Synthesis, X-ray Crystal Structure and Biological Activities of α-Phenoxyl-1,2,3-thiadiazoleacetamide

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Received May 13, 2003

The annelation of 1,2,3-thiadiazole rings was accomplished by the reaction of *N*-acylhydrazone **2a** bearing an adjacent **α**-methyl with thionyl chloride to give α-chloro-*N*-methyl-1,2,3-thiadiazole-4-acetamide **4** and was demonstrated by the X-ray crystal structure of its derivative **5a**. A novel series of α-substituted phenoxy-*N*-methyl-1,2,3-thiadiazole-4-acetamide **5** were synthesized through the reaction of the compound **4** and phenols. The results of bioassays show that the title compounds exhibit good anti-HBV activities. The crystal of compound **5a**, *N*-methyl-**α**-2-bromophenyl-1,2,3-thiadiazole-4-acetamide, has been prepared and determined by X-ray diffraction.

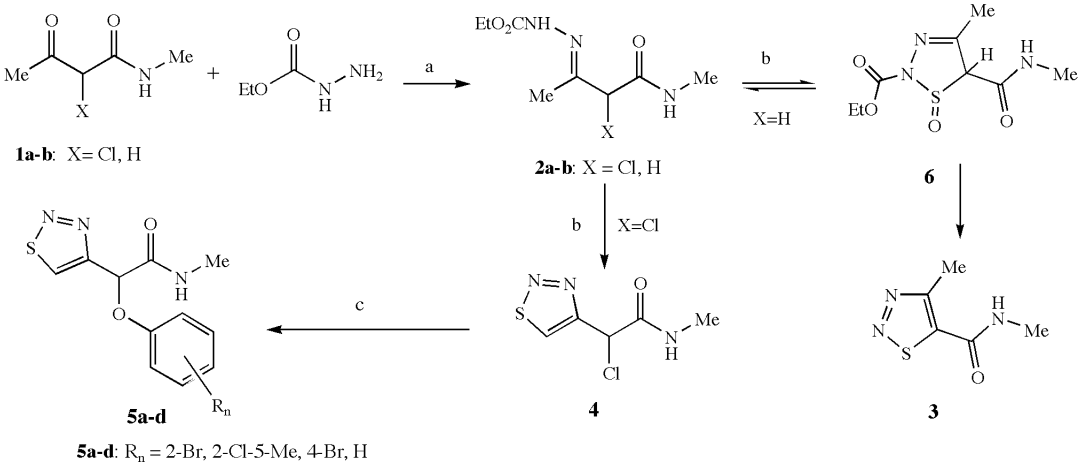
J. Heterocyclic Chem., **40**, 925 (2003).

Hepatitis B virus (HBV) is a major cause of acute and chronic liver disease worldwide. Chronic infection with this virus often leads to chronic liver disease, including cirrhosis or primary hepatocellular carcinoma [1]. Approximately 30% of the world's population, or about 2 billion persons, have serologic evidence of hepatitis B virus (HBV) infection. Of these, an estimated 350 million have chronic HBV infection, and at least 1 million chronically infected persons die each year from chronic liver disease. New infection with HBV can be prevented by vaccination. However, the present vaccination is not effective for chronic carriers worldwide. **α**-Interferon and nucleoside play important role in HBV chemotherapy. However, use of **α**-interferon is limited, the success rate is low, and serious side effects are observed [2,3]. Recently, nucleoside analogues, such as famciclovir, an analogue structurally similar to acyclovir and lamivudine have been approved for the treatment of HBV infection. Unfortunately, most patients experience a rebound in viremia once the therapy is stopped [4]. In addition, anti-HBV nucleosides are often burdened with difficult and costly synthetic preparations. There is a clear need for a non-nucleoside therapeutic for HBV infection [5].

Herein we reported a series of substituted **α**-phenoxyl-1,2,3-thiadiazoleacetamides that have been found to possess potent antihepatitis B activity and describe a facile method for synthesis of 1,2,3-thiadiazole-4-acetamide.

In the investigation of new agrochemicals, we found that 1,2,3-thiadiazole derivatives have good biological activities [6,7,8,9,10], such as, 1,2,3-thiadiazole-5-formamides possessed excellent fungicidial activity [11], We became interested in making 1,2,3-thiadiazoleacetamide analogues of the lead 1,2,3-thiadiazole-4-formamide, due to their high fungicide activity. Their antiviral activity against HBV was discovered by the random screening in the Hep-G2 cells assay. The *N*-acylhydrazone **2a**, which was easily

Scheme 1



(a) ethanol, rt, 18 h; (b) $SOCl_2$, 5 ºC, rt, 24 h, (c) NaOH, methanol, rt, 48 h

Synthesis of 1,2,3-thiadiazole-4-acetamide

prepared from commercially available *N*-methyl-α-chloroacetoacetamide and ethyl carbazate in ethanol, **2** was cyclized with three equivalents of thionyl chloride at room temperature to form α-chloro-*N*-methyl-1,2,3- thiadiazole-4-acetamide **4** in accord with the Hurd-Mori reaction conditions. Compound **4** reacted with substituted phenols in presence of inorganic base to yield α-substituted phenoxy-*N*-methyl-1,2,3-thiadiazole-4-acetamide **5**. The reaction sequence is outlined in Scheme 1.

It is known that the Hurd-Mori reaction is the most convenient methodology for construction of 1,2,3-thiadiazole rings in which α-methylene ketones or aldehydes are starting substrates [12]. Regioselectivity in ring closure plays a role in formation of 1,2,3-thiadiazole, and cyclization predominates at the more reactive methylene site rather than the methyl site [13,14]. If X = H, **2** leads exclusively to product **3**. When X = Cl, the α-chloro-1,2,3-thiadiazole-4-acetamide **4** was obtained in a good yield without by-product being detected. Hurd [15] and Butler [16] reported that an unstable intermediate **6** may be formed. We speculate that the b step is reversible. Herein the annelation of 1,2,3-thiadiazole ring by the reaction of *N*-acylhydrazone bearing an adjacent α-methyl instead of α-methylene with thionyl chloride was expected, when X = Cl. This could be confirmed by the fact that the methyl absorption peak is not observed and the hydrogen absorption peak of 1,2,3-thiadiazole ring is observed (δ = 8.67 ppm) in the ^{1}H NMR spectra of compound **4**. Furthermore, the structure is verified by X-ray crystallography of compound **5a**, derivative of compound **4**. Since knowledge of the stereochemistry is useful in the rational design of pharmaceuticals, the refined small molecule X-ray crystal structure of **5a** is shown in Figure 1. Figure 2 shows the packing diagram of compound **5a**. The crystal structure obviously indicated that annelation of the 1,2,3-thiadiazole ring occurred by the reaction of *N*-acylhydrazone bearing an adjacent α-methyl with thionyl chloride. Strange enough, neither intramolecular nor intermolecular hydrogen bonds were found in the crystal structure of compound **5a**.

The α-substituted-*N*-methyl-1,2,3-thiadiazole-4-acetamides **5** have been found to exhibit significant anti-HBV activity in human hepatoblastoma-derived liver Hep-G2 cells (2.2.15 cells). The inhibition rate of compound **5a** at 0.6 μmol/mL was 88.6% against HBsAg and 15.5%

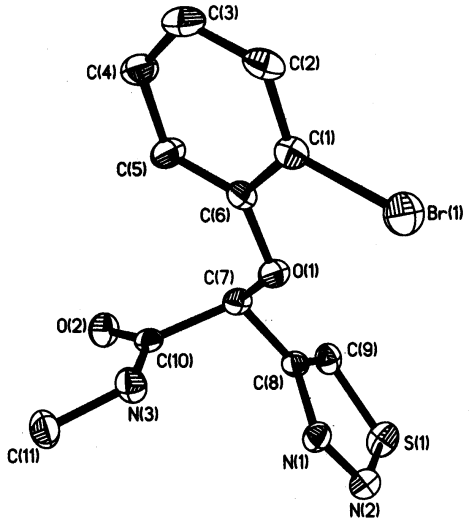


Figure 1. X-Ray crystal structure of compound **5a**.

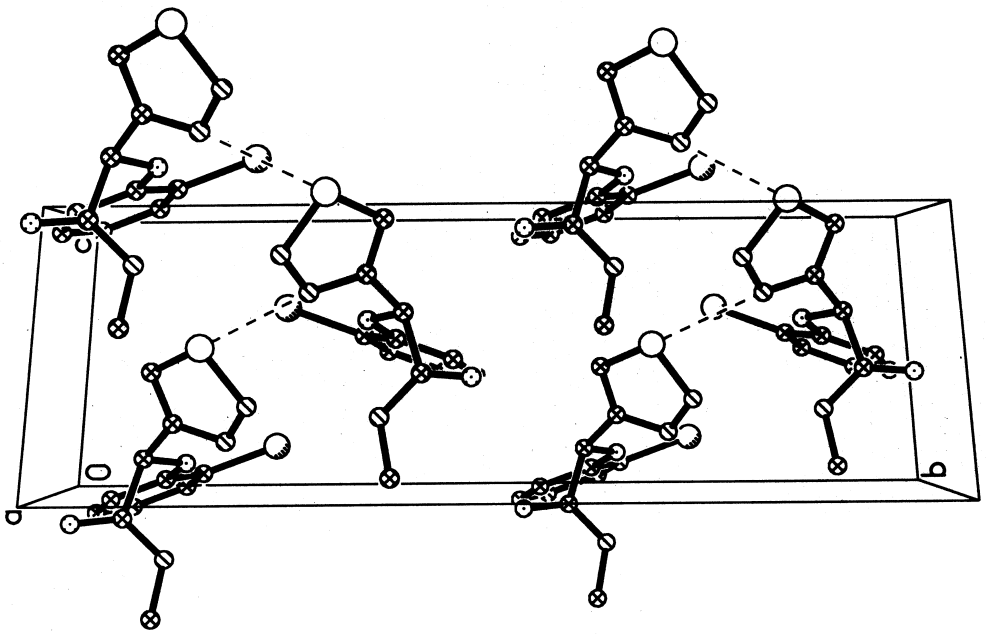


Figure 2. The packing diagram of compound **5a**.

against HBeAg. In contrast, that of purine acyclic acycolvir at 0.4 μmol/mL was 52.9% against HBsAg and 44.2% against HBeAg for comparison of their anti-HBV potency. And compound **5** was also found to have very low acute oral toxicity. The average LD_{50} values for female chmice and male chmice were 4300 and 2330 mg/Kg.

Compounds **5** were also tested against other viruses, and showed high antiviral activity against *tobacco mosaic virus*. These results are promising.

In summary, we have shown that α-substituted-*N*-methyl-1,2,3-thiadiazole-4-acetamides were potent inhibitors of HBsAg in 2.2.15 cells, and were readily synthesized from the commercially available *N*-methyl-α-chloroacetoacetamide and thionyl chloride by the annelation at the α-methyl. Low cost, low oraltoxicity and the high anti-HBsAg activities of these compounds offer significant potential for them as a non-nucleoside therapy for HBV infection. Further studies on anti-HBV activities of the title compounds **5** are under investigation and will be reported in due course.

EXPERIMENTAL

^{1}H NMR spectra were acquired using a Bruker AC-P200 spectrometer. Tetramethylsilane (TMS) was used as an internal standard. Elemental analyses were carried out on a Yanaco MT-3 instrument. Melting points were determined with a model Yanaco MP-500 apparatus and are uncorrected. Mass spectra were recorded on a HP 5989 instrument (EI). IR spectra were obtained on a Shimadzu-435 spectrometer.

The Synthesis of Compound **2a**.

A solution of ethyl carbazate (0.125 mol) in 10 mL anhydrous ethanol was added dropwise to a solution of α-chloro-*N*-methyl-acetoacetamide **1** (0.125 mol) in 60 mL anhydrous ethanol. The mixture was stirred at room temperature for 18 h and the solvent was evaporated. The resultant solid was recrystallized from THF to give compound **2a**, m.p. 120-121°, yield 77%; ^{1}H NMR ($CDCl_3$): δ 1.30 (t, *J* =7.2 Hz, 3H, CH_3), 1.90 (s, 3H, CH_3C=N), 2.87 (d, *J* =4.6 Hz, 3H, NCH_3), 4.25 (q, *J* =6.9Hz, 2H, CH_2), 5.71 (s, 1H, CHCl), 6.76 (br., 1H, NH), 7.92 (br., 1H, NH).

Anal. Calcd. for $C_8H_{14}ClN_3O_3$: C, 40.77; H, 5.99; N, 17.83. Found: C, 40.78; H, 5.58; N, 17.64.

α-Chloro-*N*-methyl-1,2,3-thiadiazole-4-acetamide (**4**).

Compound **2a** (0.089 mol) was added in portion to 25 mL thionyl chloride at 5°. After 2 hours, the mixture was stirred at room temperature over night. The mixture was added dropwise to 500mL saturated sodium bicarbonate solution, the precipitate was collected by filtration and then recrystallized from methanol to give compound **4**, m.p. 144-145°, yield 85%, ^{1}H NMR ($CDCl_3$): δ 2.93 (d, *J* =4.8Hz, 3H, NCH_3), 5.91 (s, 1H, CHCl), 6.96 (br. 1H, NH), 8.67 (s, 1H, ring-H).

Anal. Calcd. for $C_5H_6ClN_3OS$: C, 31.34; H, 3.16; N, 21.93; Found: C, 31.45; H, 2.89; N, 21.77.

The Synthesis of α-Substitued Phenyloxy-*N*-methyl-1,2,3-thiadiazole-4-acetamides **5a-d**.

The compound **4** (3.6 mmol) was added to a mixture of sodium hydroxide (4 mmol), the appropriate substituted phenol (4 mmol) and methanol (15 mL). The reaction mixture was stirred for 48 hours at room temperature. After removal of solvent, the residue was chromatographed on silica gel column eluting with ethyl acetate/petroleum ether (1/3) to give pure products.

For compound **5a** a suitable single crystals for X-ray diffraction were grown from anhydrous alcohol by slow evaporation at room temperature; m.p. 148-149°, yield 39%; ^{1}H NMR ($CDCl_3$): δ 2.95 (d, *J* =5.0, 3H, NCH_3), 6.24 (s, 1H, CH), 6.91-7.58 (m, 4H, Ar-H), 7.28 (br. 1H, NH), 8.63 (s, 1H, ring-H).

Anal. Calcd. for $C_{11}H_{10}BrN_3O_2S$: C, 40.26; H, 3.07 N, 12.80. Found: C, 40.29; H, 3.08; N, 12.90.

Compound **5b** was obtained in 25 % yield, m.p. 134-135°; ^{1}H NMR ($CDCl_3$): δ 2.89 (s, 3H, CH_3), 2.91 (d, *J* =5.0Hz, 3H, NCH_3), 6.11 (s, 1H, CH), 6.74-7.24 (m, 3H, Ar-H), 6.98 (br. 1H, NH), 8.62 (s, 1H, ring-H); MS (12eV): m/z 299 (M^+, 12, ^{37}Cl), 297 (M^+, 34, ^{35}Cl), 242 (4), 240 (11), 142 (100), 128 (82).

Anal. Calcd. for $C_{12}H_{12}ClN_3O_2S$: C, 48.40; H, 4.06; N, 14.11. Found: C, 48.54; H, 3.91; N, 13.90.

Compound **5c** was obtained in 46% yield, m.p. 140-142°; ^{1}H NMR ($CDCl_3$): δ 2.91 (d, *J* =4.8 Hz, 3H, NCH_3), 6.12 (s, 1H, CH), 6.85-7.39 (q, 4H, Ar-H), 6.93 (br. 1H, NH), 8.63 (s, 1H, ring-H); MS (12eV): m/z 329 (M^+, 13, ^{81}Br), 327 (M^+, 13, ^{79}Br), 272 (7), 270 (8), 174 (29), 172 (31), 128 (100).

Anal. Calcd. for $C_{11}H_{10}BrN_3O_2S$: C, 40.26; H, 3.07 N, 12.80. Found: C, 40.41; H, 2.77; N, 12.61.

Compound **5d** was obtained in 29% yield, m.p. 140-141°; ^{1}H NMR ($CDCl_3$): δ 2.91 (d, *J* =5.0Hz, 3H, NCH_3), 6.16 (s, 1H, CH), 6.95-7.30 (m, 5H, Ar-H), 6.98 (br. 1H, NH), 8.62 (s, 1H, ring-H).

Anal. Calcd. for $C_{11}H_{11}N_3O_2S$: C, 53.00; H, 4.45; N, 16.86. Found: C, 53.18; H, 4.46; N, 16.60.

Crystal Structure Determinations and Refinements.

A single crystal with dimensions of $0.30 \times 0.25 \times 0.20$ mm was selected. Lattice constant of compound **5a** and diffracted intensities were measured with a Bruker Smart 1000 CCD area detector system using graphite monochromatized Mo-*K*α radiation (λ = 0.71073Å). An ω scan mode was employed for the collection of data in the range $2.00° \le \theta \le 26.42°$. A total of 2892 independent reflections were collected, of which 1949 reflections with $I > 2\sigma(I)$ were considered to be observed and were used in the succeeding refinement. The structure was solved by direct methods and refined on F^2 by full-matrix least-squares procedures using the SHELXL-97 package. The crystal structure ($C_{11}H_{10}BrN_3O_2S$, Mr = 327.18) is monoclinic, space group Cc, with unit cell parameters *a* = 9.262(3), *b* = 20.328(7), *c* = 7.661(3) Å, α = 90°, β = 120.413(5)°, γ = 90°, *Z* = 4, *V* = 1244.0(7) Å^3, *F* (000) = 652. Goodness-of-fit on F^2 is 0.925. The measure minimum and maximum transmission factors form corrections are 0.4226 and 0.5438, respectively. The maximum peak in the final difference Fourier map is 0.326 and the minimum one -0.310 e/Å^{-3}. All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were located in successive difference Fourier syntheses and refined. The final refinement including hydrogen atoms was converged to *R* = 0.0377 and *w*R = 0.0664. The atomic coordinate and equivalent isotropic displacement parameters are listed in Table 1. The interatomic bond distances and bond angles are given in Table 2 and Table 3, respectively.

Table 1

Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$)

Atom	x	y	z	U_{eq}
Br(1)	1774(1)	7615(1)	6491(1)	58(1)
S(1)	9710(2)	8221(1)	10115(2)	49(1)
O(1)	3970(5)	8564(2)	6027(6)	36(1)
O(2)	5689(5)	9710(2)	4107(6)	45(1)
N(1)	7108(6)	8002(2)	6921(7)	36(1)
N(2)	8533(6)	7738(2)	8128(7)	45(1)
N(3)	4453(7)	8721(2)	2787(7)	42(1)
C(1)	1247(7)	8477(3)	5498(8)	36(1)
C(2)	-289(8)	8735(3)	4925(9)	44(2)
C(3)	-683(9)	9374(3)	4183(10)	49(2)
C(4)	493(8)	9739(3)	4058(10)	50(2)
C(5)	2058(8)	9490(3)	4650(9)	43(2)
C(6)	2455(7)	8856(3)	5378(8)	32(1)
C(7)	5307(7)	8977(2)	6266(8)	32(1)
C(8)	6890(7)	8603(3)	7527(7)	31(1)
C(9)	8244(7)	8801(3)	9303(9)	39(2)
C(10)	5156(7)	9171(3)	4231(8)	32(1)
C(11)	4244(9)	8842(3)	787(9)	55(2)

U_{eq} is defined as one third of the trace of the orthogonalized U_{ij} tensor

Table 2

Bond Lengths (Å)

Bond	Dist.	Bond	Dist.
Br (1)-C (1)	1.874(5)	C (1)-C (2)	1.363(7)
S (1)-C (9)	1.663(6)	C (1)-C (6)	1.399(7)
S (1)-N (2)	1.670(5)	C (2)-C (3)	1.391(8)
O (1)-C (6)	1.365(7)	C (3)-C (4)	1.360(9)
O (1)-C (7)	1.429(6)	C (4)-C (5)	1.377(9)
O (2)-C (10)	1.223(6)	C (5)-C (6)	1.379(7)
N (1)-N (2)	1.284(6)	C (7)-C (8)	1.493(7)
N (1)-C (8)	1.357(6)	C (7)-C (10)	1.545(7)
N (3)-C (10)	1.326(7)	C (8)-C (9)	1.363(7)
N (3)-C (11)	1.464(7)		

Table 3

Bond Angles (°)

Bond angles	(°)	Bond angles	(°)
C (9)-S (1)-N (2)	92.5 (3)	O (1)-C (6)-C (1)	116.5 (5)
C (6)-O (1)-C (7)	117.1 (4)	C (5)-C (6)-C (1)	118.9 (5)
N (2)-N (1)-C (8)	114.0 (4)	O (1)-C (7)-C (8)	106.7 (4)
N (1)-N (2)-S (1)	111.7 (4)	O (1)-C (7)-C (10)	113.1 (4)
C (10)-N (3)-C (11)	120.4 (5)	C (8)-C (7)-C (10)	109.9 (4)
C (2)-C (1)-C (6)	120.4 (5)	N (1)-C (8)-C (9)	112.7 (5)
C (2)-C (1)-Br (1)	120.3 (4)	N (1)-C (8)-C (7)	120.3 (5)
C (6)-C (1)-Br (1)	119.2 (4)	C (9)-C (8)-C (7)	127.0 (5)
C (1)-C (2)-C (3)	120.4 (6)	C (8)-C (9)-S (1)	109.2 (4)
C (4)-C (3)-C (2)	118.9 (6)	O (2)-C (10)-N (3)	126.9 (5)
C (3)-C (4)-C (5)	121.6 (6)	O (2)-C (10)-C (7)	118.3 (5)
C (4)-C (5)-C (6)	119.8 (6)	N (3)-C (10)-C (7)	114.9 (5)
O (1)-C (6)-C (5)	124.6 (5)		

Acknowledgments.

The authors are indebted to Dr. Fajun Nan, Ms. Zhen Qian and National Center for Drug Screening of China for their technical assistance in performing the biological assays, and the National Natural Science Foundation of China (20202003) and "863" program (2001AA235011) and Nankai University for financial support.

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