CHARACTERIZATION AND ANTIMALARIAL ACTIVITY OF FOUR ACETYLATED PYRIMETHAMINES

Nancy Acton and Arnold Brossi*

Medicinal Chemistry Section, Laboratory of Chemistry, NIAMDD,

National Institutes of Health, Bethesda, Maryland 20205

and

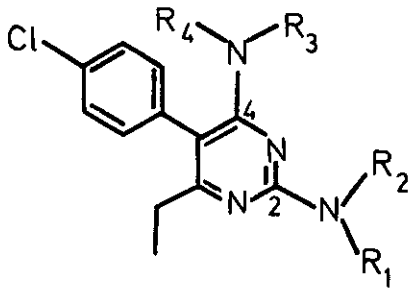
David E. Davidson, Jr., and Thomas R. Sweeney

Walter Reed Army Institute of Research, Walter Reed Army

Medical Center, Washington, D. C. 20012

Abstract - Acetylation of the antimalarial drug pyrimethamine afforded four different N-acetyl derivatives which were characterized. These Derivatives do not show sustained release activity but they are active antimalarials per se.

Although 2,4-diamino-pyrimidines constitute valuable chemotherapeutic agents, relatively little is known about the chemical and biological properties of N-acyl derivatives.[1,2] We now would like to report the preparation and characterization of four different N-acetyl derivatives of the antimalarial drug pyrimethamine (1) belonging to this class of compounds. A summary regarding the in vivo antimalarial activity of these N-acetyl derivatives is included.



Pyrimethamine	1:	$R_1 = R_2 = R_3 = R_4 = H$
Diacetyl-Py	2:	$R_1 = R_3 = H$, $R_2 = R_4 = Ac$
Acetyl-Py	3:	$R_1 = R_3 = R_4 = H$, $R_2 = Ac$
Triacetyl-Py	4;	$R_1 = H$, $R_2 = R_3 = R_4 = Ac$
Tetraacetyl-Py	5:	$R_1 = R_2 = R_3 = R_4 = Ac$

When pyrimethamine (1) was refluxed in a mixture of acetic acid and acetic anhydride (1:1) the diacetyl derivative 2 was obtained. When 1 was treated with acetic anhydride and pyridine (1:1) at room temperature, a mixture of the monoacetyl derivative 3 and the triacetyl derivative 4 was obtained, and separated into the individual compounds by crystallization. A mixture of 3 with 4 kept in pyridine and acetic anhydride (1:1) for a long period of time afforded the tetraacetyl derivative 5. The four acetylated pyrimethamines could be interconverted as follows: Treatment of the monoacetyl derivative 3 with refluxing acetic anhydride-acetic acid mixture afforded the diacetyl derivative 2, which could be converted in a separate treatment with acetic anhydride-pyridine into the triacetyl derivative 4. Hydrolysis of triacetyl derivative 3 and tetraacetyl derivative 5 with basic alumina in CH_2Cl_2 solution at room temperature afforded the diacetyl derivative 2. The latter represents, therefore, the easiest acetyl derivative of pyrimethamine to be obtained.

The four acetylated pyrimethamines (2-5) have sharp melting points, gave correct combustion analyses (C,H,N,Cl) and dissolve - much better than pyrimethamine - in a variety of organic solvents such as ethyl acetate, chloroform and acetone. Their structures were secured by the following methods: The similarity between the UV absorption of 1 (EtOH 290 nm, ε = 9700 and 210 nm, ε = 22,000) and the absorptions of the acetylated derivatives (see exp. part) made it unlikely that ring acetylation had occured. This was further substantiated by the easy interconversions of 3 with 2 and 4 upon further acetylation and 4 and 5 with 2 by mild hydrolysis.[3] The mass and nmr spectra of 2-5 (see exp. part) substantiated the degree of acetylation which had occurred. It is noteworthy that monoacetylation in 2-position gave rise to a singlet at 2.6 ppm (2,3 and 4), lowered by about 0.2 ppm to 2.4 ppm by N-diacetylation (5). A derivative monoacetylated in 4-position had an absorption of about 2.4 ppm (2), again lowered by 0.2 ppm to 2.2 ppm (4 and 5) by N-diacetylation. That acetylation in the monoacetyl derivative 3 had occurred at the 2-amino group was demonstrated by a comparison of the pK_a values of 3 (5.80)[4,5] with that of reference compounds: 4-Aminopyrimidine (5.64), pyrimethamine (7.32), 2,4-diamino-pyrimidine (7.23) and 2-amino-pyrimidine (3.65).

The triacetyl derivative had acetyl singlets at δ 2.18 (6 protons) and at 2.58 (3 protons), whereas the acetyl singlet in 3 was at δ 2.62.

This established structure 4 for the triacetyl analog.

Biological Data - The four acetylated pyrimethamines were compared with pyrimethamine (1) in mice infected with a lethal inoculum of Plasmodium berghei (KBG 173), using the screening method of Osdene, Russell and Rane.[6] All four derivatives (2-5) exhibited antimalarial activity by the oral and subcutaneous routes of administration.[7] They were, like pyrimethamine, less toxic and more active by the subcutaneous route. Subcutaneously, all acetylated derivatives were curative, but not quite as active as pyrimethamine itself. Orally, none of the acetylated

derivatives were curative, but all produced significant prolongation of the survival time indicating antimalarial activity. The reduced toxicity and antimalarial activity suggested that perhaps these derivatives were more slowly released from the subcutaneous injection site. When given orally and subcutaneously to mice on day zero and challenged with parasitized erythrocytes obtained from Plasmodium berghei (KBG 173) at 7 or 14 days, none of these compounds displayed respository antimalarial activity, compared with the positive results obtained with 2,4-diamino-6-(2-napthylsulfony)-quinazoline (WR158,122), an antifolate that has fair repository antimalarial activity.

Although it seems unlikely that simple N-acylation of 2,4-diaminopyrimidines, such as pyrimethamine, will afford compounds showing sustained release, it seems noteworthy that antimalarial activity is retained. This together with the finding that the solubilities are drastically changed upon acetylation, makes N-acylation of this class of compounds an interesting tool.

Experimental - Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were performed by the Microanalytical Laboratory of NIAMDD,NIH. *NMR spectra were obtained with a Varian HR 220 spectrometer.* A Beckman IR 4230 spectrophotometer was used to obtain infrared spectra. Mass spectra were determined on a Finnegan 1015 D (chemical ionization) instrument. Ultraviolet-visible spectra were measured using a Beckman DB-G grating spectrophotometer.

Diacetyl-Py 2 - Pyrimethamine (3.0 g, 12.1 mmol) in 15 ml acetic acid and 15 ml acetic anhydride was stirred at reflux for 1.5 hr. Excess acetic acid - acetic anhydride was removed under reduced pressure, and the residue washed with ether, then recrystallized from ethyl acetate to give 2.3 g (57%) of diacetate, mp 174-176°. Anal. ($C_{16}H_{17}ClN_4O_2$) C,H,Cl,N. Pnmr (220 MHz, $CDCl_3$): δ 1.14 tr, J=7.5 Hz (3.07 H, CH_3); 2.36 s (2.82 H, CH_3CO); 2.46 d, J=7.5 Hz (1.99 H, CH_2); 2.59 s (3.15 H, CH_3CO); 7.16 d, J=8.5 Hz (2.04 H, Ar); 7.44 d, J=8.5 Hz (2.04 H, Ar); 8.06 br s (0.91 H, NH); 8.99 br s (0.95 H, NH). IR (cm^{-1}, $CHCl_3$): 3425 w, 3390 m, 2985 w, 1715 sh, 1689 s, 1684 s, 1580 s, 1464 s, 1418 m, 1372 s, 1279 s, 1091 m, 1036 w, 1008 m, 996 m. λ_{max} (EtOH): 280 sh (ε10,330), 247 sh (ε20,100), 225 (ε34,410) 205 sh (ε17,300). Mass (CI, NH_3): M^+ = 333-335.

Diacetyl-Py 2 - (604 mg) was dissolved in warm pyridine (5 ml) and acetic anhydride (5 ml). After 43 hr at room temperature, residual pyridine and acetic anhydride were removed by addition of toluene followed by evaporation under reduced pressure (2 times). Recrystallization of the residue from ethyl acetate gave 400 mg of triacetyl-Py mp 168 - 170.5°, identical with 4 by IR and by TLC ($CHCl_3$ - MeOH, 10:1).

Mono and Triacetyl-Py 3 and 4:- Pyrimethamine (5.0 g, 20 mmol) was mixed with 20 ml acetic anhydride and 20 ml pyridine. After warming to dissolve the amine, the solution was left overnight at room temperature. Solvents were stripped at reduced pressure, and the residue triturated with ether. After standing for 3 days, the crystalline material was collected, washed with ether, and recrystallized from ethyl acetate - chloroform (9:1) to give 1.84 g (31.5%) of monoacetyl pyrimethamine, mp 205 - 208°. The ethereal mother liquor was concentrated and let stand overnight to give an additional 1.6 g of crystalline material. Recrystallization of this fraction from ethyl acetate gave 987 mg (13%) of triacetyl pyrimethamine, mp 171 - 172°.

Monoacetyl-Py 3:

Anal. ($C_{14}H_{15}ClN_4O$) C,H,Cl,N.

Pnmr (220 MHz, $CDCl_3$): δ 1.11 tr, J=7.5 Hz (3.02 H, CH_3); 2.33 q, J=7.5 Hz (1.96 H, CH_2); 2.62 s (2.99 H, CH_3CO): 7.18 d, J=8.5 Hz (1.99 H, Ar); 7.46 d, J=8.5 Hz (2.18 H, Ar); 10.04 br s (0.87 H, NH).

IR (cm^{-1}, $CHCl_3$): 3509 w, 3436 w, 3311 w, 3205 w, 2985 w, 1715 sh, 1667 s, 1634 m, 1610 m, 1587 s, 1570 s, 1481 w, 1460 m, 1372 m, 1323 s, 1087 m, 1010 w, 990 w.

λ_{max} (EtOH): 285 nm (ε9,900), 250 (ε13,200), 220 (ε29,900). Mass (CI, NH_3): M^+ = 291-293.

Monoacetyl pyrimethamine (193 mg) in 5 ml acetic anhydride and 5 ml acetic acid was stirred at reflux for 1.5 hr. Solvents were stripped, and last traces removed by addition of toluene followed by stripping (2 times). The residue was washed with ether yielding 89 mg of diacetyl pyrimethamine identical with 2 by IR and by TLC. Concentration of the ether filtrate gave an additional 90 mg of less pure 2.

Triacetyl-Py 4:

Anal. ($C_{18}H_{19}ClN_4O_3$)C,H,Cl,N.

Pnmr (220 MHz, $CDCl_3$): δ 1.18 tr, J=7.5 Hz (3.07 H, CH_3); 2.18 s (5.66 H, di-CH_3CO); 2.58 s superimposed on q, J=7.5 Hz (5.28 H, CH_2 and CH_3CO); 6.82 d, J=8.5 Hz (2.02 H, Ar); 7.18 d, J=8.5 Hz (2.02 H, Ar); 8.02 br s (0.96 H, NH). IR (cm^{-1}, $CHCl_3$): 3413 m, 2985 w, 1718 s, 1695 sh, 1582 s, 1543 m, 1477 s, 1412 m, 1372 s, 1092 m, 1013 m, 998 w, 983 w. λ_{max} (EtOH): 280 nm sh (ε6,320), 243 (ε24,280), 208 (ε14,580). Mass (CI, NH_3): M^+ = 375-377.

A mixture of 116 mg (0.31 mmol) of triacetyl pyrimethamine and 1.5 g of Woelm basic alumina in 20 ml of dichloromethane was stirred at room temperature for 5 hr. The mixture was filtered, and the alumina washed several times with chloroform. Evaporation of the filtrate left 101 mg (98%) of diacetyl pyrimethamine, mp 166.5 - 170°, identical with 2 by IR and by TLC (10:1 $CHCl_3$:MeOH).

Tetraacetyl-Py 5 - A mixture of mono- and triacetyl pyrimethamines (6.2 g) was dissolved in 25 ml pyridine and 25 ml acetic anhydride. After standing at room temperature for 2 months, solvents were stripped, and the residue washed with ether. Recrystallization from ether-acetone gave 4.0 g of tetraacetyl pyrimethamine, 5 mp 164 - 166°. Anal. ($C_{20}H_{21}ClN_4O_4$)C, H,Cl,N. Pnmr (220 MHz, $CDCl_3$): δ 1.22 tr, J=8 Hz (2.98 H, CH_3): 2.20 s (5.82 H, di-CH_3CO); 2.39 s (5.82 H, di-CH_3CO): 2.68 q, J=8 Hz (2.17 H, CH_2); 7.12 d, J=8.5 Hz (2.12 H, Ar); 7.70 d, J=8.5 Hz (2.04 H, Ar).

IR (cm^{-1}, $CHCl_3$): 2975 w, 1725 s, 1555 m, 1527 m, 1395 s, 1370 s, 1210 s, 1095 m, 1001 m, 975 m. λ_{max} (EtOH): 285 nm sh (ε2,350), 250 sh (ε9,400), 210 (ε22,400). Mass (CI, NO-N_2): M^+ = 416-418.

A solution of 164 mg of 5 in 20 ml CH_2Cl_2 was stirred with 837 mg of Woelm basic alumina. After 3 days at room temperature, TLC indicated partial conversion to diacetyl pyrimethamine. An additional 1.6 g of alumina was added, and the mixture stirred at reflux for 24 hr. The mixture was filtered, and the filtrate stripped to gave 70 mg of material identical with 2 by IR and by TLC ($CHCl_3$ - MeOH, 10:1).

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