Mesoionic Isoxazolo[2,3-*a*]pyrimidinediones and 1,3,4-Oxadiazolo[3,2-*a*]pyrimidinediones as Potential Adenosine Antagonists

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Several derivatives of two novel mesoionic ring systems, *i.e.*, isoxazolo[2,3-*a*]pyrimidinedione and 1,3,4-oxadiazolo[3,2-*a*]pyrimidinedione, were prepared for evaluation as adenosine antagonists. Whereas both ring systems are relatively stable when the 6-position (*i.e.*, that position corresponding to the purine 1-position) is substituted by an alkyl group, neither ring system is stable when this position is unsubstituted. An example of a 6-unsubstituted non-mesoionic isoxazolopyrimidinedione exhibited similar behavior. As adenosine antagonists, the mesoionic compounds were found to be less potent than their previously evaluated mesoionic thiadiazolo[3,2-*a*]pyrimidinedione counterparts.

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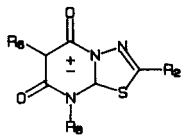
Theophylline, caffeine, and certain other structurally-related alkyl xanthines act as antagonists at adenosine receptors [1]. Several mesoionic xanthine analogs (*e.g.* **1**) share this property [2]. However, whereas the substitution of an alkyl or aryl group at the 8-position of the xanthines increases potency, this property is not shared by the mesoionic xanthines [2]. We postulated that at the receptor, the sharper C-S-C bond angle of **1** might orient the R_2 substituents somewhat differently relative to those of the non-mesoionic xanthines [2]. Thus, it was desired to prepare and evaluate examples of novel mesoionic ring systems such as **2** and **3** where the sulfur atom is replaced by either carbon or oxygen.



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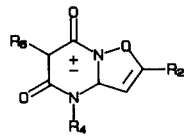

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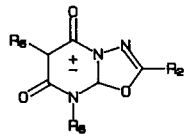

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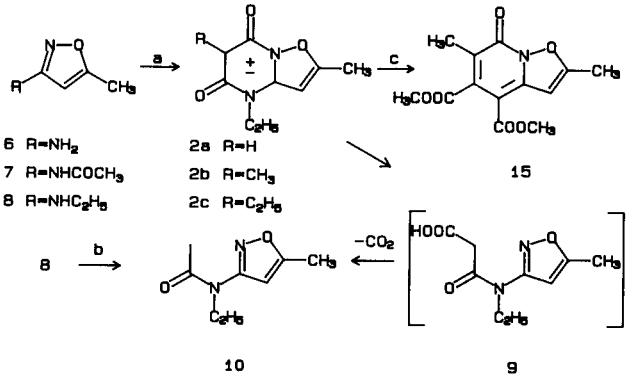
Results and Discussion.

Mesoionic compounds such as **1** are typically prepared by fusion of the appropriately substituted aminoheterocycle with bis-2,4,6-trichlorophenyl esters of malonic acid (*i.e.* **4a**) or of alkyl-substituted malonic acids such as methylmalonic acid or ethylmalonic acid (*i.e.*, **4b** and **4c**, respectively). This method is commonly referred to as the "TCP malonate fusion method". This was the general approach envisioned for use in the present study. However, fusion of **8** with **4** failed to afford the desired products and resulted in decomposition. The situation could not be remedied by variation of the fusion time or temperature. Huhn and co-workers [3] have reported the preparation and application of several bis-pentachlorophenyl esters of malonic acid (*i.e.*, **5**) and have found that these esters are quite reactive at room temperature. The preparation of these esters is briefly described in the patent literature [3] and, to our knowledge, their use in the synthesis of mesoionic compounds such as those described herein has not been previously reported. To assess the application of these esters to the synthesis of mesoionic compounds, two known derivatives of **1** (*i.e.* **1a** and **1b**, where R_6 = H and methyl, respectively and R_2 = H and R_8 = ethyl in both cases) were first synthesized. Due to the success of these reactions, the pentachlorophenyl (PCP) malonate esters were then used in the preparation of **2**. Reaction of **8** with **5b** at room temperature afforded the desired **2b**. Compound **2c** was prepared in like manner using the appropriately substitued ester **5c** (Scheme 1). The PCP malonate condensation method did not work for the preparation of **2a**. Compound **2a** was successfully prepared by the condensation of **8** with carbon suboxide. Bubbling carbon suboxide into an ethereal solution of **8** resulted in the immediate precipitation of a white crystalline material that was homogeneous by thin layer chromatography. Al-

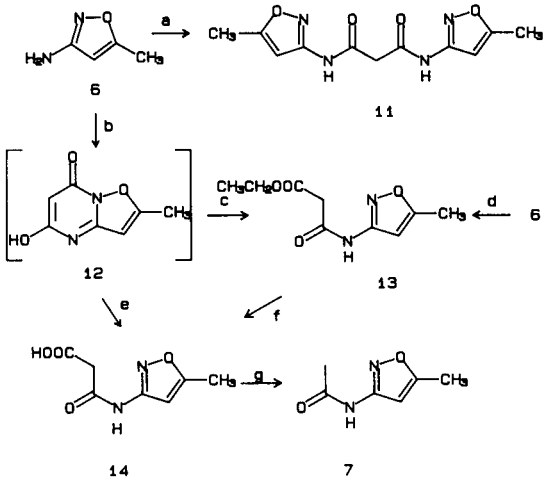


Scheme 1. a = carbon suboxide (for **2a**) or PCP-malonate esters (where PCP = 2,3,4,5,6-pentachlorophenyl) **5b** and **5c** (for **2b** and **2c**, respectively), b = acetyl chloride, triethylamine, c = dimethyl acetylene dicarboxylate.

though spectral data indicated the formation of **2a**, its proposed structure was not supported by elemental analysis. Upon standing (exposed to the atmosphere) overnight, **2a** was slowly converted to an oily substance. This decomposition could be retarded if **2a** was stored in a dessicator (over phosphorus pentoxide) or under nitrogen. Attempted distillation of this oil afforded **10**, whose structure was confirmed by independent synthesis. Presumably, **2a** is unstable and slowly undergoes hydrolytic ring-opening to **9** (see related comments regarding **12**, below); heating of **9** results in decarboxylation to afford **10**.

Fused mesoionic compounds such as **1** typically undergo 1,4-dipolar cycloaddition reactions with, for example, dimethyl acetylene dicarboxylate. Consequently, **2b** was allowed to react with this ester to afford the isoxazolopyridine **15** (Scheme 1).

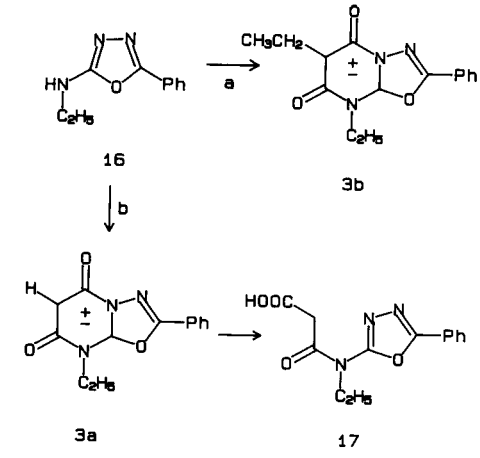
In order to compare the instability of **2a** with that of its non-mesoionic counterpart, attempts were made to prepare **12**. Condensation of **6** with **4a** or with carbon suboxide gave the dimer **11** as the sole product (Scheme 2). Condensation of **6** with the pentachlorophenyl malonate ester **5a** gave a mixture of two products that could be easily separated by their solubility characteristics in acetone. The acetone-soluble material was found to be identical with **11** and the acetone-insoluble material was assigned the structure **12** based on the infrared and magnetic resonance spectra of the crude material. After a single recrystallization from ethanol, however, a new product was obtained which was distinctly different from **12** with respect to spectral and chromatographic properties. For example, the infrared spectrum of crude **12** displayed a single carbonyl band at 1680 cm^{-1} whereas the new product displayed carbonyl bands at 1620 and 1730 cm^{-1}.



Scheme 2. a = TCP-malonate ester (where TCP = 2,4,6-trichlorophenyl) or carbon suboxide, b = PCP-malonate ester, c = ethanol, d = ethyl malonyl chloride, e = 0.01 *N* hydrochloric acid, f = 0.01 *N* sodium hydroxide, g = heat.

Subsequently, this new product (based on analogy to the formation of **9**) was assigned structure **13** and was further identitied by independent synthesis (Scheme 2). Interestingly, treatment of crude **12** with dilute hydrochloric acid affords **14**, which could also be obtained by the hydrolysis of **13**; heating **14** (neat) in an oil bath resulted in decarboxylation to afford **7**. Thus, the behavior of the non-mesoionic isoxazolopyrimidine **12** is quite reminiscent of that of the mesoionic counterpart **2a** and suggests that it is not the mesoionic nature of **2a** that is responsible for its instability.

The mesoionic oxadiazolopyrimidine **3b** was prepared by the malonate fusion method condensing **16** with **4c** (Scheme 3). The malonate fusion method was also successful for the preparation of **3a** (in 31% yield), but **3a** could be prepared in higher yield (70%) by condensation of **16** with carbon suboxide. As with **2a** and **12**, **3a** was found to be unstable and standing exposed to the atmosphere for 24 hours resulted in the formation of **17**. (Alternatively, it is possible that ring-opening could have occurred at the 7-position to give the 3-substituted 2-imino-1,3,4-oxadiazole derivative. It was not possible to gain further insight as to the product of the ring opening reaction by magnetic resonance or ultraviolet studies and structure **17** was assigned based on analogy with **9** and **14**. Attempts to decarboxylate **17** resulted in decomposition).



Scheme 3. a = TCP-malonate ester, b = PCP-malonate ester or carbon suboxide (Ph = phenyl).

A_1-Adenosine receptor (inhibition of tritiated cyclohexyladenosine binding in rat cerebral cortical membranes) and A_2-adenosine receptor (inhibition of 2-chloroadenosine-stimulated accumulation of cyclic adenosine-3′, 5′-monophosphate in tritiated adenine-labelled guinea pig cerebral cortex slices) [2] binding data were obtained for **2b** and **3b**. Both compounds were, as were derivatives of **1**, inactive at the A_2 receptor (at 300 μM) and were less potent (20% inhibition at 300 μM) than the corresponding derivatives of **1** at A_1 sites.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared and ^{1}H-nmr spectra were obtained using a Perkin-Elmer 257 spectrophotometer and a JEOL FX 90Q spectrometer with tetramethylsilane as an internal standard. Mass spectra were obtained using a Finnigan 4015 (70 eV) gas chromatograph/mass spectrometer data system. Elemental analyses were performed by Atlantic Microlab Inc., Atlanta, GA. The synthesis of the bis 2,4,6-trichlorophenyl esters of malonic acid, methylmalonic acid, and ethylmalonic acid (**4a-c**, respectively) [4] and the preparation of carbon suboxide [5] have been previously reported.

Anhydro 8-Ethyl-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-*a*]pyrimidinium Hydroxide (**1a**, $R_2 = R_6 = H$, R_8 = ethyl).

2-Ethylamino-1,3,4-thiadiazole (1.29 g, 1 mmole) and triethylamine (1.5 g, 1.5 mmoles) were added at room temperature to a suspension of **5a** (3.75 g, 6 mmoles) in acetone (25 ml). The mixture, which clarified within 1 minute, was allowed to stir for 1 hour. The solvent was removed under reduced pressure and the residue was triturated with anhydrous ether (20 ml) to give a white crystalline solid which was collected by filtration. Recrystallization from acetonitrile afforded 1.4 g (68%) of **1a** as white crystals, mp 207° (lit [6] mp 208-209°).

Anhydro 8-Ethyl-5-hydroxy-6-methyl-7-oxo-1,3,4-thiadiazolo-[3,2-*a*]pyrimidinium Hydroxide (**1b**, $R_2 = H$, R_6 = methyl, R_8 = ethyl).

The title compound was prepared from 2-ethylamino-1,3,4-thiadiazole (1.29 g, 1.0 mmole) and **5b** (4.3 g, 7 mmoles) using the same method used for the synthesis of **1a** to afford 0.8 g (36%) of **1b** as white crystals after recrystallization from 2-propanol, mp 214-215° (lit [6] mp 214-215°).

Anhydro 4-Ethyl-5-hydroxy-2-methyl-7-oxoisoxazol[2,3-*a*]pyrimidinium Hydroxide (**2a**).

Carbon suboxide [5] was bubbled into a solution of **8** (0.14 g, 1 mmole) in anhydrous ether (10 ml) until no further percipitate formed (*ca* 3 minutes). The white crystalline material was collected by filtration and was washed well with anhydrous ether to afford 0.1 g (52%) of **2a**, mp 135°; ir (potassium bromide): 1690-1630 cm^{-1} (C=O); ^{1}H-nmr (deuteriochloroform): 1.4 (t, 3H, ethyl-CH_3), 2.7 (s, 3H, C2-CH_3), 4.2 (q, 2H, ethyl-CH_2), 5.0 (s, 1H, C6-H), 6.5 (s, 1H, C3-H).

On standing overnight at room temperature, **2a** decomposed to an oil. Heating this oil (0.07 g) under reduced pressure in a Kugelrhor apparatus (in an attempt to effect distillation) resulted in solidification; a crude cream-colored material (mp 185-190°) was isolated. Recrystallization of this product from 95% ethanol afforded 0.03 g of a material (mp 193°) that was found to be identical (mixture melting point, ^{1}H-nmr, ir, thin-layer chromatography) with an authentic sample of **10**.

Anhydro 2,6-Dimethyl-4-ethyl-5-hydroxy-7-oxoisoxazolo[2,3-*a*]pyrimidinium Hydroxide (**2b**).

Compound **8** (0.14 g, 1 mmole) and triethylamine (0.2 g, 2 mmoles) were added at room temperature to a stirred suspension of **5b** (0.61 g, 1 mmole) in acetone (10 ml). The mixture, which clarified within 1 minute, was allowed to stir for 1 hour. The solvent was removed under reduced pressure and the gummy residue was triturated with anhydrous ether to give a white solid. The solid material was collected by filtration and was recrystallized from ethyl acetate to afford 0.04 g (18%) of **2b** as white needles, mp 139-141°; ir (potassium bromide): 1680-1650 cm^{-1} (C=O); ^{1}H-nmr (deuteriochloroform): 1.45 (t, 3H, ethyl-CH_3), 2.05 (s, 3H, C6-CH_3), 2.7 (s, 3H, C2-CH_3O), 4.15 (q, 2H, N-CH_2-), 6.5 (s, 1H, C3-H); ms: m/z 208.

Anal. Calcd. for $C_{10}H_{12}N_2O_3 \cdot 0.5H_2O$: C, 55.29; H, 6.03; N, 12.90. Found: C, 55.48; H, 5.69; N, 12.69.

Anhydro 4,6-Diethyl-5-hydroxy-2-methyl-7-oxoisoxazolo[2,3-*a*]pyrimidinium Hydroxide (**2c**).

Compound **2c** was prepared in the same manner as **2b** (using **5c** in place of **5b**) to afford 0.03 g (15%) of a white crystalline product after recrystallization from ethyl acetate, mp 136-137°; ir (potassium bromide): 1670, 1640 cm^{-1} (C=O); ^{1}H-nmr (deuteriochloroform): 1.1 (t, 3H, C6-CH_2-CH_3), 1.4 (t, 3H, N-CH_2-CH_3), 2.5 (q, 2H, C6-CH_2-), 2.7 (s, 3H, C2-CH_3), 4.15 (q, 2H, N-CH_2-), 6.5 (s, 1H, C3-H).

Anal. Calcd. for $C_{11}H_{14}N_2O_3 \cdot 0.25H_2O$: C, 58.26; H, 6.44; N, 12.36. Found: C, 58.30; H, 6.40; N, 12.18.

Anhydro 8-Ethyl-5-hydroxy-2-phenyl-7-oxo-1,3,4-oxadiazolo-[3,2-*a*]pyrimidinium Hydroxide (**3a**) and *N*-Ethyl-*N*-(5-phenyl-1,3,4-oxadiazol-2-yl)malonamic Acid (**17**).

Method A.

Carbon suboxide [5] was bubbled into a solution of 2-ethylamino-5-phenyl-1,3,4-oxadiazole (**16**) (0.2 g, 1 mmole) in anhydrous ether (40 ml) until there was no further formation of a precipitate. The product was collected by filtration and was washed thoroughly with anhydrous ether to afford 0.18 g (70%) of **3a** as a white powder, mp 168-170° (decomposes).

Method B.

A mixture of **16** (0.2 g, 1 mmole) and **4a** (0.46 g, 1 mmole) was heated neat in an oil bath (160°) until a clear melt resulted (*ca* 1 minute). A slow stream of nitrogen was passed over the reaction mixture during the heating period to aid in the removal of 2,4,6-trichlorophenol. When cool, the resultant residue was triturated with anhydrous ether (20 ml) until crystallization occurred. The solid was collected by filtration and was recrystallized from an ethyl acetate/methanol (8:2) mixture to afford 0.08 g (31%) of **3a**, mp 170° dec. The products obtained by the two methods of preparation were identical; ir (potassium bromide): 1700 and 1640 cm^{-1} (C=O); ^{1}H-nmr (deuteriochloroform) 1.55 (t, 3H, N-CH_2-CH_3), 4.35 (q, 2H, N-CH_2-), 5.1 (s, 1H, C6-H), 7.7-8.2 (m, 5H, phenyl).

Upon standing overnight, solid **3a** (0.08 g) decomposed to **17**. Recrystallization of **17** from xylenes gave 0.05 g (58%) of the product as a white powder, mp 175-177°; ir (potassium bromide): 3000 cm^{-1} (broad band, COOH), 1660 cm^{-1} (C=O); ^{1}H-nmr (deuteriochloroform): 1.25 (t, 3H, N-CH_2-CH_3), 3.85 (s, 2H, -CH_2), 4.3 (q, 2H, N-CH_2-), 7.7-8.0 (m, 5H, phenyl), 9.3 (s, 1H, COOH).

Anal. Calcd. for $C_{13}H_{13}N_3O_4$: C, 56.72; H, 4.76; N, 15.26. Found: C, 56.69; H, 4.79; N, 15.17.

Anhydro 6,8-Diethyl-5-hydroxy-2-phenyl-7-oxo-1,3,4-oxadiazolo[3,2-*a*]-pyrimidinium Hydroxide (**3b**).

Compound **3b** was prepared using the same trichlorophenyl malonate fusion method used in the preparation of **3a** (*i.e.* Method B) except that ester **4a** was replaced with ester **4c**. Compound **3b** was obtained in 31% yield, after recrystallization from ethyl acetate, mp 162°; ir (potassium bromide): 1690 and 1660 cm^{-1} (C=O); ^{1}H-nmr (deuteriochloroform): 1.0 (t, 3H, C6-CH_2-CH_3), 1.5 (t, 3H, N-CH_2-CH_3), 2.4 (q, 2H, C6-CH_2-), 4.35 (q, 2H, N-CH_2-), 7.7-8.1 (m, 5H, phenyl).

Anal. Calcd. for $C_{15}H_{15}N_3O_3 \cdot H_2O$: C, 59.41; H, 5.61; N, 13.85. Found: C, 59.68; H, 5.23; N, 13.61.

Bis(2,3,4,5,6-pentachorophenyl) Malonate Esters (**5**).

Method A.

Methylmalonic acid (2.4 g, 20 mmoles) or ethylmalonic acid (3.2 g, 24 mmoles) and thionyl chloride (40 mmoles) were heated at 60° for 30 minutes; pentachlorophenol (10.6 g, 40 mmoles) was added and the reaction mixture was allowed to heat at 80-90° for an additional 4 hours. The solution was allowed to cool to room temperature and the solid product was triturated with acetone (40 ml), collected by filtration, and washed with 95% ethanol to afford 3.5 g (28%) of **5b**, mp 163-164° or 3.5 g (23%) of **5c**, mp 146-148° (lit [3] mp 165-168° and 145-148°, respectively).

Method B.

Compound **5c** was also prepared by a second method. Ethylmalonic acid (3.2 g, 24 mmoles) was added in small portions to a suspension of phosphorus pentachloride (8.9 g, 43 mmoles) in dry benzene (30 ml). The

mixture was allowed to stir at 50° for 2 hours at which time the benzene was removed by distillation *in vacuo*. The residue was dissolved in carbon tetrachloride (30 ml) and added in a dropwise manner to a solution of pentachlorophenol (10.6 g, 40 mmoles) and pyridine (3.2 g) in carbon tetrachloride (30 ml). The reaction mixture was allowed to stir at room temperature overnight and the solid product was collected by filtration and was washed thoroughly with 95% ethanol to afford 2.6 g (17%) of **5c**, mp 147-148°. This latter method was also used to prepare 7 g (24%) of **5a** from malonic acid, mp 198° (lit [3] mp 197°).

3-Ethylamino-5-methylisoxazole (**8**).

A solution of 3-acetamido-5-methylisoxazole (**7**) [7] (5 g, 36 mmoles) in freshly distilled tetrahydrofuran (150 ml) was added dropwise with stirring to a suspension of lithium aluminum hydride (3.5 g, 92 mmoles) at 0°. The mixture was heated at reflux for 5 hours and was then allowed to stir at room temperature overnight (16 hours). A solution of water in tetrahydrofuran (20%, 10 ml), followed by water (10 ml), was added to the stirred mixture at 0° until the evolution of hydrogen ceased. The mixture was filtered and the filtrate was dried (magnesium sulfate) and evaporated to dryness to yield 0.5 g (11%) of **8** as a colorless oil after distillation *in vacuo*, bp 45-50°/0.25 mm Hg. The oily product solidified on standing, mp 58-59°; ir (chloroform): 3300 cm^{-1} (NH); ^{1}H-nmr (deuteriochloroform): 1.1 (t, 3H, $-CH_3$), 2.15 (s, 3H, aromatic-CH_3), 3.15 (q, 2H, $-CH_2-$), 4.95 (br s, 1H, NH exchangeable with deuterium oxide), 5.6 (s, 1H, aromatic-H).

Anal. Calcd. for $C_6H_{10}N_2O$: C, 57.11; H, 7.99; N, 22.20. Found: C, 56.90, H, 8.02; N, 22.13.

N-Ethyl-*N*-(5-methylisoxazol-3-yl)acetamide (**10**).

Acetyl chloride (0.07 g, 1 mmole) was added slowly with stirring to a solution of **8** (0.14 g, 1 mmole) and triethylamine (0.1 g, 1 mmole) in tetrahydrofuran (10 ml) at 0°. The reaction mixture was allowed to stir at room temperature for 16 hours and the solid material was removed by filtration. The filtrate was dried (magnesium sulfate) and evaporated to dryness under reduced presure to afford a white solid product. Recrystallization from 95% ethanol gave 0.06 g (36%) of **10** as white crystals, mp 193-195°; ir (potassium bromide): 1630 cm^{-1} (C=O); ^{1}H-nmr (DMSO-d_6): 1.0 (t, 3H, $-CH_2-CH_3$), 2.0 (s, 3H, $-CH_3$), 2.3 (s, 3H, $-CH_3$), 3.65 (q, 2H, $-CH_2-$), 6.5 (s, 1H, C3-H).

Anal. Calcd. for $C_8H_{12}N_2O_2$: C, 57.12; H, 7.18; N, 16.65. Found: C, 57.45; H, 7.33; N, 16.93.

N,N'-Di(5-methylisoxazol-3-yl)malonamide (**11**).

Method A.

Carbon suboxide [5] was bubbled into a solution of **6** (0.2 g, 2 mmoles) in anhydrous ether to give an instantaneous white precipitate. The solid material was collected by filtration and was recrystallized from methanol to yield 0.1 g (20%) of **11** as white needles, mp 227-229°; ir (potassium bromide): 1700 cm^{-1} (C=O); ^{1}H-nmr (DMSO-d_6): 2.3 (s, 6H, aromatic-CH_3), 3.45 (s, 2H, $-CH_2-$), 6.5 (s, 2H, aromatic-H), 11.0 (br s, 2H, exchangeable with deuterium oxide); ms: m/z 264.

Method B.

A mixture of **6** (0.2 g, 2 mmoles) and bis(2,4,6-trichlorophenyl) malonate (**4a**) (0.94 g, 2 mmoles) was heated neat in an oil bath (160°) until a clear melt resulted (*ca* 30 seconds). When cool, the solid material was triturated with anhydrous ether (10 ml), collected by filtration, and recrystallized from methanol to yield 0.08 g (16%) of **11** as white needles, mp 225-226°. This material was identical with that prepared by Method A.

Anal. Calcd. for $C_{11}H_{12}N_4O_4$: C, 50.00; H, 4.54; N, 21.21. Found: C, 49.96; H, 4.64; N, 21.11.

7*H*-5-Hydroxy-2-methylisoxazolo[2,3-*a*]pyrimidin-7-one (**12**).

Amine **6** (0.2 g, 2 mmoles) and triethylamine (0.4 g, 4 mmoles) were added at room temperature to a stirred suspension of **5a** (1.25 g, 2 mmoles) in acetone (20 ml). Although an immediate precipitate was formed, the reaction mixture was allowed to stir for an additional 30 minutes. The solid material was collected by filtration (evaporation of the filtrate afforded **11**) and was washed thoroughly with acetone to yield 0.08 g (24%) of crude **12**, mp 135°; ir (potassium bromide): 1680 cm^{-1} (C=O); ^{1}H-nmr (DMSO-d_6): 2.35 (s, 3H, $-CH_3$), 4.0 (br s, 2H), 6.5 (s, 1H, aromatic-H). Compound **12** decomposed on standing. Attempts to recrystallize **12** from ethanol afforded **13**.

Ethyl *N*-(5-Methylisoxazol-3-yl)malonamate (**13**).

Method A.

Ethyl malonyl chloride (1.5 g, 10 mmoles) was added in a dropwise manner to a stirred solution of **6** (1.0 g, 10 mmoles) and triethylamine (0.2 g, 20 mmoles) in tetrahydrofuran (20 ml) at 0°. The reaction mixture was allowed to stir overnight (18 hours) and was filtered; the filtrate was dried (magnesium sulfate) and evaporated to dryness under reduced pressure to afford a cream-colored solid. Recrystallization from absolute ethanol/ether (7:3) yielded 0.8 g (38%) of **13** as shiney white crystals, mp 125-127°; ir (potassium bromide): 1730 and 1620 cm^{-1}; ^{1}H-nmr (deuteriochloroform): 1.3 (t, 3H, $-CH_3$), 2.4 (s, 3H, C5-CH_3), 3.5 (s, 2H, $-CH_2-$), 4.2 (q, 2H, $-CH_2$), 6.6 (s, 1H, aromatic-H), 10.1 (br s, 1H, NH exchangeable with deuterium oxide).

Anal. Calcd. for $C_9H_{12}N_2O_4 \cdot 0.5H_2O$: C, 48.86; H, 5.92; N, 12.67. Found: C, 48.85; H, 5.58; N, 12.66.

Method B.

In an attempt to recrystallize **12** from 95% ethanol, **13** was identified by thin layer chromatography. Crude **13** was purified by column chromatography (glass column: 14 x 1 cm; silica gel with chloroform/ethyl acetate (1:1) as eluent) in approximately 40% yield (based on the amount of material placed on the column), mp 123-125°. This product was spectrally and chromatographically identical with that prepared above.

N-(5-Methylisoxazol-3-yl)malonamic Acid (**14**).

Method A.

A solution of **13** (0.1 g, 0.5 mmole) in aqueous 0.01 *N* sodium hydroxide (5 ml) was allowed to stir at room temperature overnight (14 hours). At this time, the reaction mixture was neutralized by the addition of dilute hydrochloric acid and extracted with ether (2 x 15 ml). The combined ethereal extracts were dried (magnesium sulfate) and evaporated to dryness to give crude **14**. Recrystallization of this material from aqueous ethanol (60%) afforded 0.07 g (76%) of **14** as a white powder, mp 160-162°; ir (potassium bromide): 3000 cm^{-1} (broad band, COOH), 1710 and 1680 cm^{-1} (C=O); ^{1}H-nmr (DMSO-d_6): 2.4 (s, 3H, C5-CH_3), 3.4 (s, 2H, $-CH_2$), 4.3 (s, 1H, NH exchangeable with deuterium oxide), 6.6 (s, 1H, aromatic-H), 11.0 (s, 1H, COOH exchangeable with deuterium oxide).

Method B.

A solution of crude **12** (0.05 g) in 0.01 *N* hydrochloric acid (3 ml) was allowed to stir at room temperature. The precipitate which formed within 15 minutes was collected by filtration and recrystallized from aqueous ethanol to afford 0.03 g of **14**, mp 160°. This product was identical with that prepared in Method A above.

Compound **14** (0.1 g) was heated neat in an oil bath (160°) for several minutes until effervescence ceased. The product was recrystallized from ethanol to yield 0.05 g (66%) of a white crystalline solid, mp 183°, which was identified as **7** [7].

2,6-Dimethyl-4,5-dicarbomethoxyisoxazolo[2,3-*a*]pyridin-7-one (**15**).

A solution of **2b** (0.05 g, 0.2 mmole) and dimethyl acetylene dicarboxylate (0.07 g, 0.5 mmole) in chloroform (10 ml) was heated at reflux for 20 hours. The solvent was removed under reduced pressure and the resultant oily residue was triturated with petroleum ether and allowed to stand overnight. The resultant cream-colored solid was collected by filtration and recrystallized from 2-propanol to yield 0.03 g (54%) of **15** as white crystals, mp 122-125°; ir (chloroform): 1720-1700 cm^{-1} (ester

C=O), 1630 cm^{-1} (C=O); ^{1}H-nmr (deuteriochloroform): 1.4 (s, 3H, C6-CH_3), 2.1 (s, 3H, C2-CH_3), 3.9 (s, 6H), 5.3 (s, 1H, aromatic-H).

Anal. Calcd. for $C_{13}H_{13}NO_6$: C, 55.91; H, 4.69; N, 5.02. Found: C, 55.92; H, 4.51; N, 4.96.

2-Ethylamino-5-phenyl-1,3,4-oxadiazole (**16**).

The title compound was prepared by the method of Gehlen and Moeckel [8]. In addition, the title compound was also prepared using a method that parallels that of Hoggarth [9,10] for the synthesis of 2-amino-5-phenyl-1,3,4-oxadiazole; neither of the two intermediates isolated in this procedure were characterized other than by their melting points. 4-Ethyl-3-thiosemicarbazide (5 g, 42 mmoles) and benzoyl chloride (6.3 g, 45 mmoles) were combined and allowed to stir at 0° for 15 minutes. The reaction mixture was warmed to 100° and this temperature was maintained for 1 hour. When cool, the reaction mixture was filtered and the solid material was recrystallized from 95% ethanol to yield 2 g (22%) of 1-benzoyl-4-ethyl-3-thiosemicarbazide as a white crystalline solid, mp 189-192° (lit [11] mp 192-193°). A solution of this material in methanol (50 ml) was stirred in a sealed flask with methyl iodide (2.2 g, 15 mmoles) at 60° for 30 minutes. Methanol was removed under reduced pressure; the solid material was dissolved in water (25 ml) and the solution was adjusted to *p*H 8 by the portionwise addition of solid sodium bicarbonate. The precipitated solid was collected by filtration and recrystallized from an ethyl acetate/ethanol (8:2) mixture to afford 0.5 g (22%) of the *S*-methyl derivative, mp 133-134°. Heating the *S*-methyl compound (1 g, 4.2 mmoles) in an oil bath (90°) for 3 hours resulted in cyclization with loss of methanethiol. The solid residue was recrystallized from benzene to yield 0.5 g (62%) of **16** as shiney, colorless needles, mp 129° (lit [8] mp 128-129°).

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REFERENCES AND NOTES

[1] R. F. Bruns, J. W. Daly and S. H. Snyder, *Proc. Nat. Acad. Sci.* (USA), **77**, 5547 (1980).

[2] R. A. Glennon, S. M. Tejani-Butt, W. Padgett and J. W. Daly, *J. Med. Chem.*, **27**, 1364 (1984).

[3] M. Huhn, E. Somfai, G. Szabo, G. Resofszki and L. Gneth, German Patent 2,627,709, 1976; *Chem. Abstr.*, **86**, 89426 (1977).

[4] T. Kappe and W. Lube, *Monatsh. Chem.*, **102**, 781 (1971).

[5] R. A. Glennon and S. M. Tejani, *Nucleosides Nucleotides*, **3**, 389 (1984).

[6] R. A. Coburn, R. A. Glennon and Z. F. Chmielewicz, *J. Med. Chem.*, **17**, 1025 (1974).

[7] M. M. El-Kerdawy, S. M. Bayomi, I. A. Shehata and R. A. Glennon, *J. Heterocyclic Chem.*, **24**, 501 (1987).

[8] H. Gehlen and K. Moeckel, *Ann. Chem.*, **660**, 144 (1962).

[9] E. Hoggarth, *J. Chem. Soc.*, 1949, 1918.

[10] E. Hoggarth, *J. Chem. Soc.*, 1949, 1163.

[11] M. H. Shah, M. Y. Mhasalkar, V. M. Patki, C. V. Deliwala and U. K. Sheth, *J. Pharm. Sci.*, **58**, 1398 (1969).