A Versatile Route to Potential Dihydrofolate Reductase Inhibitors *via* the Hitherto Unknown 6-Benzyl-2-(*O*-methyl)uracils: Synthesis of Isotrimethoprim

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The synthesis of 2,4-diamino-6-(3,4,5-trimethoxybenzyl)pyrimidine (isotrimethoprim) has been accomplished starting from 2-(*O*-methyl)-6-(3,4,5-trimethoxybenzyl)uracil by standard procedures. The benzyluracil derivative has been obtained by reacting *O*-methylisourea sulphate and ethyl 3,4,5-trimethoxyphenacetylacetate.

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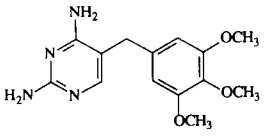
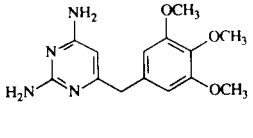
Analogues of trimethoprim (**1**) [1] having the benzyl side-chain attached at 6-position of pyrimidine ring have not yet been studied due to the limited availability of synthetic methods directed towards 6-benzyluracils, key intermediates to the related 2,4-diamino-6-benzylpyrimidines. On the other hand, researches on such diaminopyrimidines are highly desireable to better define the structure-activity relationships in this class of dihydrofolate reductase inhibitors. Prompted by this reason, we decided to synthesize 2,4-diamino-6-(3,4,5-trimethoxybenzyl)pyrimidine (**2**, isotrimethoprim).



1 Trimethoprim

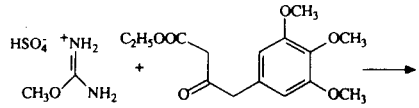


2 Isotrimethoprim

In previous papers [2] one of us described a single high-yielding procedure to obtain 6-substituted uracils starting from a β-ketoester and *O*-methylisourea sulphate. This reaction proved to be general for 6-alkyluracils but failed to give the corresponding 6-phenyl derivative when ethyl benzoylacetate was used as a substrate.

For our purposes we now tried to condensate *O*-methylisourea sulphate with ethyl 3,4,5-trimethoxyphenacetylacetate (**3**) [3] under the same conditions described for the synthesis of 6-alkyluracils [2]. In the present case condensation occurred easily and gave high yields of 6-(3,4,5-trimethoxybenzyl)-2-methoxy-4(3*H*)-pyrimidinone (**4**).

Treatment of **4** with the Vilsmeier-Haack reagent [4] led to the formation of 4-chloro-2-methoxy-6-(3,4,5-trimethoxybenzyl)pyrimidine (**5**), which was then transformed into **2** on reacting with 32% ammonium hydroxide at reflux.



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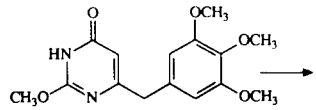

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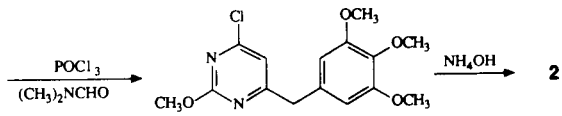

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Derivative **2** is now under investigation to test its potential antibacterial activities in comparison with trimethoprim (**1**).

EXPERIMENTAL

Melting points were determined on an Electrothermal IA 6304 apparatus and are uncorrected. Infrared spectra were run on a Perkin-Elmer 297 spectrophotometer. The ^{1}H-nmr spectra were recorded on a Varian EM-390 spectrometer with TMS as internal standard. Silica gel Merck (70-230 mesh ASTM) was used for chromatographic purifications. Mycroanalyses were performed by A. Pietrogrande, Padova, Italy.

Ethyl 3,4,5-Trimethoxyphenacetylacetate (**3**).

To 20 ml of anhydrous THF under a nitrogen atmosphere with stirring 1 g (7.6 mmoles) of monoethyl malonate and several mg of 2,2'-bipyridyl as an indicator were added. After cooling to −70°, *n*-butyllithium (13.8 ml of 1*M* solution in *n*-hexane, 15 mmoles) was dropped slowly while allowing the temperature to rise to *ca.* −5° near the end of the addition. After the pink indicator persisted at −5° the heterogeneous solution was recooled to −65° and the 3,4,5-trimethoxyphenacetyl chloride (1.86 g, 7.6 mmoles) in THF (60 ml) was added dropwise over 5 minutes. The ice bath was then removed and the reaction mixture was stirred for 1 more hour. The reaction solution was poured into 40 ml of ethyl ether and 20 ml of 1*N* hydrochloric acid was added. After mixing and separating the aqueous layer, the organic phase was washed with saturated sodium bicarbonate (2 x 100 ml) and with water (10 ml), dried over anhydrous sodium sulfate and concentrated *in vacuo*. Purification by chromatography on silica gel column (eluting with *n*-hexane/ethyl acetate 8:2) gave 1 g (46%) of the pure β-ketoester **3**, mp 69-70°; ir (carbon tetrachloride): ν 1720 (CO ketone) and 1745 cm^{-1} (CO ester); ^{1}H-nmr (deuteriochloroform): δ 6.5 (s, 2H, aromatic protons), 3.98-4.32 (q, 2H, J_1 = 7.2 Hz, J_2 = 21 Hz, OCH_2CH_3), 3.84 (s, 2H, benzylic protons) and 1.1-1.35 ppm (t, 3H, J = 7.2 Hz, OCH_2CH_3).

Anal. Calcd. for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80. Found: C, 60.62; H, 6.93.

6-(3,4,5-Trimethoxybenzyl)-2-methoxy-4(3*H*)-pyrimidinone (**4**).

To a well stirred suspension of 440 mg (2.5 mmoles) of *O*-methylisourea bisulfate and 222 mg (3.0 mmoles) of calcium hydroxide in water (5 ml) (basic *p*H), 500 mg (1.7 mmoles) of ethyl 3,4,5-trimethoxyphenacetylacetate (**3**) in ethanol (8 ml) were added. The resulting mixture was stirred at room temperature for 36 hours. Neutralization of the reaction mixture with 1*N* hydrochloric acid, evaporation of most the solvent under reduced pressure and extraction with chloroform of the residue furnished a solid which was purified by chromatography on a silica gel column. Elution with chloroform/methanol (9.5/0.5) afforded 596 mg (78%) of pure **4**, mp 172-174° (chloroform/*n*-hexane); ir (chloroform): ν 3400 (NH), 1660 (CO), 1610 cm^{-1} (CN); ^{1}H-nmr (deuteriochloroform): δ 6.54 (s, 2H, aromatic protons), 5.9 (s, 1H, C_5-H), 4.0 (s, 3H, C_2-OCH_3, 3.85 (s, 9H, aromatic OCH_3), 3.75 ppm (bs, 2H, benzylic protons).

Anal. Calcd. for $C_{15}H_{18}N_2O_5$: C, 58.82; H, 5.92; N, 9.15. Found: C, 58.68; H, 5.96; N, 9.10.

4-Chloro-2-methoxy-6-(3,4,5-trimethoxybenzyl)pyrimidine (**5**).

A mixture of **4** (420 mg, 137 mmoles), anhydrous chloroform (18 ml), anhydrous DMF (0.14 ml) and phosphoryl chloride (0.13 ml, 1.37 mmoles) was stirred under nitrogen atmosphere for 24 hours. When the substrate disappeared (tlc analysis on silica gel with chloroform/methanol:9.9/0.1) the reaction mixture was diluted with chloroform, washed with saturated sodium bicarbonate and brine. The aqueous layer was then extracted with chloroform and the combined extracts were dried over sodium sulfate and evaporated to dryness. Purification of the residue by column chromatography on silica gel (elution with chloroform/methanol:9.9/0.1) gave **5** as an oil (297 mg, 67%); ^{1}H-nmr (deuteriochloroform): δ 6.68 (s, 1H, C_5-H), 6.4 (s, 2H, aromatic protons), 3.98 (s, 3H, C_2-OCH_3), 3.85 (bs, 2H, benzylic protons), 3.75 ppm (s, 9H, aromatic OCH_3).

Anal. Calcd. for $C_{15}H_{17}ClN_2O_4$: C, 55.48; H, 5.28; N, 8.63. Found: C, 55.43; H, 5.31; N, 8.59.

2,4-Diamino-6-(3,4,5-trimethoxybenzyl)pyrimidine (Isotrimethoprim) (**2**).

To a solution of 200 mg (0.6 mmole) of **5** in anhydrous DMF (0.5 ml) concentrated ammonium hydroxide (5 ml) was added. The mixture was refluxed for 12 hours, occasionally saturating it with gaseous ammonia. The reaction mixture was then extracted with chloroform, the organic layer was dried over sodium sulfate and the solvent evaporated *in vacuo*. Purification over preparative tlc (solvent chloroform/methanol:9.5/0.5) gave **2** (142 mg, 82%) as pure crystals, mp 245-246° (from chloroform-*n*-hexane); ir (chloroform): ν 3700 (NH); ^{1}H-nmr (deuteriochloroform): δ 8.94 (bs, 2H, NH_2), 8.71 (bs, 2H, NH_2), 6.4 (s, 2H, aromatic protons), 5.5 (s, 1H, C_5-H), 3.78 (s, 9H, aromatic OCH_3), 2.6 ppm (s, 2H, benzylic protons).

Anal. Calcd. for $C_{14}H_{18}N_4O_3$: C, 57.92; H, 6.25; N, 19.30. Found: C, 57.88; H, 6.28; N, 19.21.

Acknowledgement.

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