Synthesis and Immunomodulating Activity of 5*H*-Thiazolo[2,3-*b*]quinazolin-3(2*H*)-one

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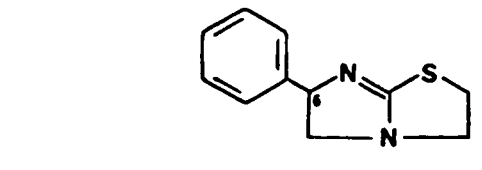
Abstract □ The synthesis and immunomodulating activity of 5*H*-thiazolo[2,3-*b*]quinazolin-3(2*H*)-one (**7**) are described. When tested in the Kennedy plaque assay, **7** exerted immunosuppressive activity on IgM production in female C3H mice sensitized with sheep erythrocytes.

For the last decade or so, the immunopotentiating activity of levamisole (**1**) has been extensively studied.[1] Chemically, levamisole represents an imidazo[2,1-*b*]thiazole ring system having a phenyl ring attached at C-6. Recently,[2] we reported the preparation and biological activity of a series of structurally related analogues, the 2-substituted 2,3-dihydro-5*H*-thiazolo[2,3-*b*]quinazoline derivatives **2** and **3**.

As seen from structures **2** and **3**, the order of the three heteroatoms remains the same as in levamisole (**1**). However, contrary to the latter, derivatives **2** and **3** contain a fused phenyl ring that provides for a rigid tricyclic system. When tested for biological activity, a number of the 2-substituted 2,3-dihydro-5*H*-thiazolo[2,3-*b*]quinazolines **2** and **3** showed anti-inflammatory activity in the carrageenin-induced rat paw edema test.[2] In addition, some of derivatives **2** and **3** were found to display immunomodulating activity in the Kennedy plaque assay. For example,[2] **2a** (**2**: R = $NHCH_2C_6H_5$) and **3a** (**3**: R = OCH_3) significantly suppressed the humoral immunocompetence at doses of 1.56, 6.25, and 25.0 mg/kg; **2b** (**2**: R = 1-adamantanemethylamino) exerted immunosuppressive activity only at the highest dose level (25.0 mg/kg), whereas analogue **2c** [**2**: $N(CMe_3)CH_2C_6H_5$] exhibited a significant immunopotentiation at a dose of 1.56 mg/kg and a significant immunosuppression at the 25.0-mg/kg dose level. Furthermore, both derivatives **2d** (**2**: R = OC_2H_5) and **2e** (**2**: R = $NHCH_2CH{=}CH_2$), although not statistically significant ($p \leq 0.050$), also displayed a tendency towards immunosuppression at a dose of 25.0 mg/kg. From the reported experimental data[2] it becomes evident that changing the 6-phenylimidazo[2,1-*b*]thiazole ring system of levamisole (**1**) to the tricyclic 2,3-dihydro-5*H*-thiazolo[2,3-*b*]quinazoline system of **2** and **3**, resulted in a change in the nature of the immunomodulating activity—from immunopotentiating (in the case of levamisole) to the predominantly immunosuppressive activity of derivatives **2** and **3**.

In consideration of the latter results, it became of interest to us to learn what, if any, change in the degree of immunosuppressive activity of derivatives **2** and **3** would occur from removing the substituent at C-2. In the present communication, we wish to disclose the synthesis and immunomodulating activity of 5*H*-thiazolo[2,3-*b*]quinazolin-3(2*H*)-one (**7**). The preparation of **7** was straightforward and involved an initial lithium aluminum hydride reduction of 2-aminobenzamide (**4**) to provide the corresponding 2-aminobenzylamine (**5**). Following treatment of derivative **5** with thiophosgene, the 3,4-dihydro-2(1*H*)-quinazolinethione (**6**) was obtained.[3] Condensation of the latter with chloroacetic acid in aqueous sodium bicarbonate-*N*,*N*-dimethylformamide solution yielded the 5*H*-thiazolo[2,3-*b*]quinazolin-3(2*H*)-one (**7**; Scheme I).



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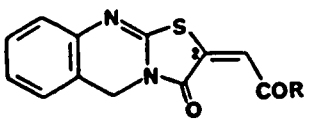

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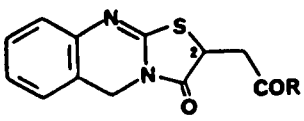


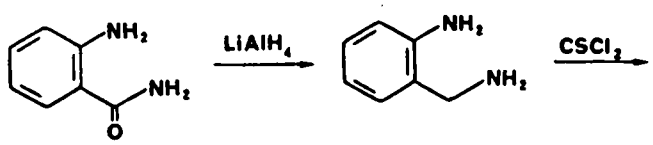
3

Experimental Section

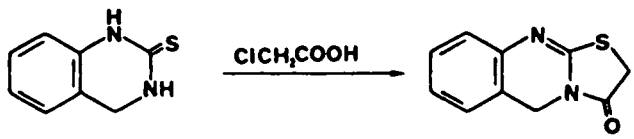
Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The infrared (IR) spectra of the compounds as KBr discs were obtained on a Nicolet MX-1 FT spectrometer. The proton nuclear magnetic resonance (^1H NMR) spectra were obtained on a Varian EM-360A (60 MHz) spectrometer using tetramethylsilane as an internal standard. All spectra were consistent with the assigned structures.

2-Aminobenzylamine (5)—Compound **5** was prepared according to the procedure of Grosso et al.[3] in 95% yield by reducing 2-aminobenzamide (**4**) with lithium aluminum hydride in tetrahydrofuran solution (reflux for 26 h, followed by a workup); mp 59–59.5 °C (lit. mp 58.5–60.5 °C).

3,4-Dihydro-2(1*H*)-quinazolinethione (6)—Derivative **6** was obtained according to the procedure of Grosso et al.[3] by treating a mixture of 2-aminobenzylamine (**5**) and triethylamine in ether



4 **5**



6 **7**

Scheme I

0022-3549/87/0800-0633$01.00/0

solution with thiophosgene at −78 °C; mp 210–212 °C (aqueous ethanol) [lit. mp 204–207 °C (aqueous ethanol)].

5*H*-Thiazolo[2,3-*b*]quinazolin-3(2*H*)-one (7)—A mixture of 1.0 g (6 mmol) of 3,4-dihydro-2(1*H*)-quinazolinethione (**6**), 0.57 g (6.5 mmol) of chloroacetic acid, and 0.54 g (6.5 mmol) of sodium bicarbonate in 31.25 mL of water was heated overnight at 90 °C. Then, 12.5 mL of *N,N*-dimethylformamide was added and the reaction mixture was refluxed for 6.5 h. After cooling, the precipitated solid was filtered off and recrystallized from anhydrous ethanol yielding 0.50 g (41%) of compound 7; mp 203–205 °C; IR (KBr): 3078 and 3045 (=CH), 1724 (with a shoulder at 1710 cm^{-1}; lactam C=O), 1614 (C=N), 1610, 1594, and 1574 (C=C), and 774 cm^{-1} (=CH, phenyl 1,2-substitution); ^1H NMR ($CDCl_3$-TFA): 7.10–7.45 (cm, 4H, aromatic), 5.06 (s, 2H, N-CH_2), and 4.30 ppm (s, 2H, S-CH_2).

Anal.—Calc. for $C_{10}H_8N_2SO$: C, 58.81; H, 3.95; N, 13.72; S, 15.70. Found: C, 59.03; H, 4.12; N, 13.70; S, 15.79.

Kennedy Plaque Assay—The humoral immunocompetence was ascertained using the Kennedy plaque (antibody-forming cells) assay according to Garvey et al.[4] For the IgM production in female C3H mice, the T-dependent antigen sheep erythrocytes (SRBC; 0.2 mL of a 20% suspension) were administered intraperitoneally (ip) on day 0. The mice were treated with 7 (1.56–25.0 mg/kg/d, ip), once daily on days +1, +2, and +3. Twenty four hours after final doses were implemented, the animals were sacrificed, the spleens were removed aseptically, and the number of plaque-forming cells (PFC) were enumerated. The statistical significance (p value) was determined by the analysis of variance, followed by the Newman–Keuls test.[5]

Table I—Immunosuppressive Activity of Compound 7[a]

Compound[b]	Dose, mg/kg/d	Number of mice	Number of Plaque-Forming Cells (PFC)/10^5 Splenocytes ± SE	Response, %
Control (SRBC)	—	16	79.9 ± 2.0	100
7	1.56	8	57.7 ± 5.1	76
	6.25	8	54.6 ± 1.5	72[c]
	25.0	8	61.5 ± 6.1	81
6-Mercaptopurine	60.0	8	27.4 ± 2.5	36.1[c]

[a] Kennedy plaque assay. [b] Compounds were administered intraperitoneally; the observed activity was expressed as a percentage of the control response. [c] The response decreased significantly when compared with the control group; $p \leq 0.050$ using analysis of variance.

Results and Discussion

The 5*H*-thiazolo[2,3-*b*]quinazolin-3(2*H*)-one (7) was evaluated for immunomodulating activity in the Kennedy plaque assay using T-dependent antigen sheep erythrocytes (Table I). As seen from Table I, although 7 exerted a statistically significant ($p \leq 0.050$) immunosuppression only at the middle dose level (6.25 mg/kg), it also tended to suppress the humoral immunocompetence at both the 1.56- and 25.0-mg/kg dose levels, even though a statistical significance was not achieved ($p \leq 0.055$). A conservative two-tail analysis of variance was used in calculating the p values. The higher (25.0 mg/kg) and lower (1.56 mg/kg) doses are not considered statistically significant since their p values were at the 0.055 level and just over the accepted limit of $p = 0.050$.[6] The corresponding p value of the standard immunosuppressive drug 6-mercaptopurine is also listed in Table I. The observed immunosuppressive activity of 7 was approximately equipotent to that of the previously studied 2-substituted 2,3-dihydro-5*H*-thiazolo[2,3-*b*]quinazoline derivatives 2 and 3.[2] Hence, the lack of a substituent at the C-2 position of analogue 7 did not prove to be of significant importance for imparting immunosuppressive activity.[7]

References and Notes

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6. A comparison of the observed immunosuppression at the three dose levels using the analysis of variance and the Newman–Keuls test showed that, in fact, the biological activity of 7 at all three levels may be considered quantitatively similar and thus not dose related.
7. This communication represents part 10 of the series "Drug-Induced Modifications of the Immune Response."