X^3=Me$

7: $X^1=X^2=X^3=H$
8: $X^1=Cl, X^2=X^3=H$
9: $X^1=X^2=Cl, X^3=H$
11: $X^1=X^2=X^3=Me$

10: $X^1=X^2=Cl, X^3=H$
12: $X^1=X^2=X^3=Me$

Next, we turned our attention to the preparation of 4-aryl-1,2,5-oxadiazole-3-yl *N,N*-dialkylcarbamates from furoxans. 4-Nitoro compounds (**7** and **9**) were respectively converted to the 4-hydroxy derivatives (**13**) {mp. 130-134°C, (lit.[7] mp. 133°C), 93% yield} and (**14**) (mp. 155-156°C, 82% yield) by treatment with sodium hydroxide in tetrahydrofuran . The introduction of a carbamoyl moiety at the hydroxyl group was achieved by treatment with sodium hydride and *N*-methyl-*N*-propylcarbamoyl chloride. Under these reaction conditions, the carbamoyl group was site-selectively introduced to produce **15** and **16**, whose structures were confirmed based on X-ray crystallography of **16**.[11] After several attempts we found that the *N*-oxide in **15** could be removed by treatment with triethylphosphite[12] (neat) at 150°C to furnish the 1,2,5-oxadiazole (**17**) in 58% yield. By employing the same reaction conditions, the oxygen atom at the *N*-2 position in **16** could not be reduced, resulting in the recovery of the starting material, probably due to steric hindrance by the 2',6'-dichloro substituents.

Table 1 summarizes the anti-HIV-1 activity and cytotoxicity of the compounds synthesized in this study and of the related compounds. MT-4 cells and HIV-1 (HTLV-IIIB strain) were used in the anti-HIV-1 assay. 3-Phenylfuroxan derivative (**15**) exhibited antiviral activity (50% effective concentration (EC_{50})=20.0 μM). By removal of the *N*-2 oxygen in furoxan (**15**), the activity increased 10-fold (**17**: EC_{50}=2.0 μM), which is comparable to that of the corresponding thiadiazole derivative (**18**). Similar the TDA series, the introduction of the chlorine atom on the benzene ring significantly enhanced the activity (**16**: EC_{50}=0.12 μM). These results indicate that oxadiazole derivatives together with the TDA family may contribute to the development of potent anti-HIV chemotherapeutics. Further studies are in progress in our laboratories to gain a more precise structure-activity relationship for this class of compounds.



13: X=H
14: X=Cl

15: X=H
16: X=Cl

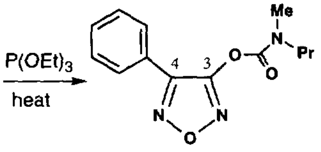


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Table 1. Inhibition of HIV-1 Replication in MT-4 Cells

compounds	EC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]
15	20.0	25.0	1
17	2.0	13.0	6.5
16	0.12	8.6	72
18	5.6	46.8	8
19	0.013	131	10000
AZT	0.004	3.2	800

[a] 50% effective concentration based on the inhibition of HIV-1-induced cytopathic effect in MT-4 cells. [b] 50% cytotoxic concentration based on the reduction of viability in mock-infected MT-4 cells. [c] selectivity index based on the ratio of CC_{50} to EC_{50}. All data represent mean values of at least two separate experiments.

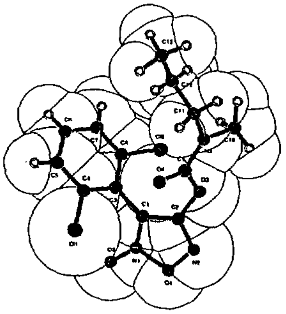
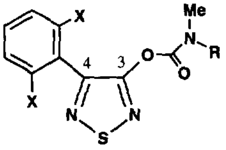


Fig. 1 X-ray structure of compound **16**



18: X=H, R=Me
19: X=Cl, R=Pr

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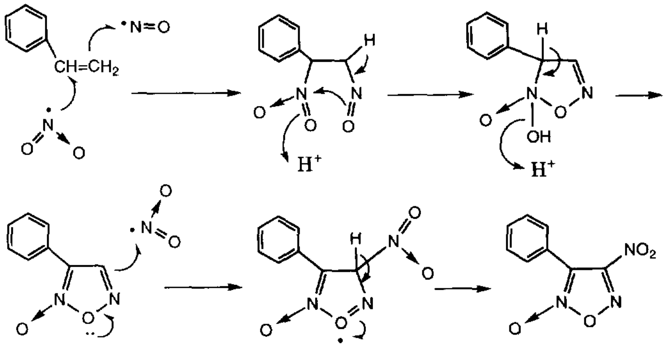
9. A plausible mechanism for the formation of furoxan from styrene.

$$NaNO_2 \xrightarrow{H^+} 2HNO_2 \longrightarrow \dot{N}O + \dot{N}O_2 + H_2O$$



Nitrofuroxan

10. All new compounds gave satisfactory spectral and analytical (combustion or high resolution mass spectral) data consistent with the structures shown.
11. Crystallographic data for compound **10** (mp. 64-65°C, $C_8H_5Cl_2NO_2$). A colorless prismatic crystal was mounted on a glass fiber. The space group is $P2_1/c$. Cell constants are a=3.847(2), b=19.876(3), c=11.968(2)Å, β=90.64(2)°, V=914.9(5)Å^3, Z=4. All measurements were made using a Rigaku AFC5S diffractometer with graphite monochromated Cu-Kα radiation with the ω-2θ scan technique to a maximum 2θ value of 120.1°. Of the 1649 reflections which were collected, 1418 were unique. The structure was solved by direct methods (SHELXS86) and expanded using a Fourier technique (DIRDIF92). The final R factor is 0.078 (Rw=0.112). Crystallographic data for compound **16** (mp. 85-86°C, $C_{13}H_{13}Cl_2N_3O_4$). A colorless prismatic crystal was mounted on a glass fiber. The space group is P1. Cell constants are a=8.464(1), b=11.490(2), c=8.067(1)Å, α=91.03(1)°, β=90.64(2)°, γ=83.61(1)°, V=779.3(2)Å^3, Z=2. All measurements were made using a Rigaku AFC5S diffractometer with graphite monochromated Cu-Kα radiation with the ω-2θ scan technique to a maximum 2θ value of 131.9°. Of the 2519 reflections which were collected, 2332 were unique. The final R factor is 0.057 (Rw=0.059).The refined fractional atomic coordinates, the bond lengths, the bond angles, and thermal parameters of compounds **10** and **16** have been deposited at the Cambridge Crystallographic Data Centre.
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